THE PROGNOSTIC SIGNIFICANCE OF P53, BCL2 AND MIB1 EXPRESSIONS RELATED WITH OTHER CLINICOPATHOLOGICAL VARIABLES IN SEROUS OVARIAN CARCINOMAS. A CLINICOPATHOLOGICAL STUDY IN PERITONEAL FLUIDS

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THE PROGNOSTIC SIGNIFICANCE OF P53, BCL2 AND MIB1 EXPRESSIONS RELATED WITH OTHER CLINICOPATHOLOGICAL VARIABLES IN SEROUS OVARIAN CARCINOMAS. A CLINICOPATHOLOGICAL STUDY IN PERITONEAL FLUIDS (Abstract): Objective: The first cytological study examining the expression of P53, BCL2 and MIB1 expressions in correlation with other clinicopathological parameters in ascitic fluids of patients with serous ovarian carcinomas. Materials and Methods: Fifty women 35-75 years old were diagnosed cytologically and confirmed histologically after operation in the University Hospital of Crete. All carcinomas were serous type and eight(8) of grade I, eighteen(18) of grade II and twenty two (22) of grade III. All carcinomas were staged according to the Figo criteria. Fifteen (15) were of Figo stage III and thirty five (35) were of Figo stage IV. For p53 and bcl-2, staining was evaluated on a semiquantitative scale depending on the number of cells showing positivity. For MIB1, the percentage of positive nuclei was calculated. Main outcome measure(s): The expression of P53, BCL2 and MIB 1 (Ki 67) correlated with tumor grade and Figo stages were estimated by chi-square (x²). Results: The expression of P53 and MIB1 were found to be statistically significant (p<0.005) correlated with Figo stage and tumor grade. A statistical significant correlation was also found between BCL2 expression and tumor Grade (p<0.005) but not between BCL2 expression and Figo Stage. The study found a high expression of P53 (64%) and MIB1 (72%) and an expression of BCL2 (48%) in ascitic fluid of patients with ovarian carcinoma. A statistically significant correlation between P53 and MIB1 expression correlated with tumor grade and Figo stage (p<0.005) and a statistically significant correlation between BCL2 expression and tumor grade but no with the Figo stage was found (p<0.005).There was a positive correlation between P53 and MIB1 .No significant association was found between P53 and BCL2 expression or MIB1 labeling index. Conclusion(s): Our data show significant differences in the expression of these markers in ovarian tumors and suggest a possible role for these tumor-associated genes as supplemental tools in prognosis and further definition of the biologic potential of these tumors. Keywords: OVARIAN CANCER, P53, BCL2, MIB1, APOPTOSIS.

Cancer of the ovary is one of the most common causes of cancer deaths in women and continues to present a challenge despite advances in our knowledge of the disease.
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don the past 20 years. The etiologic factors involved in the pathogenesis of ovarian cancer are poorly understood (1).

Recent studies have emphasized the importance of programmed cell death or apoptosis in the maintenance of tissue homeostasis and pathogenesis of tumors (2). Tissue homeostasis is maintained by the balance between cell proliferation and cell loss. There is evidence that much of the cell loss observed is due to programmed cell death or apoptosis (3). Apoptosis is a distinct form of cell death that can result from the activation of a genetically regulated cell suicide program or from cell injury induced by various stimuli. Types of cell damage that initiate apoptosis include: 1) exposure of cells to ionizing radiation 2) hyperthermia 3) toxic exposures 4) viral infections 5) cytokines 6) chemotherapeutic agents.

Numerous genetic factors have been identified as important contributors to the regulation of both cell proliferation and apoptosis. Aberrant regulation of both these processes, which stems from the malfunctioning of such genes and their products, plays an important role in tumor growth and development. The P53 protein produced in response to DNA damage restrains cellular proliferation by binding to specific regions of DNA and regulates the expression of other genes responsible for the cell cycle arrest, DNA repair, and initiation of apoptosis. The P53 gene also regulates expression of the anti-apoptotic BCL2 gene (4). Mutations of the P53 gene constitute the most common molecular genetic change associated with many cancers, including ovarian cancers (5, 6). The BCL2 gene is located on chromosome 18, and encodes the BCL2 protein (7). BCL2 is unique among the proto-oncogenes, as it is located on the mitochondrial membranes and interferes with programmed cell death independently of its ability to promote cell division (8). BCL2 belongs to a still growing family, the members of which are able to form homo- or heterodimers with one another. The most important regulators of apoptosis are found among these dimmers (9, 10).

Proliferating cell nuclear antigen (PCNA) or Ki-67 (MIB 1) is an axillary protein of DNA polymerase, an enzyme involved in the catalysis of DNA synthesis and repair. It has been shown that the total proliferative compartment of the tumor can be determined by the expression of this protein (11).

The present study examined the extent of apoptosis, apoptosis regulatory proteins P53 and BCL2 as well as the extent of tumor cell proliferation reflected by MIB1 expression in serous ovarian tumors.

MATERIAL AND METHODS

We studied, retrospectively, 50 women 35-75 years old (mean age 55 years) with serous ovarian carcinoma. All patients were referred and treated surgically at the University hospital of Crete in the Department of Gynecology and Obstetrics, diagnosed cytologically and confirmed histologically in the Department of Pathology-Cytology.

The cytological; histological determination was done by cytologist and pathologist and histological type and grade assigned according to the World Health Organisation Classification (12). All carcinomas were serous type and eight (8) of grade I, eighteen (18) of grade II and twenty two (22) of grade III, as determined by histopathology. All carcinomas were staged according to the Figo criteria. Fifteen (15) were of Figo stage III and thirty five (35) were of Figo stage IV.
For the immunocytochemical staining the following primary monoclonal antibodies were used. Monoclonal mouse anti-human P53 protein (DAKO) that labels wild type and mutant type P53 protein, monoclonal mouse anti-human BCL2 oncoprotein, clone 124 (DAKO) and Ki-67(MIB1), rabbit monoclonal expressed in G1-S-, M-, and G-2 phases of the cell-cycle( Thermo Scientific), at a dilution of 1/50, 1/50 and 1/200, respectively. A standard alkaline phosphatase method (APAAP) was used with 1-h incubation in a microwave oven at 750 watts, 3 times. A very light hematoxylin counterstaining was performed before cell counting. Tissues from the respective primary serous tumors were used as positive controls. Peritoneal fluids with endometriosis from Yale series (University-based endometriosis referral center) were used as negative controls too. In each specimen (at least 500 cells) in ten high power fields (x 400) were examined (light microscopy).For the statistical analysis chi-square ($x^2$) test was used.

**RESULTS**

P53 accumulation was documented in 32 (62%) of the 50 cases showed a nuclear accumulation in more than 10% of neoplastic cells. Cytoplasm immunoreactivity was never encountered. P53 accumulation was positively correlated with grade (grade I 2/8, grade II 10/18, and grade III 20/24) ( p<0,005 ) and Figo stage ( stage III 6/15 and stage IV 26/35) ( p<0,005 ) (tab. I).

**BCL2 immunoreactivity**

BCL2 immunoreactivity was observed in 24 (48%) of the 50 cases: it was restricted to the cell cytoplasm in more than 10 % of neoplastic cells. An inverse correlation, with grade was found (grade I 8/8, grade II 9/18 and grade III 7/24) p<0,005 but no any correlation with Figo stage (stage III 7/15 and stage IV 17/35) (tab. II).

**TABLE I**

<table>
<thead>
<tr>
<th>Tumor Grade</th>
<th>P53 expression and relationship with other clinical and pathological variables in ovarian carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2/8(25%)</td>
</tr>
<tr>
<td>II</td>
<td>10/18(55,6%)</td>
</tr>
<tr>
<td>III</td>
<td>20/24(83,4%)</td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>6/15(40%)</td>
</tr>
<tr>
<td>IV</td>
<td>26/35(74,3%)</td>
</tr>
</tbody>
</table>

**TABLE II**

<table>
<thead>
<tr>
<th>Tumor Grade</th>
<th>BCL2 expression and relationship with other clinical and pathological variables in ovarian carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8/8(100%)</td>
</tr>
<tr>
<td>II</td>
<td>9/18(50%)</td>
</tr>
<tr>
<td>III</td>
<td>7/24(29%)</td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>7/15(46,6%)</td>
</tr>
<tr>
<td>IV</td>
<td>17/35(48,5%)</td>
</tr>
</tbody>
</table>
The prognostic significance of P53, BCL2 and MIB1 expressions related with other clinical and pathological variables in serous ovarian carcinomas

**Proliferative fraction**

MIB 1 labeling index (L.I.) in 36 (72%) of the 50 cases. The expression was nuclear in more than 30% of the neoplastic cells. The MIB 1 L.I. was positively correlated with grade (grade I 2/8, grade II 12/18 and grade III 22/24) and Figo stage (stage III 5/15 and stage IV 21/35) (p<0.005) (tab. III).

Correlations among P53, BCL2 and MIB1 immunostaining

There was a positive correlation between P53 and MIB1 expressions whereas no significant correlation was found between P53 and BCL2 or BCL2 and MIB1 (tab. IV).

**TABLE 3**

<table>
<thead>
<tr>
<th>Tumor Grade</th>
<th>MIB 1 expression and relationship with other clinical and pathological variables in ovarian carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2/8 (25%) p&lt;0.005</td>
</tr>
<tr>
<td>II</td>
<td>12/18 (66.6%)</td>
</tr>
<tr>
<td>III</td>
<td>22/24 (90%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FIGO Stage</th>
<th>MIB 1 expression and relationship with other clinical and pathological variables in ovarian carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>5/15 (33.3%) p&lt;0.005</td>
</tr>
<tr>
<td>IV</td>
<td>21/35 (60%)</td>
</tr>
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</table>

**TABLE 4**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference value</th>
<th>RR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 accumulation</td>
<td>&lt;10% stained cells</td>
<td>2.769</td>
<td>0.0005</td>
</tr>
<tr>
<td>BCL2</td>
<td>&lt;10%</td>
<td>1</td>
<td>0.676</td>
</tr>
<tr>
<td>MIB 1</td>
<td>&lt;30%</td>
<td>2.499</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The prognosis of advanced-stage ovarian cancer patients has remained largely unchanged over the past 20 years (13). The available clinical and pathological prognostic factors have thus far proven insufficient to clearly define prognostic subgroups of patients.

Several studies have been devoted to identifying novel prognostic indicators for ovarian carcinomas, but the actual prognostic and/or predictive implications of a variety of biological parameters (P53 gene aberration and protein accumulation, BCL2, tumor growth fraction and ploidy) in these malignancies are still highly controversial (14). It is exceedingly difficult to ascertain from the currently available data whether and/or the extent to which evaluation of the above parameters contributes to a better definition of the biology and clinical behavior of ovarian carcinomas, in addition to the information already provided by the conventional clinical (age, performance status) and pathological (histological type, grade and stage of the neoplasms, presence of ascites) prognostic indicators. The aim of this study was to access the prognostic and predictive implications of P53 accumulation, BCL2 expression as regulator of apoptosis and tumor proliferative fraction evaluated by the MIB 1 monoclonal antibody against the Ki-67 antigen in a series of 50 ovarian...
carcinoma patients correlated with the grade and stage (15).

Our results in accord with previous reports (14,16,17,18,19,20,21) nuclear P53 accumulation was ascertained in more than 50% of ovarian carcinomas and was found to be statistical correlated with the tumor grade and Figo stage. The possible prognostic value of P53 aberrations in ovarian carcinomas has been addressed in several studies, many of which documented an adverse prognostic effect of P53 protein accumulation and that nuclear accumulation of P53 protein in more than 10% of neoplastic cells was significantly correlated with worse disease-free and overall survival (16).

In our study BCL 2 expression was found in 48% of our cases and in more than 10% of neoplastic cells was significantly correlated with the grade of the tumors but not with the Figo stage. In other studies BCL 2 immunoreactivity was not correlated with grade or stage of ovarian tumors (14, 1) but was related to histopathologic subtype, and positive staining was seen most frequently in serous (77%) and endometrioid (56%) carcinomas, but was less common in mucinous carcinomas (20%) (1).

This study clearly showed that MIB 1 was positively correlated with grade and stage of the tumors expressed in 72% of our cases and in more than 30% of the neoplastic cells. A positive correlation between MIB 1 labeling index and P53 accumulation was ascertained, whereas no significant association was found between BCL2 immunoreactivity and P53 accumulation or MIB 1 labeling index in accord with another previous study. (14)

Both P53 accumulation and MIB 1 immunoreactivity correlated significantly with a worse prognosis and a reduced overall survival but BCL2 expression has no independent effect on the survival of patients.

The molecular characterization of ovarian cancer in different subtypes is the cornerstone to build a personalized approach in the era of personalized medicine (22, 23, 24, 25, 26, 27, 28, 29, 30, 31).

**CONCLUSIONS**

Our results indicate that the simultaneous evaluation of p53 accumulation and MIB 1 labeling index (L. I.) has independent prognostic indications in serous ovarian carcinomas. Our data show significant differences in the expression of P53, BCL 2, and MIB1 in ovarian tumors and suggest a possible role for these tumor-associated genes as supplemental tools in prognosis and further definition of the biologic potential of these tumors.

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