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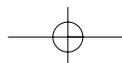
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◆ MANUSCRITO ORIGINAL

Detection of the risk of adenocarcinoma in Barrett's esophagus by means of tumor markers (p53 and ki67)

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Summary

The rising incidence of adenocarcinoma in Barrett's esophagus has intensified the research into methods of early recognition of cancer risk, detecting cytological and architectural changes (dysplasia) or using biomarkers as predictive tests. The aim of this paper is to evaluate the involvement of two tumor markers: p53 (tumor suppressor gene) and Ki67 (proliferation marker), by means of immunohistochemical analysis with monoclonal antibodies designed for the specific localization of p53 and Ki67 antigens, in esophageal biopsies with columnar metaplasia of patients with and without dysplasia and adenocarcinoma, and to anticipate which ones are liable to suffer it in the future. Both markers were positive in all intestinal metaplasia patients with high-grade dysplasia and adenocarcinoma, and even in some cases with low grade or without dysplasia. In contrast, in those who have gastric metaplasia, tumor markers were negative. Expression of biomarkers next to dysplasia reduces interobserver variation. Patients with these abnormalities have to be included into a surveillance protocol.

Key words. Barrett's esophagus; dysplasia; adenocarcinoma; tumor markers (p53, Ki67).

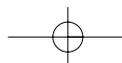
Detección del riesgo de desarrollar adenocarcinoma en el esófago de Barrett por medio de marcadores tumorales (p53 y ki67)

Resumen

El aumento de la incidencia del adenocarcinoma en el esófago de Barrett ha intensificado la investigación hacia métodos para el diagnóstico temprano del riesgo de cáncer, detectando cambios citológicos y arquitecturales (displasia) o utilizando marcadores tumorales como pruebas predictivas. El objetivo de este trabajo es evaluar el comportamiento de dos marcadores tumorales, el p53 (gen supresor tumoral) y el Ki67 (marcador de proliferación), por medio del análisis inmunohistoquímico con anticuerpos monoclonales diseñados para la localización específica de los antígenos p53 y Ki67, en biopsias con metaplasia columnar esofágica de pacientes con y sin displasia y adenocarcinoma, tratando de anticipar cuales son los posibles candidatos a sufrirlo en el futuro. Ambos marcadores resultaron positivos en todos los pacientes con displasia de alto grado y adenocarcinoma, y aún en algunos casos sin displasia y con displasia de bajo grado. Por el contrario, en aquellos que tuvieron metaplasia gástrica, los marcadores resultaron negativos. Los pacientes con tales anomalías deben ser incluidos en protocolos de vigilancia.

Palabras claves. Esófago de Barrett, displasia, adenocarcinoma, marcadores tumorales (p53,



**Abbreviations:***BE: Barrett's Esophagus.**GM: gastric metaplasia.**IM: intestinal (specialized) metaplasia.**GERD: gastro-esophageal reflux disease.**ACa: adenocarcinoma.**ND: negative for dysplasia.**IND: indedfinite for dysplasia.**LGD: low-grade dysplasia.**HGD: high-grade dysplasia.**SE: sensitivity.**SP: specificity.**PPV: positive predictive value.**NPV: negative predictive value.**AC: accuracy.**CI: confidence interval.**Mx: maximum.**Mn: minimum.*

Barrett's esophagus (BE) is defined endoscopically as columnar metaplasia lining the distal esophagus. The normal mucosa is replaced by a mosaic of diverse types of epithelium: cardiac (junctional) or gastric fundic with parietal (oxyntic) cells, designated as gastric metaplasia (GM+), or intestinal specialized metaplasia with goblet cells (IM+).¹⁻³ Columnar lined esophagus is a histological consequence of persistent gastro-esophageal reflux disease (GERD) and an intermediate step in the development of adenocarcinoma (ACa). The molecular mechanisms responsible for progression towards carcinoma are mostly unknown.^{4,5}

The rising incidence of ACa in BE has intensified the research into methods of early detection of cancer risk, spotting cytological and architectural changes (dysplasia) and/or using biomarkers as predictive tests.

Alterations of the p53 tumor suppressor gene and the Ki67 proliferation marker, detected by immunohistochemistry in tissue samples, combined with the presence of dysplasia, appear to be the most frequent association to detect possible progression to ACa in BE,⁶⁻⁹ with respect to the number of biomarkers in use.^{10,11} The presence of these biomarkers increases the risk of progression to ACa; the lack of this mutation does not avoid the risk.^{12,13}

cate esophageal ACa in BE and to anticipate which cases are liable to suffer it in the future.

Materials and Methods**Patients**

Retrospectively, 176 patients with IM+ and 16 with GM+ were evaluated at Departments of Gastroenterology and Pathology (School of Medicine, Catholic University of Córdoba, Argentina). As to the 176 patients with IM+, 63.07% were male (n=111) and 36.93% female (n=65) (Table 1). As to the 16 patients with GM+, 62.5% were male (n=10) and 37.5% female (n=6) (Table 2).

Table 1. Age and sex in 176 patients with BE and IM+.

| | Total n=176 | Males n=111 | Females n=65 |
|-------------|----------------|----------------|-----------------|
| Mean age | 58.72 | 54.02 | 66.76 |
| Maximum age | 87 | 84 | 87 |
| Minimum age | 24 | 24 | 46 |

Table 2. Age and sex in 16 patients with BE and GM+.

| | Total n=16 | Males n=10 | Females n=6 |
|-------------|---------------|---------------|----------------|
| Mean age | 52.06 | 48 | 58.83 |
| Maximum age | 75 | 71 | 75 |
| Minimum age | 30 | 30 | 46 |

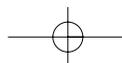
Upper endoscopy

Columnar metaplasia of distal esophagus was identified endoscopically by means of the Prague (C & M) criteria.^{9,14} Short (< 3 cm) and long (> 3 cm) segments of BE were included in the IM+ group. Short segments of BE were clearly identified, since the biopsies obtained in the upper stomach showed no intestinal metaplasia. Meanwhile, only long segment BE cases were considered in the GM+ group.

Histology

Biopsy samples were identified from patients with BE and graded according to the published modified criteria established for inflammatory bowel disease in negative for dysplasia (ND), indefi-





(LGD), high-grade dysplasia (HGD) and adenocarcinoma (ACa).^{9,15,16} Sections were stained with H&E and alcian blue.

Immunostaining

Immunohistochemical analysis with monoclonal antibodies designed for the specific localization of p53 and Ki67 antigens in formalin-fixed, paraffin-embedded tissue sections were used: Super Sensitive Ready-to-Use Antibody, AM239-5M for p53 and AM297-5M for Ki67 (BioGenex®, San Ramon, CA, USA). Histological samples with more than 10% positive nuclei were considered as positive.⁸

Statistical analysis

Data were collected in Microsoft Excel® 2003 tables, and analyzed by means of SPSS 15.0 program for Windows®. The Irwin-Fisher test for a 2x2 table was used. Besides, the sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), accuracy (AC) and the J Youden index were calculated. Also the standard deviations for a 95% confidence interval (CI) were established by means of Wilson method.

Results

Total mean age, maximum and minimum distributed by sex, are shown in Tables 1 and 2, concerning to patients with BE in relation to IM+ and GM+. In Table 3, p53 and Ki67 tumor markers corresponding to 176 patients with BE and IM+ are shown, focused on different degrees of dysplasia and ACa. Same data concerning 16 patients with BE and GM+ are shown in Table 4.

Table 3. Tumor markers in 176 patients with Barrett's Esophagus with specialized intestinal columnar metaplasia (IM+).

| TUMOR MARKERS | ND n=111 | IND. n=4 | LGD n=20 | HGD n=11 | ACa n=30 |
|---------------|-------------|----------|----------|-----------|-----------|
| p53 + | 20 (18.02%) | 0 | 15 (75%) | 11 (100%) | 30 (100%) |
| p53 - | 91 (81.98%) | 4 (100%) | 5 (25%) | 0 | 0 |
| Ki67 + | 74 (66.66%) | 3 (75%) | 16 (80%) | 11 (100%) | 30 (100%) |
| Ki67 - | 37 (33.33%) | 1 (25%) | 4 (20%) | 0 | 0 |

ND: negative for dysplasia, IND: indefinite for dysplasia, LGD: low-grade dysplasia.

Table 4. Tumor markers in 16 patients with Barrett's Esophagus with junctional (cardiac) or gastric-fundic columnar metaplasia (GM+).

| TUMOR MARKERS | ND n=16 | IND. n=0 | LGD n=0 | HGD n=0 | ACa n=0 |
|---------------|------------|----------|---------|---------|---------|
| p53 + | 0 | 0 | 0 | 0 | 0 |
| p53 - | 16 (100%) | 0 | 0 | 0 | 0 |
| Ki67 + | 14 (87.5%) | 0 | 0 | 0 | 0 |
| Ki67 - | 2 (12.5%) | 0 | 0 | 0 | 0 |

ND: negative for dysplasia, IND: indefinite for dysplasia, LGD: low-grade dysplasia, HGD: high-grade dysplasia, ACa: adenocarcinoma.

Discussion

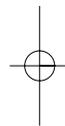
The vast majority of our patients with IM+ were male and the mean age at the time of diagnosis was lower in this group (Table 1). Comparable data go on GM+ (Table 2).

In the IM+ group, both markers' positivity increases according to severity of dysplasia, and were all positive in HGD and ACa, highly positive in LGD, and were also positive in some of our patients without dysplasia (Table 3), in agreement with some other papers.¹⁷⁻¹⁹ On the contrary, this feature was not observed by others.^{4,20-24} Meanwhile in the GM+ group, no dysplasia or ACa were positive and only Ki67 was positive in ND patients (Table 4). We assume that only IM in Barrett's is a premalignant condition.

The statistical analyses (Table 5) contribute to understand the clinical value of these biomarkers in our patients with BE. p53 emerges as an enhanced test to identify the possible advance to cancer in dysplastic and non-dysplastic tissue, with a high sensitivity, moderate specificity and PPV, good accuracy and an excellent J Youden index. The high NPV is also significant to discard those patients with low cancer risk. Dysplasia vs. p53 has an excellent Fisher test.

Ki67 appears to be lower than p53, despite a good SE, NPV and Fisher test. J Youden index is low. We presume that Ki67 as a proliferation marker, perceives inflammatory changes better than malignancy, and p53 is relevant as a tumor marker.

These markers can not be consistently used alone to predict progression to cancer.²⁰ The presence of dysplasia with positive tumor markers validates the presence of changes in gene structure, gene expression and protein structure. When positivity



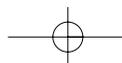


Table 3. Tumor markers in 176 patients with Barrett's Esophagus with specialized intestinal columnar metaplasia (IM+).

| | SE CI | SP CI | PPV CI | NPV CI | AC CI | J Youden Index | P |
|------|------------------|--------------------|--------------------|------------------|--------------------|-------------------|-----------------------|
| p53 | 100% 91.4–100 | 54.1% 66.1–80.7 | 53.9% 42.8–64.7 | 100% 96.3–100 | 81.1% 73.6–85.3 | 0.7 | D vs. p53 <0.0001 |
| Ki67 | 100% 91.4–100 | 31.1% 23.9–39.4 | 30.6% 23.4–38.8 | 100% 91.6–100 | 47.2% 39.9–54.5 | 0.3 | D vs. Ki67 <0.0001 |

CI: confidence interval; SE: sensitivity; SP: specificity; PPV: positive predictive value; NPV: negative predictive value; AC: accuracy; D: dysplasia

changes may be considered. Long term evaluation is necessary to determine the number of patients with tumor marker abnormalities and non-dysplastic intestinal metaplasia who will progress to dysplasia or carcinoma.²⁵

These patients must be included in long term follow up according to established guidelines.^{3,16} The follow up of GM patients is less essential for the reason that only IM is a premalignant condition, but the liability to develop cancer in some stage of the metaplasia-dysplasia-carcinoma sequence has been mentioned.²

Expression of biomarkers next to dysplasia in tissue samples may have a predictive value for cancer risk and reduces interobserver variation.²⁶ Increasing grades of dysplasia showed progressively more p53 accumulation^{24,26} and was accompanied by an augmented Ki67 labeling index.^{7,19,20}

We conclude that the vast majority of patients who have BE will never develop malignancy, and the columnar lining in their esophagus will not reduce their life expectancy. Dysplasia stays on the gold standard in the histological diagnosis of premalignant conditions, including BE. Tumor markers, particularly p53, facilitate the understanding of the progress of columnar metaplasia towards different grades of dysplasia and Aca. Biomarkers' positivity, in addition to dysplasia, moves closer to the diagnosis of malignancy and reduces interobserver variation. Our results suggest that this procedure, which is technically easy, economical and quick, could play a role in the evaluation and follow-up of patients with Barrett's esophagus with and without dysplasia. We assume that patients with IM and positive biomarkers must be included

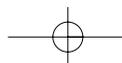
consideration the presence of dysplasia and tumor markers concomitantly positive. Perhaps equally important, if not more so, these markers may also be able to define a benign subset of patients with a low risk of developing cancer.

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