

# Prevalence of p53, bcl-2, and Ki-67 Immunoreactivity and of Apoptosis in Normal Oral Epithelium and in Premalignant and Malignant Lesions of the Oral Cavity

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**Purpose:** Loss of normal p53 is correlated to the progression of several preneoplastic lesions to neoplasms, and overexpression of bcl-2 determines an alteration of programmed cell death. There is an increased awareness of the importance of apoptosis in cancerogenesis, and a strong correlation of Ki-67 with high tumor grade has been reported.

**Materials and Methods:** The aim of our study was to investigate immunohistochemically the expression and relationship of p53, bcl-2, MIB-1, and the apoptotic index (AI) in normal oral epithelium, leukoplakia, dysplasia, and oral squamous cell carcinoma.

**Results:** A strong correlation was found between p53 overexpression and cell proliferation (MIB-1) and the AI. An inverse relationship was found between bcl-2 expression and MIB-1 and AI. A significant inverse relationship was found between p53 and bcl-2. A good positive correlation was present between AI and MIB-1 expression.

**Conclusions:** Apoptosis could be important to help to understand oral carcinogenesis.

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There is an increased awareness of the importance of apoptosis in cancerogenesis.<sup>1</sup> Studies of human B-cell

lymphomas (Bcl) led to the discovery of proto-oncogene bcl-2, which is situated on chromosome 18. This 25-kd protein is also located at the mitochondrial outer membrane and in the nuclear envelope, plasma membrane, and endoplasmic reticulum.<sup>2</sup> bcl-2 expression was first described in follicular lymphomas with a chromosomal translocation; later studies also found it in lymphomas and epithelial tumors without this translocation.<sup>2</sup> Overexpression of bcl-2 results in an alteration of programmed cell death with persistence of cells that fail to die.<sup>3</sup> In normal proliferating epithelium, bcl-2 is expressed in stem cell zones such as the basal layers, where it acts to prevent the death of cells in the regenerative compartment.<sup>3,4</sup> Bcl-2 protein overexpression has been found in the early phase of epithelial cancerogenesis.<sup>1</sup> Bcl-2 was mainly expressed in well-differentiated transitional cell carcinomas, whereas it was absent in high-grade transitional cell carcinomas.<sup>5</sup> This loss may reflect a deregulation of the mechanisms that control bcl-2 expression.<sup>5</sup> Little is known about the presence of bcl-2 in normal, dysplastic, or neoplastic oral epithelium.<sup>6,7</sup> Loss of normal p53 function is correlated to the progression

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of several preneoplastic lesions to neoplasms and with a shortened survival in several malignancies.<sup>5</sup> Moreover, p53 expression showed a statistically significant correlation with tumor grade and stage.<sup>5</sup>

The detection of p53 in preinvasive areas adjacent to squamous cell carcinoma and in dysplastic oral epithelium suggests that p53 abnormalities may constitute an early event in the natural history of oral cancer.<sup>8-11</sup> p53 safeguards genomic stability by blocking the entry of a DNA-damaged cell from the G1 phase to the S phase and by activating apoptosis through a down-regulation of bcl-2.<sup>12</sup>

The p53 gene is thus involved in the apoptotic pathway.<sup>1</sup> The majority of oral squamous cell carcinomas (SCCs) (up to 80%) carry p53 mutations, and this fact shows that oral mucosa is one of the most common targets for p53 mutations.<sup>13</sup> A strong correlation of Ki-67 with high tumor grade has been reported,<sup>5</sup> and MIB-1 is regarded as a reliable marker of proliferating cells.<sup>14</sup> The aims of our study were: 1) to investigate, in normal oral epithelium, leukoplakia, dysplasia and oral SCC, the prevalence of p53, bcl-2, and Ki-67 immunoreactivity and of apoptosis; and 2) to determine the relationship between them.

## Materials and Methods

A total of 70 biopsy samples were analyzed. The following formalin-fixed, paraffin-embedded tissues were used in this study: normal oral mucosa obtained during third molar removal (10 cases), leukoplakia (12 cases), epithelial dysplasia (12 cases: 6 mild and 6 severe dysplasia and carcinoma in situ), invasive carcinoma well differentiated (G1) (12 cases), invasive carcinoma moderately differentiated (G2) (12 cases), and invasive carcinoma poorly differentiated (G3) (12 cases). The age of the patients ranged from 48 to 67 years (mean, 55 years). All selected samples had been routinely fixed in 10% neutral formalin (24 to 48 hours), dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin. The hematoxylin-eosin slides were all reviewed, the diagnosis was confirmed, and the slides for the quantitative evaluation were selected. Epithelial dysplasia was diagnosed according to the criteria and the definition proposed by Pindborg et al<sup>15</sup>: *mild dysplasia* was considered mild expansion of the proliferative zone, with mitotic activity, hyperchromatism, and nuclear variability; *moderate dysplasia* indicated these same features were present in up to half of the thickness of the epithelium; and *severe dysplasia* indicated the atypical proliferative zone encompassed up to three fourths of the epithelium.

Immunohistochemical staining for bcl-2 protein was performed using the following antigen retrieval system. Sections were deparaffinized in 2 changes of

xylene for 10 minutes each and then were rehydrated through graded alcohols and immersed in 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Sections were then washed in phosphate-buffered saline (PBS). The tissue sections were put in a microwave oven (Cooktyronic M720, 700 W; Philips, Eindhoven, The Netherlands) in a plastic Coplin jar filled with 10 mmol/L sodium citrate buffer (pH 6.0) at 5-minute intervals for a total of 10 minutes. At each 5-minute interval, the fluid in the Coplin jar was removed from the microwave oven and allowed to cool. Slides were incubated overnight in a 1:60 dilution of the primary mouse anti-human bcl-2 monoclonal antibody (124; DAKO, Glostrup, Denmark). A biotin streptavidin detection system was used with diaminobenzidine as the chromogen.

Slides were washed twice with PBS and incubated with the linking reagent (biotinylated anti-immunoglobulins) for 15 minutes at room temperature. After being rinsed in PBS, the slides were incubated with the peroxidase-conjugated streptavidin label for 15 minutes at room temperature. The sections were again rinsed in PBS and incubated with diaminobenzidine for 10 minutes in the dark. After chromogen development, slides were washed in 2 changes of water and counterstained with a 1:10 dilution of hematoxylin. The sections were then dehydrated, cleared in xylene, and mounted. A negative control was performed in all cases by omitting the primary antibody, which in all instances resulted in negative immunoreactivity. Sections from a lymph node with follicular lymphoma were used as positive controls. Normal lymphocytes present in the tissues represented an internal positive control for bcl-2 immunostaining. In all positive cases, immunoreactivity was restricted to the cytoplasm. The percentage of bcl-2-positive cells was evaluated from a minimum of 1000 cells in each case, and bcl-2 immunostaining was scored using a 3-tiered scale in which minus indicates less than 5% cells, plus indicates 5% to 50% cells, and double-plus indicates more than 50% cells.

Descriptive statistical analysis was performed for each group of lesions.

Immunohistochemical staining for p53 protein was performed using the following antigen retrieval system. Sections were deparaffinized in 2 changes of xylene for 10 minutes each and then were rehydrated through graded alcohols and immersed in 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Sections were then washed in PBS. The tissue sections were put in a microwave oven (Cooktyronic M720, 700 W; Philips) in a plastic Coplin jar filled with 10 mmol/L sodium citrate buffer (pH 6.0) at 5-minute intervals for a total of 10 minutes. Slides were incubated overnight with a 1:50 dilution of the primary mouse anti-human p53

monoclonal antibody (DO-7; DAKO). A biotin-streptavidin detection system was used with diaminobenzidine as the chromogen. Slides were washed twice with PBS and incubated with the linking reagent (biotinylated anti-immunoglobulins) for 15 minutes at room temperature. After being rinsed in PBS, the slides were incubated with the peroxidase-conjugated streptavidin label for 15 minutes at room temperature. The sections were again rinsed in PBS and incubated with diaminobenzidine for 10 minutes in the dark. After chromogen development, slides were washed in 2 changes of water and counterstained with a 1:10 dilution of hematoxylin. The sections were then dehydrated, cleared in xylene, and mounted. A negative control was performed in all cases by omitting the primary antibody, which in all instances resulted in negative immunoreactivity. p53 expression and location were evaluated on histologic sections using a Leitz Orthoplan microscope (Wetzlar, Germany) equipped with a  $\times 63$  objective and an eyepiece graticule. Only nuclear staining of epithelial cells was observed, and the nuclei with a clear brown color, regardless of staining intensity, were regarded as p53 positive. The percentage of p53-positive cells was evaluated from a minimum of 1,000 cells in each case, and p53 immunostaining was scored using a 3-tiered scale, as for bcl-2 immunostaining. The cells were evaluated in both the basal and parabasal layers. Descriptive statistical analysis was performed for each group of lesion, and the  $\chi^2$  test was used to determine statistically significant differences between the groups.

For MIB-1 immunostaining, the slides were pretreated with 3-aminopropyltriethoxysilane (APES; Sigma Chemical Co, St Louis, MO), which avoided the separation of the section from the slide during the incubation in the microwave oven. For each case, a 5- $\mu$ m section was cut and placed onto a pretreated slide. The staining protocol of this slide consisted of the applications of a series of reagents in the following manner: overnight drying at 37°C, dewaxing and rehydration, immersion in a plastic box containing 0.01 mol/L citrate buffer at pH 6.0, incubation for 5 minutes in a microwave oven initially at 750 W until boiling began and then at 350 W for the remaining time, incubation for 5 minutes in a microwave oven at 350 W, cooling for 20 minutes at room temperature, washing in running water and then in distilled water for 5 minutes, washing in Tris-buffered saline (TBS) for 5 minutes, removal of any excess TBS, addition of primary monoclonal mouse anti-ki-67 antibody (Immunotech, Marseille, France) diluted 1:25 in TBS, and overnight incubation at 4°C in a humidified room. Next, the protocol consisted of washing in TBS for 5 minutes (3 times), addition of secondary prediluted biotinylated anti-mouse antibody (LSAB; DAKO) and incubation

for 10 min at room temperature, washing in TBS for 5 minutes (3 times), addition of prediluted streptavidin-peroxidase complex (LSAB; DAKO) and incubation for 10 minutes at room temperature, and washing in TBS for 5 minutes (3 times). The protocol continued with immersion in 0.05% DAB and 0.01% H<sub>2</sub>O<sub>2</sub> in TBS for 2 to 3 minutes at room temperature, washing in running water and then in distilled water for 5 minutes, counterstaining with ethyl green for 30 minutes, washing in distilled water for 30 seconds, washing in buthanol I for 5 seconds, washing in buthanol II for 30 seconds, and dehydration and mounting in Permount.

The positivity to MIB-1 was evaluated by counting the number of positive cells per 1000 cells, and the values were expressed in an percentage. The presence of apoptotic bodies was evaluated in a random fashion in the parabasal layer in 20 high-power fields.

Mann-Whitney *U*, Kruskal-Wallis, and  $\chi^2$  tests were used to evaluate the presence of statistically significant differences. Simple regression analysis was used to evaluate the correlation between features.

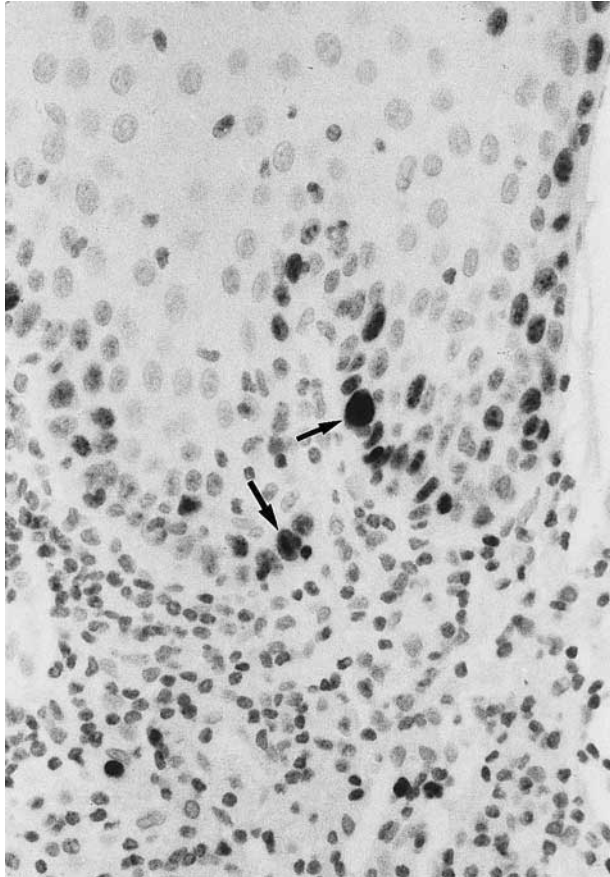
## Results

### p53

p53 was present in the basal layer in normal oral epithelium; in the basal and parabasal layers in leukoplakia, dysplasia, and carcinoma in situ; and in central and peripheral regions in invasive carcinoma. In normal oral epithelium in only 1 sample, the positivity was between 5% and 50%, whereas all of the other specimens showed a positivity of less than 5% (Fig 1). In leukoplakia, in 9 samples, the positivity was less than 5%, and in 3 samples, it was between 5% and 50%. In mild dysplasia, in 4 samples, the positivity was less than 5%, and in 2 samples, it was between 5% and 50%. In severe dysplasia, 5 samples had a positivity between 5% and 50% (Fig 2), and 1 sample had more than 50%. In well-differentiated carcinoma, 6 samples had a positivity of less than 5%, 5 samples had between 5% and 50%, and 1 sample had more than 50%. In moderately differentiated carcinoma, 5 samples had a positivity of less than 5%, 5 samples had between 5% and 50%, and 2 samples had more than 50%. In poorly differentiated carcinoma, 5 samples had a positivity of less than 5%, 4 samples had between 5% and 50%, and 3 samples had more than 50%.

### bcl-2

In normal oral epithelium and in leukoplakia, bcl-2 protein expression was seen in the basal layer, and in dysplasia, it was evaluated in the basal layer and rarely in the parabasal layer. In carcinoma, it was present in



**FIGURE 1.** Normal oral mucosa, showing a few basal cells positive to p53 (arrows) (p53 immunostaining alkaline phosphatase antialkaline phosphatase, original magnification  $\times 40$ ).

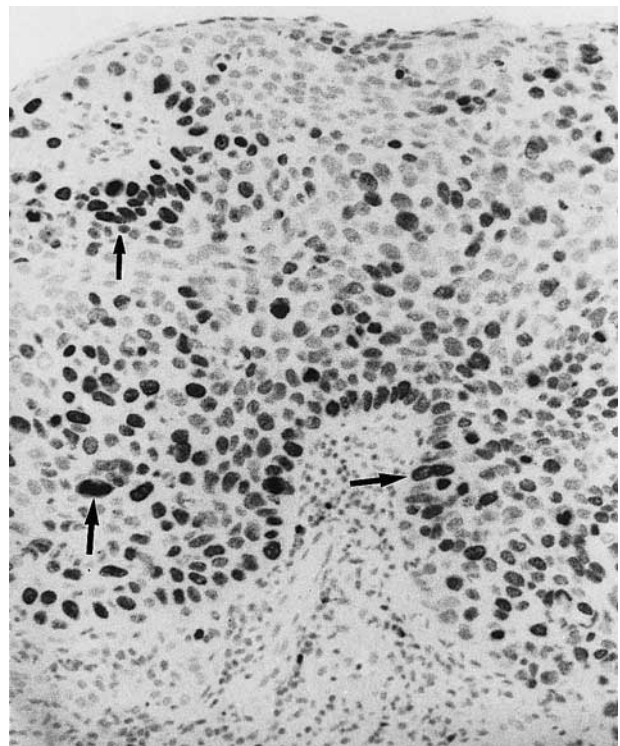
the central and peripheral areas of the tumor. In normal oral epithelium, in 3 samples, the positivity was less than 5%, in 6 samples, it was between 5% and 50%, and in 1 sample, it was more than 50% (Fig 3). In leukoplakia, 8 samples showed a positivity of less than 5%, and 4 samples had between 5% and 50%. In mild and moderate dysplasia, 3 samples had a positivity of less than 5% (Figs 4, 5), and 3 samples had between 5% and 50%. In severe dysplasia and carcinoma in situ, in all 6 samples, the positivity was less than 5%. In well-differentiated carcinoma, in 8 samples, the positivity was less than 5%, in 3 samples, it was between 5% and 50%, and in 1 sample, it was more than 50%. In moderately differentiated carcinoma, in 9 samples, the positivity was less than 5%, in 2 samples, it was between 5% and 50% (Fig 6), and in 1 sample, it was more than 50%. In poorly differentiated carcinoma, in 6 samples, the positivity was less than 5%, in 2 samples, it was comprised between 5% and 50%, and in 4 samples, it was more than 50%.

#### MIB-1

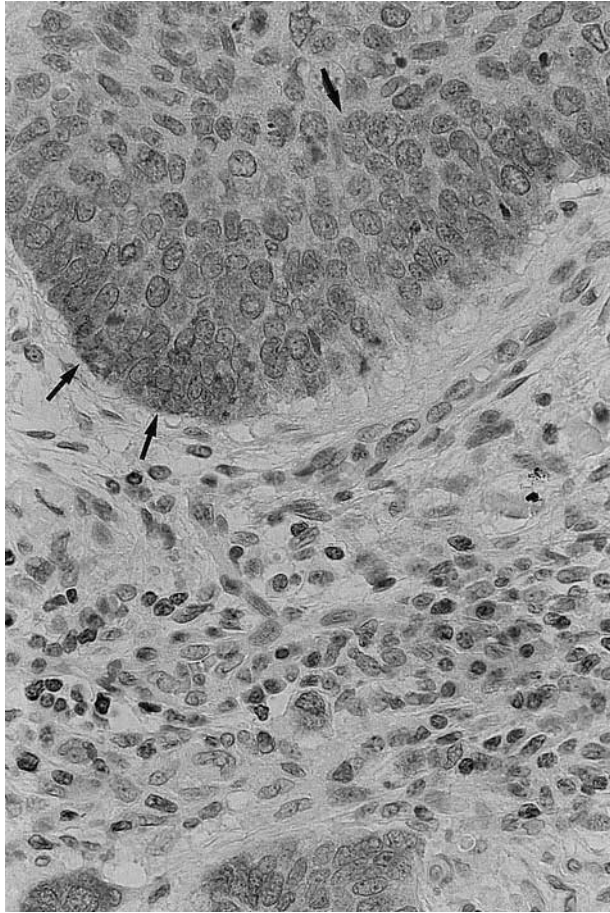
The expression of MIB-1 was very similar to that of p53. In normal oral mucosa, the value was 5.2%

( $\pm 2.20\%$ ); in leukoplakia, 9.0% ( $\pm 3.89\%$ ); in mild dysplasia, 15.3% ( $\pm 5.61\%$ ); in severe dysplasia and carcinoma in situ, 28.6% ( $\pm 6.21\%$ ) (Fig 7); in well-differentiated carcinoma, 18.8% ( $\pm 6.20\%$ ); in moderately differentiated carcinoma, 20.8% ( $\pm 6.06\%$ ); and in poorly differentiated carcinoma, 24.2% ( $\pm 6.0081\%$ ) (Figs 8, 9)

The mean values for all types of carcinoma was 21.6% ( $\pm 6.81\%$ ). With the Mann-Whitney *U* test, statistically significant differences were found between normal oral mucosa and leukoplakia ( $P = .0161$ ), normal oral mucosa and mild dysplasia ( $P = .0017$ ), normal oral mucosa and severe dysplasia ( $P = .0011$ ), and normal oral mucosa and carcinomas ( $P < .0001$ ). Statistically significant differences were found, moreover, between leukoplakia and mild dysplasia ( $P = .039$ ), leukoplakia and severe dysplasia ( $P = .0007$ ), and leukoplakia and carcinoma ( $P < .0001$ ). Statistically significant differences were also found between mild and severe dysplasia ( $P = .0065$ ), mild dysplasia and carcinoma ( $P = .0455$ ), and severe dysplasia and carcinoma ( $P = .0455$ ). Kruskal-Wallis test showed no statistically significant differences among well-differentiated, moderately differentiated, and poorly differentiated carcinoma ( $P = .1209$ ).



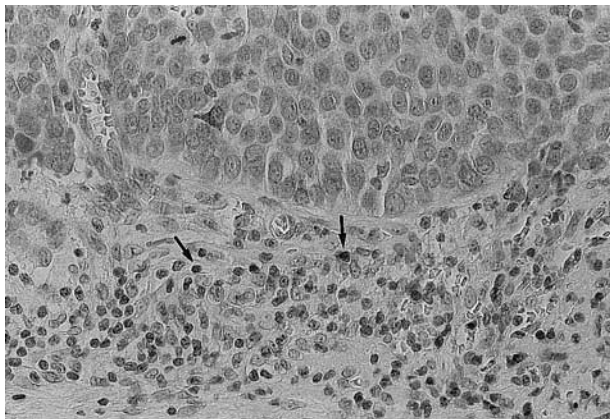
**FIGURE 2.** Severe dysplasia, showing strong positivity to p53 is seen in about 5% to 10% of epithelial cells (arrows) (p53 immunostaining alkaline phosphatase antialkaline phosphatase, original magnification  $\times 20$ ).



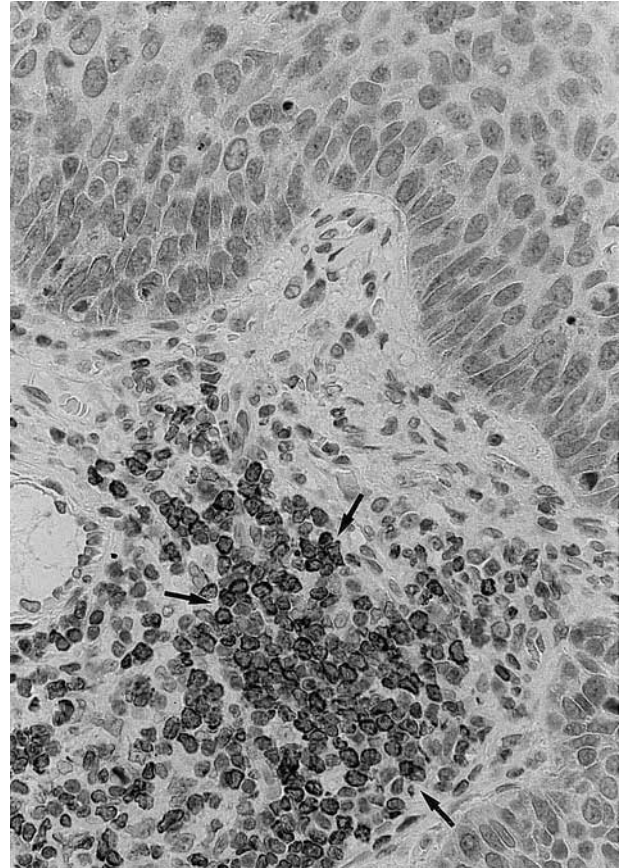
**FIGURE 3.** Normal oral mucosa, showing diffuse positivity of the basal and parabasal cell layers to bcl-2 protein (arrows) (bcl-2 immunostaining alkaline phosphatase antialkaline phosphatase, original magnification  $\times 40$ ).

#### APOPTOTIC INDEX

In normal oral mucosa, the apoptotic index (AI) was  $0.09 (\pm 0.07)$ ; in leukoplakia,  $0.083 (\pm 0.08)$ ; in

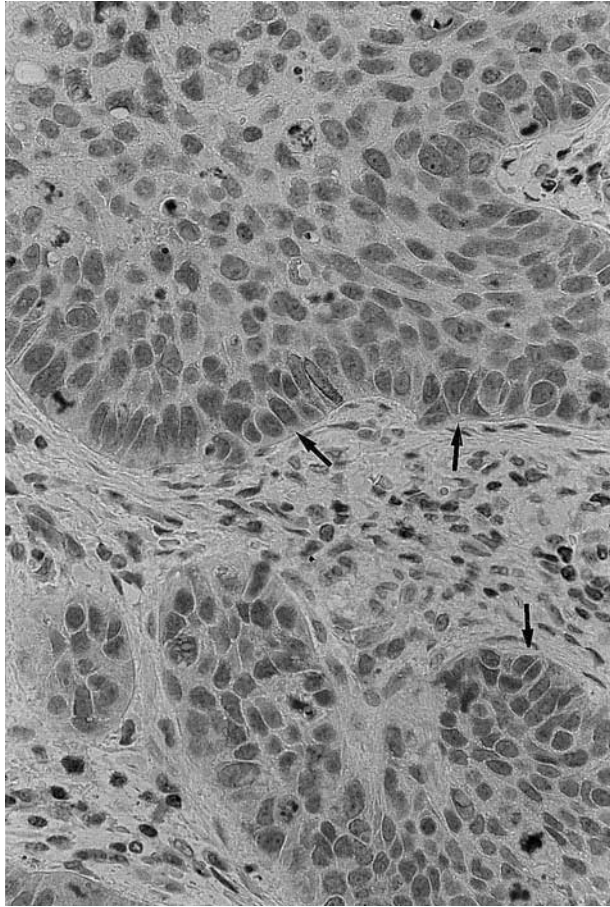


**FIGURE 4.** Mild dysplasia, showing negativity of the basal cell layers to bcl-2 and positivity of the lymphocytes (arrows) (bcl-2 immunostaining alkaline phosphatase antialkaline phosphatase, original magnification  $\times 40$ ).



**FIGURE 5.** Moderate dysplasia, showing complete negativity to bcl-2 of the basal cell layer and positivity of the lymphocytes (arrows) (bcl-2 immunostaining alkaline phosphatase antialkaline phosphatase, original magnification  $\times 40$ ).

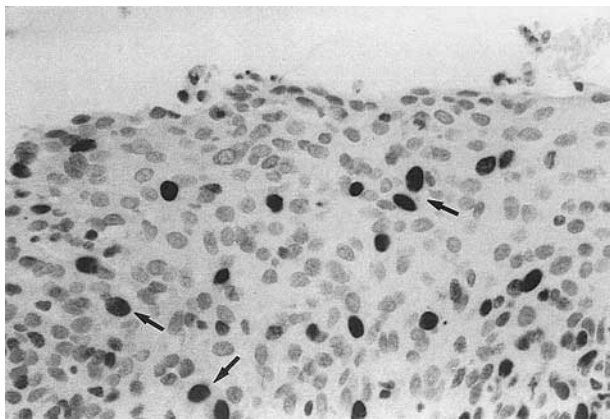
mild dysplasia,  $0.133 (\pm 0.10)$ ; in severe dysplasia and carcinoma in situ,  $0.317 (\pm 0.12)$ ; in well-differentiated carcinoma,  $0.242 (\pm 0.12)$ ; in moderately differentiated carcinoma,  $0.367 (\pm 0.23)$ ; and in poorly differentiated carcinoma,  $0.433 (\pm 0.25)$  (Fig 10). With the Mann-Whitney *U* test, statistically significant differences were found between normal oral mucosa and severe dysplasia ( $P = .0024$ ), leukoplakia and severe dysplasia ( $P = .0020$ ), and mild and severe dysplasia ( $P = .025$ ). Moreover, a statistically significant difference was found between well-differentiated carcinoma and normal oral mucosa ( $P = .002$ ), leukoplakia ( $P < .001$ ), and mild dysplasia ( $P = .0103$ ). Kruskal-Wallis test has been used to evaluate the differences of the AI of well-differentiated, moderately differentiated, and poorly differentiated carcinoma: these differences were not statistically significant ( $P = .1286$ ). Moreover, no statistically differences were found between normal oral mucosa and leukoplakia ( $P = .8175$ ), normal oral mucosa and mild dysplasia ( $P = .4477$ ), leukoplakia and mild dysplasia ( $P = .3490$ ), and severe dysplasia and carcinoma ( $P = .8939$ ).



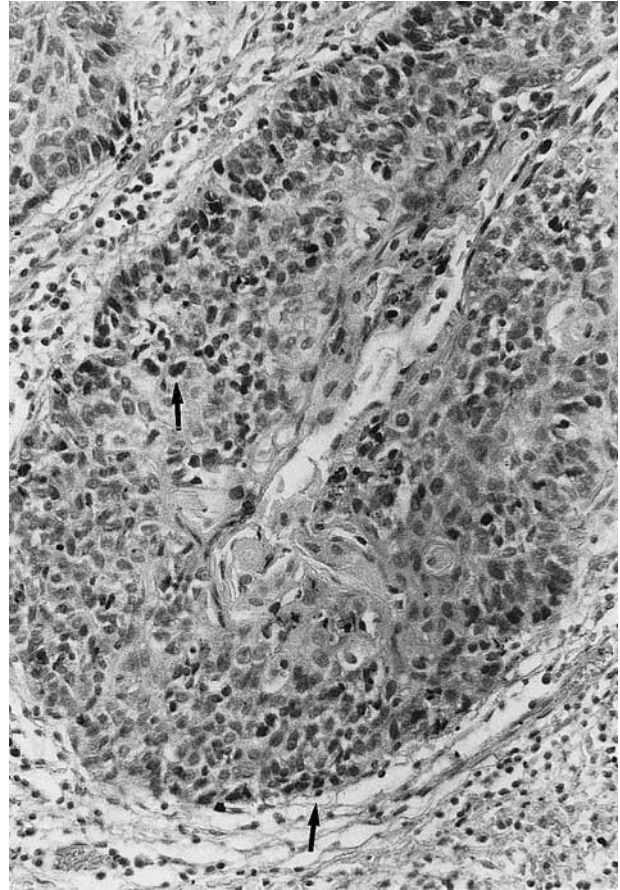
**FIGURE 6.** Moderately differentiated carcinoma, showing nonhomogeneous, diffuse (<50%) positivity of the neoplastic cells to bcl-2 (arrows) (bcl-2 immunostaining alkaline phosphatase antialkaline phosphatase, original magnification  $\times 40$ ).

**CORRELATION BETWEEN FEATURES**

p53 protein was negative in 16 bcl-2-negative cases (42.1%), in 16 bcl-2-positive (+) cases (42.1%), and in



**FIGURE 7.** Severe dysplasia, showing strong positivity of basal, parabasal, and superficial layers to MIB-1 (arrows) (MIB-1 immunostaining alkaline phosphatase antialkaline phosphatase, original magnification  $\times 40$ ).

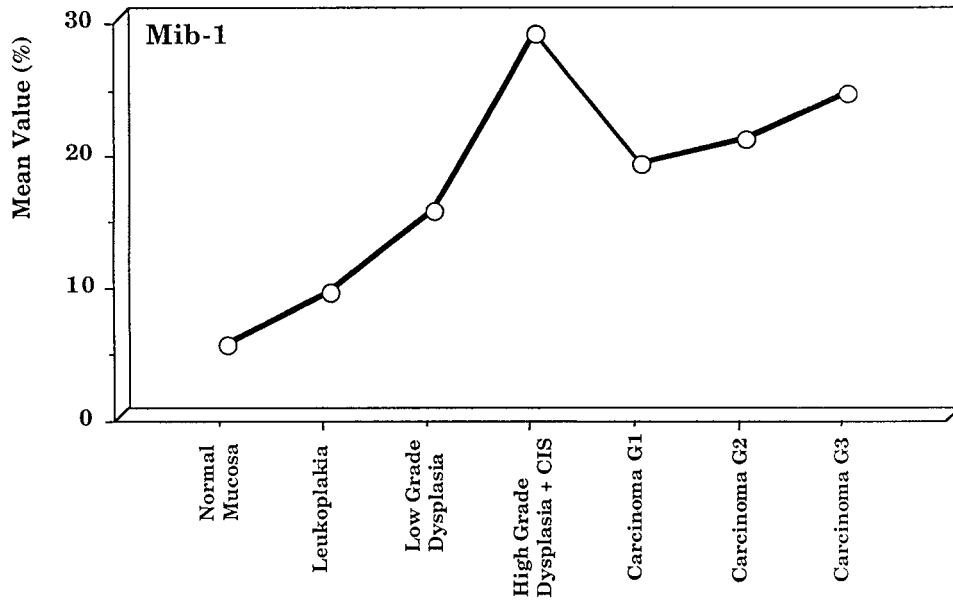


**FIGURE 8.** Poorly differentiated carcinoma, showing positivity (<50%) of the neoplastic cells (arrows) (MIB-1 immunostaining alkaline phosphatase antialkaline phosphatase, original magnification  $\times 20$ ).

6 bcl-2-positive (++) ones (15.8%). p53 was positive (+) in 20 bcl-2-negative cases (80%), in 4 bcl-2-positive (+) cases (16%), and in 1 bcl-2-positive (++) case (4%). All of the 7 p53-positive (++) cases were bcl-2 negative. There was a negative significant statistical correlation between p53 and bcl-2 protein ( $P = .001$ ). In the 43 bcl-2-negative cases, the mean value of AI was 0.314 ( $\pm 0.217$ ), whereas in the 27 bcl-2-positive (+ and ++) cases, the mean value was 0.133 ( $\pm 0.130$ ); the difference between the 2 groups was statistically significant ( $P < .0001$ ). A good, but negative, correlation between these 2 features was present. Finally, considering all of the 70 cases, the simple regression analysis (Fig 11) showed that there was a good positive correlation ( $r = 0.694$ ,  $P < .0001$ ) between AI and MIB-1 expression.

**Discussion**

Ramsay et al<sup>3</sup> showed that compared with nevi, in which the bcl-2 protein immunoreactivity was present in all cells, melanomas exhibited a progressive loss of the protein expression with increased



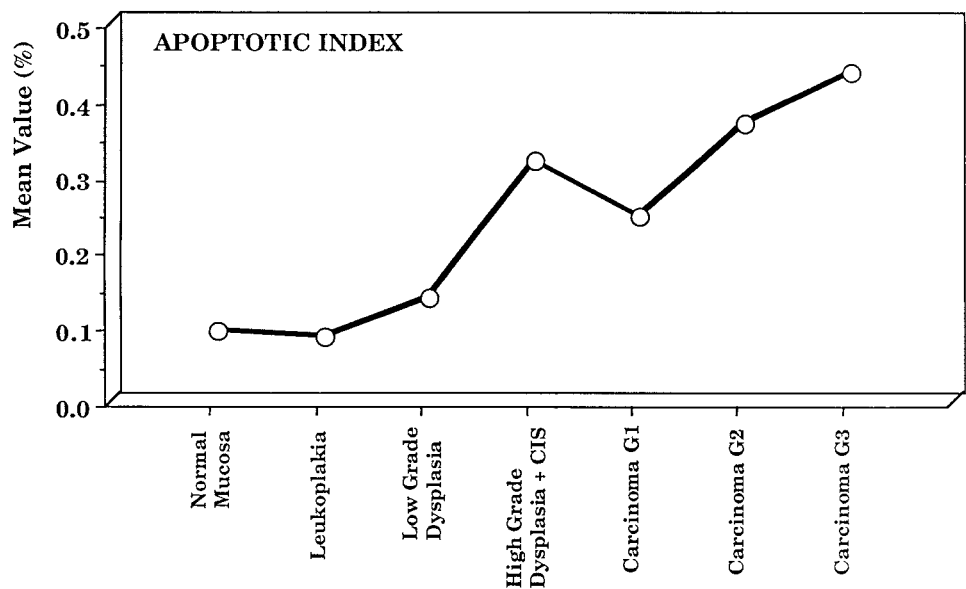
**FIGURE 9.** Mean values of the MIB-1-positive cells. (CIS, carcinoma in situ.)

levels of malignancy. These data suggest, perhaps, that *bcl-2* loss is associated with or reflects an increased malignant potential<sup>5</sup>. The presence of *bcl-2* seems to be associated with a better prognosis in some tumors but not in others.<sup>2,3,16</sup> High levels of *bcl-2* protein correlated with lower rates of complete remission and shorter survival in patients with acute myeloid leukemia.<sup>17,18</sup> On the other hand, the *bcl-2* expression in breast cancer seems to be predictive of a positive response to endocrine therapy and correlates with improved survival, whereas in prostate cancer, *bcl-2* expression is related to androgen-independent tumor growth and chemoresistance.<sup>12,16,17</sup> In thyroid cancer, *bcl-2* expression seems to be corre-

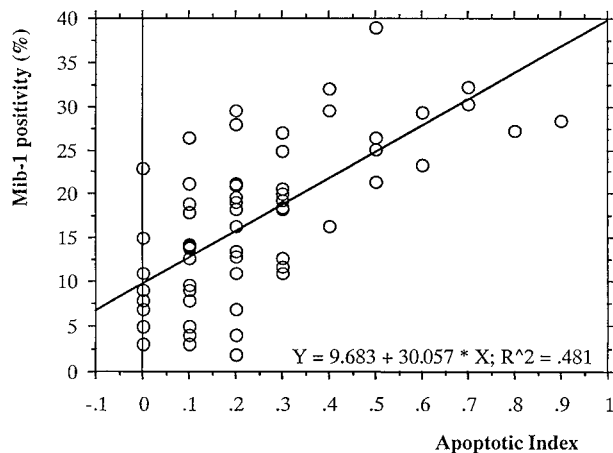
lated with the differentiation of the tumors, and undifferentiated tumors do not express the protein.<sup>16,18</sup>

In neuroblastoma, *bcl-2* expression does not seem to influence prognosis.<sup>16</sup> Tjalma et al<sup>2</sup> found a strong relationship between *bcl-2* expression and prognosis in patients with carcinoma of the uterine cervix. Flohil et al,<sup>16</sup> in a study that compared hyperplastic polyps, adenomas, and carcinomas of the colon, found that most carcinomas did not present a *bcl-2* immunoreactivity.

The expression of *bcl-2* may provide data about individual tumor cell dynamics that in the future will most probably be very important for therapy and prognosis.<sup>17</sup> A significant inverse relationship be-



**FIGURE 10.** Mean values of the apoptotic index. (CIS, carcinoma in situ.)



**FIGURE 11.** Simple regression analysis considering apoptotic index and MIB-1 positivity. A good, positive correlation is evident ( $r = 0.694$ ,  $P < .0001$ ).

tween bcl-2 and p53 was found in our specimens: similar data were reported by Harn et al<sup>1</sup> in breast carcinoma and by other researchers in carcinomas of the thyroid, colorectum, stomach, and esophagus and in gastric lymphoma.<sup>12</sup> Studies in human breast cancer and in cancer cell lines have shown that p53 can down regulate bcl-2 expression<sup>4</sup> and that apoptosis induced by p53 can be blocked by bcl-2 in cultured cancer cells.<sup>4</sup> p53 has been shown to down-regulate bcl-2 via binding to a negative regulatory element outside the bcl-2 gene promoter.<sup>19</sup> A significant inverse relationship was found between bcl-2 expression and the pathologic stage of esophageal tumors, and bcl-2 was found more frequently in early than in advanced stages.<sup>12</sup> Our results also provide evidence of an interaction between bcl-2 and MIB-1: the highest expression of MIB-1-positive cells was observed in severe dysplasia and carcinoma in situ, where the bcl-2 protein expression was lowest or even absent. A possible influence of bcl-2 expression in the down-regulation of a proliferation marker was reported by Konstantinidou et al,<sup>20</sup> who found that bcl-2-positive meningiomas presented a lower growth fraction rate and had a significantly higher proportion of proliferating cell nuclear antigen (PCNA)-strongly positive nuclei than the bcl-2-negative subgroup.

We found, on the contrary, an inverse relationship between bcl-2 and MIB-1, and the loss of bcl-2 expression in our specimens of severe dysplasia and carcinoma in situ probably reflects a deregulation of the mechanisms that control bcl-2 expression.<sup>5</sup> Our data show that a bcl-2 down-regulation was strongly associated with peaks in MIB-1 and AI in these lesions. Ravi et al<sup>9</sup> reported an increased bcl-2 expression in oral dysplasia and carcinoma. bcl-2 expression inversely correlated with the degree of differentiation between CIN I/II and III in tumors of the uterine

cervix.<sup>21</sup> We found that p53 was expressed in supra-basal layers in leukoplakia, dysplasia, and carcinoma in situ; Cruz et al<sup>13</sup> demonstrated that p53 suprabasal expression was significantly associated with the development of carcinoma. Our results are fully in accordance with the results reported by Murti et al,<sup>8</sup> who found that the overexpression of p53 protein was significantly more common in severe than in mild epithelial dysplasia and that p53 expression peaked close to the time of transition from the precancer state to cancer rather than earlier in the natural history of oral precancer. A fairly close relationship between p53 and AI was found in our specimens. Birchall et al<sup>6</sup> found that apoptosis increased from normal through dysplastic epithelium to reach a maximum in carcinoma in situ, whereas in invasive SCC AI fell to normal values<sup>6</sup>; it also increased with increasing degrees of dysplasia. A strong relationship was found in our specimens between AI and the degree of dysplasia and the onset of SCC; completely different results were reported by Birchall et al,<sup>6,7</sup> who found no such association.

A diminution of apoptosis was reported in gastric carcinoma, whereas esophageal SCC, prostatic adenocarcinoma, brain tumors, and non-Hodgkin lymphomas showed an increased AI in relationship to less tumor differentiation and increased mitotic activity.<sup>22,23</sup> Our results show a close correlation between AI and MIB-1, whereas Birchall et al<sup>6,7</sup> found that the AI was highly correlated with mitotic index but not with PCNA: these authors failed to demonstrate an increased expression of PCNA in dysplastic epithelium. Apoptosis can be important in tumor growth and prognosis; in colorectal carcinoma, Langlois et al<sup>24</sup> found that tumors with higher apoptotic counts seemed to have a good prognosis, and this may reflect that neoplasms with higher levels of apoptosis are slower growing. In renal cell carcinoma, Hindermann et al<sup>23</sup> found a decrease in cells undergoing apoptosis in less-differentiated tumors with an increase in the number of tumor cells and of tumor growth. This decrease in apoptosis was correlated with an increase in the proliferative activity. The AI was much higher in the most aggressive type of prostatic carcinoma.<sup>1</sup> The presence of Ki-67 closely coincided with p53 protein,<sup>10</sup> and a close relationship between PCNA and p53 protein was found in some oral tumors.<sup>25</sup> In nasopharyngeal carcinoma, the patients with a high PCNA had a poorer disease-free survival.<sup>26</sup>

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