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ORIGINAL ARTICLE

Human papilloma virus (HPV) status, $p16^{INK4a}$, and p53 overexpression in epithelial malignant and borderline ovarian neoplasms ☆

Giovanna Giordano[a,*], Cinzia Azzoni[a], Tiziana D'Adda[a], Alba Rocco[b], Letizia Gnetti[a], Elisabetta Froio[a], Carla Merisio[c], Mauro Melpignano[c]

[a]*Department of Pathology and Laboratory Medicine, Section of Pathology, Parma University, 43100 Parma, Italy*
[b]*Department of Clinical and Experimental Medicine, Gastroenterology Federico II Naples University, Italy*
[c]*Department of Obstetric and Gynecologic Sciences and Neonatology, Parma University, 43100 Parma, Italy*

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Abstract

This investigation is the first to evaluate simultaneously human papilloma virus (HPV) status, $p16^{INK4a}$, and p53 immunoreactivity in epithelial ovarian neoplasms. The results were analyzed and correlated with histological type, histological grade, and survival of patients. Subtypes considered are papillary serous and mucinous.

Polymerase chain reaction (PCR) analysis, performed in our previous study, had already demonstrated a small number of HPV-positive epithelial ovarian neoplasms. No significant correlation was found between the presence of HPV DNA and subtypes of ovarian neoplasms; thus, HPV cannot be considered responsible for epithelial ovarian neoplasm.

Since p16 immunoreactivity was present in many other HPV-negative cases of epithelial ovarian neoplasms, this study suggests that p16 overexpression in some neoplasms of the female genital tract is not related to HPV carcinogenesis.

A higher p53 expression rate observed between borderline and malignant serous tumors and between serous and mucinous neoplasms can confirm a recent dualistic model of ovarian carcinogenesis. According to this theory, low-grade serous carcinomas (serous intraepithelial carcinomas, serous borderline neoplasm, and ovarian mucinous neoplasms) (type I tumors) develop from mutations of KAS and BRAF, while high-grade serous carcinomas (type II tumors) develop from mutation of p53.

In malignant neoplasms, for univariate analysis, patient survival seems to be related to p53, strong and diffuse p16 overexpression, and the stage of development of neoplasms at the diagnosis.

In multinomial logistic regression, used to evaluate the role of staging, grading, p16 and p53 immunopositivity as predictor variables of unfavorable outcome of the disease, only p16 positivity was significantly related to the poor prognosis of the cancer.

Keywords: Human papilloma virus (HPV); $p16^{INK4a}$ and p53 immunoreactivity; Epithelial ovarian neoplasms

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*Corresponding author. Tel.: +39 0521 290391; fax: +39 0521 292710.
E-mail address: giovanna.giordano@unipr.it (G. Giordano).

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Introduction

p16 is the product of the *ink4a* gene and specifically binds to cyclin D–cdk4/6 complexes to control the cell cycle at the G_1–S interphase.

Practically, p16 is a tumor suppressor protein that decelerates the cell cycle by inactivating cyclin-dependent kinases that phosphorylate retinoblastoma (Rb) protein [48]. Phosphorylate pRb, which results in a conformational change, can release the associated protein E2F from the pRb; thus, protein E2F can activate the genes necessary for cell progression through the G1 phase [57].

Recent studies have revealed that p16 expression is markedly influenced by the status of Rb expression.

In fact, hypophosphorylated active Rb can repress p16 expression, whereas inactivation of pRb by phosphorylation causes p16 expression [37].

Many studies have shown $p16^{INK4a}$ overexpression in high-grade cervical squamous intraepithelial lesions associated with high-risk human papilloma virus (HPV) types, because in this instance, there is functional inactivation of pRb by HPV E7 protein [16,24–26]. Thus, the accumulation of p16 protein is the consequence of loss of the pRb function, which normally inhibits transcription of p16 [18,34].

On immunohistochemical analysis, in neoplastic cervical lesions, diffuse $p16^{INK4a}$ positivity can be regarded as a surrogate marker of the presence of HPV [24,40].

p53 is a tumor suppressor known to play an important role in the suppression of cellular growth and neoplastic transformation. It controls numerous downstream targets involved in apoptosis, growth arrest, and senescence [3].

Mutant p53 proteins generally have a longer half-life than wild-type p53 proteins and lead to nuclear accumulation [13,45]. Thus, mutant forms of p53 can be detected by immunohistochemistry as nuclear positivity [32,44].

Grace et al. [19] have found a highly significant correlation between p53 expression and HPV cervical carcinomas.

Their results, in fact, suggest that p53 protein inactivation by a complex formation with high-risk HPV subtypes may be responsible for the overexpression of p53 in cervical cancer [19].

According to Werness et al. [59], in cervical carcinoma, p53 function could be abrogated by interaction with E6 oncoprotein encoded by HPV.

Other studies have demonstrated that immunoreactivity for p53 protein can be observed in both cervical intraepithelial and invasive neoplasms [9,23,52], and overexpression of p53 and HPV was a significant predictor of poor outcome in microinvasive and invasive cervical carcinomas [21,31].

There are relatively few studies documenting p16 overexpression in ovarian neoplasms. The majority of these indicate that p16 overexpression is common in malignant ovarian neoplasms [2,7,10,14,43,50]. p53 mutations are found in 26–79% of ovarian cancers [28,30,36,38,39,41,54].

Many of these studies suggest that immunohistochemical staining may correlate with the presence of p53 missense mutations in 50–80% of ovarian tumors [30,38,54].

Because many researchers suggest that HPV may be involved in the development of epithelial ovarian neoplasms [22,33,34,61], the aim of this study is to examine p16 and p53 expression in serous and mucinous ovarian tumors, both malignant and borderline subtypes, whose HPV status has been investigated previously [18].

p16 and p53 expression in these subtypes of ovarian neoplasms were investigated immunohistochemically and correlated with HPV status. Then, the results were analyzed and correlated with histological type, histological grade, and the survival of patients affected by these subtypes of ovarian neoplasms.

Materials and methods

Tissue samples

For immunohistochemical analysis, epithelial ovarian neoplasms were collected from 73 patients who had been surgically treated between 1989 and 2001. Histological diagnoses were formulated in the Department of Pathology, Parma University. The follow-up was made in the Department of Obstetric and Gynecologic Sciences and Neonatology, Parma University.

The main pathological feature analyzed was the histological type of ovarian neoplasm. The neoplasms considered were borderline and malignant epithelial ovarian neoplasms. The grade of each malignant tumor was established according to the criteria suggested by Silverberg [51].

The stage of development of the neoplasms was evaluated according to the International Federation of Gynecologists' and Obstetricians' system (FIGO system) [4].

HPV status

HPV status was evaluated by polymerase chain reaction (PCR) according to the method used in our previous investigations [15–18].

The HPV types tested included a broad spectrum of mucosotropic genotypes (6,11,13,16,18,30–35,39,40,42,45,51–53,56,58,61,66). Most of these genotypes are correlated with lesions of high oncogenic risk (16,18,45,56,58).

Immunohistochemistry

Formalin-fixed, paraffin-embedded sections were cut (4 μm) and then deparaffinized and rehydrated through graded alcohols to water and incubated in 3% hydrogen peroxide for 5 min at room temperature to block endogenous peroxidase activity. The sections of each neoplasm were incubated overnight at 4 °C using the primary monoclonal antibody anti-p16^{INK4} (Neomarkers, Ab-7, clone 16P07, working dilution: 1/50). For antigen retrieval, slides were heated by microwave in 10 mM citrate buffer (pH 6) for three 5-min cycles. The primary antibody was detected using the ImmPress polymerized reporter enzyme staining system (ImmPRESS reagent kit, Vector Laboratories, Burlingame), according to the manufacturer's specifications. Finally, the immunostaining was visualized with 3,3-diaminobenzidine (DAB) as the substrate chromogen for 5 min, and sections were counterstained with Harris' hematoxylin.

Colon carcinomas with nuclear staining indicative of p16 expression were used as positive control.

Immunohistochemical evaluation of p53 expression was made using the primary monoclonal antibody anti-p53 (clone DO7, Dako, Glostrup, Denmark, working dilution of 1/50).

For antigen retrieval, sections were treated with 10 mM citrate at pH 6.0 in a 750-W microwave oven for three 5-min cycles. The sections were immunostained with the streptavidin–biotin kit (LSAB2, Dako) in accordance with the manufacturer's specifications and counterstained with hematoxylin. Negative controls consisted of substituting normal mouse serum for the primary antibodies.

For estimating p16 protein expression, 10 high-power fields were examined, and simultaneous staining of tumor nuclei and cytoplasms was scored as positive.

p16 staining was considered negative when $\leqslant 10\%$ of tumor cells exhibited nuclear and cytoplasmic staining. Positive p16 staining was classified in two types: type 1, positive staining with strong nuclear and cytoplasmic reactivity in more than 80% of neoplastic cells; and type 2, positive staining (focal staining) with strong nuclear and cytoplasmic reactivity in 10–80% of neoplastic elements [2].

p53 immunohistochemical staining was considered positive only when 50% and $>50\%$ of tumor cells showed nuclear staining [1].

Both p16 and p53 immunoreactivity were assessed by counting 100 tumor cells under a grid at $\times 400$ magnification and calculating the percentage of positive elements.

Expression of p16 and p53 proteins was correlated with histological subtypes of ovarian neoplasms, their grading of differentiation, HPV status, and the survival of patients.

Statistical analysis

The frequency of HPV status, p16, and p53 expression in the different subtypes of epithelial ovarian neoplasms was analyzed using X^2 and Fisher's exact tests.

Correlation between variables was examined by Spearman's nonparametric correlation test. Overall survival (OS) was defined as the interval between histological diagnosis and death from any cause, death being scored as an event, and patients who were alive being censored at the time of last follow-up. OS curves were drawn using Kaplan–Meier estimates, and were compared using log rank tests. Survival rates are presented with their 95% confidence intervals.

Multinomial logistic regression was used to evaluate the role of staging, grading, p16, and p53 immunopositivity as predictor variables of unfavorable outcome of the disease.

Data were analyzed using Prism 4 package (version 4.0) for Windows.

$P<0.05$ was taken as level of significance.

Results

HPV status

PCR study detected the presence of HPV DNA, as a weak signal, in only one out of 27 serous papillary cystadenocarcinomas (SPC) (3.70%), in 1 out of 13 borderline serous papillary neoplasms (BSPN) (7.69%), and in 1 out of 8 borderline mucinous neoplasms (BMN) (12.5%). None of the mucinous cystadenocarcinomas (MC) showed a signal for HPV DNA.

No significant correlation was found between the presence of HPV DNA and subtypes of ovarian tumors. (Spearman's correlation test $r = -0.1677$, $p = 0.1562$).

Immunohistochemical analysis of p16 and p53 expression

Immunohistochemical expression of p16 and p53 expression was examined in 73 epithelial ovarian neoplasms: 52 malignant lesions and 21 tumors of borderline malignancy. The malignant neoplasms included 27 SPC (36.9%) and 25 MC (34.3%).

Tumors of borderline malignancy included 13 serous papillary (17.9%) (BSPN) and 8 mucinous (10.9%) (BMN) neoplasms.

For p16 protein expression, two patterns of immunohistochemical staining were considered: type1 (strong immunoreactivity) and type 2 immunostaining (focal immunoreactivity), this marker was detected in 10 of 13 BSPN (77%), in 24 of 27 SPC (89%), in 2 of 8 BMN (25%), and in 9 of 25 MC (36%). A significantly higher

p16 expression rate was observed in SPC than in MC ($p < 0.0001$). p16 overexpression was also significantly higher in BSPN than in BMN ($p = 0.0318$).

A correlation between malignant and borderline neoplasms was also considered. This correlation revealed that p16 expression was significantly higher in BSPN than in MC ($p = 0.0382$).

Similarly, a significantly higher p16 expression was observed in SPC than in BMN ($p = 0.0012$).

Instead, no noteworthy correlation was found between BSPN and SPC ($p = 0.3699$), and between BMN and MC ($p = 0.6870$).

Considering only type 1 immunoreactivity (strong immunoreactivity), a significantly higher expression of p16 was found in SPC than in BSPN ($p = 0.0204$, significant value). Instead, there was no difference in p16 expression between BMN and MC ($p = 1.0000$) when considering only type 1 immunoreactivity (strong immunoreactivity).

Thus, these data demonstrate that serous papillary neoplasms show a higher significant expression of p16 than the mucinous subtype. Moreover, malignant serous neoplasms reveal strong positivity that is significantly higher than the borderline serous tumors. Instead, there is no difference in p16 expression between borderline and malignant mucinous tumors when considering only type 1 positivity.

Regarding p53 overexpression, this marker was expressed in 2 out of 13 BSPN (15.38%) (Fig. 1), in 13 out of 27 SPC (55.5%) (Fig. 2), in none of 8 BMN (0%), and in 1 out of 25 MC (Fig. 3) (8%).

A significantly higher p53 expression rate was observed in SPC than in MC ($p = 0.0004$, extremely significant value).

There was no difference in p53 expression between BSPN and MC ($p = 0.2651$, not significant value), between BSPN and BMN ($p = 0.5048$, not significant value), and between BMN and MC ($p = 1.0000$, value considered not significant, Fisher's exact test).

An almost significant p53 expression rate was observed between BSP and SPC ($p = 0.0801$, Fisher's exact test).

Thus, serous papillary neoplasms showed a significantly higher expression of p53 than the mucinous subtypes.

A correlation between grading and p16 and p53 expression was made in both SPC and MC, first considering type1 immunoreactivity (strong immunoreactivity) and type 2 immunostaining (focal immunoreactivity), and then only type 1 positivity.

Table 1 shows that type 1 p16 immunostaining was more frequent in grade 3 than in grade 2 carcinomas, and was absent in grade 1 tumors (Table 1).

Moreover, Spearman rank correlation revealing a value of $p < 0.0001$ (extremely significant value) demonstrated that less differentiated carcinomas showed a more elevated expression of p16 protein.

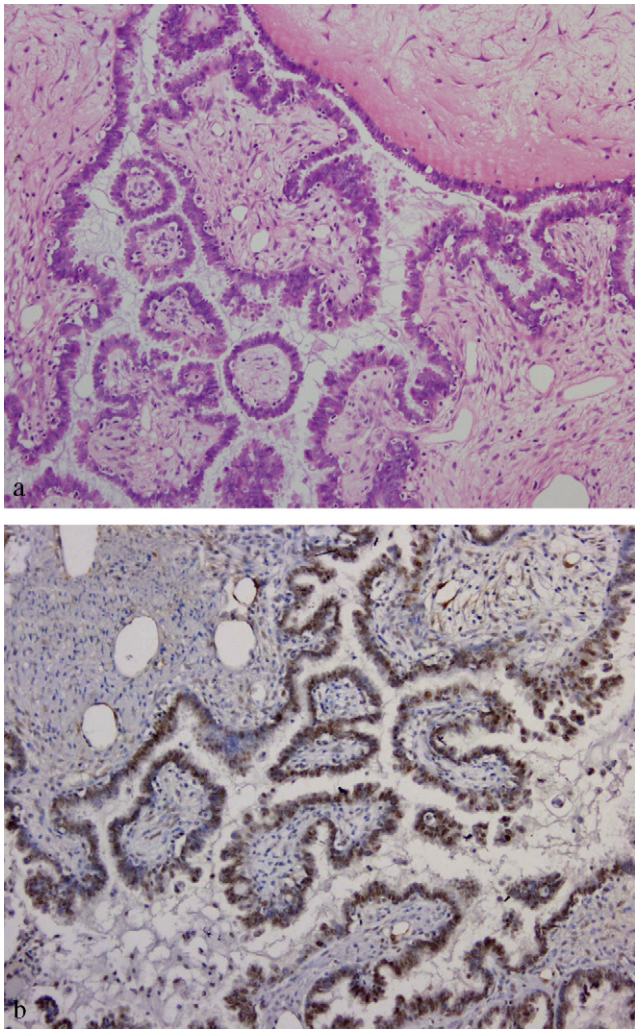

Fig. 1. Example of serous borderline ovarian neoplasm (a: hematoxylin–eosin × 100) showing p53 nuclear immunoreactivity in >50% of neoplastic cells (b: × 100).

Also, comparing only type 1 immunoreactivity between the tumor types, we observed that grade 3 of all neoplasms showed a significantly higher expression of p16 than grade 1 and 2 ($p = 0.0001$, value extremely significant).

Similarly, p53 expression was higher in less differentiated neoplasms (Table 2). Spearman rank correlation, in fact, revealed an extremely significant p-value ($p < 0.0001$).

Immunohistochemical analysis showed that all HPV-positive neoplasms are related to p16 positivity, although the pattern of immunostaining is not equal in three HPV-positive tumors. p16 immunostaining in both the case of HPV-positive SPC and HPV-positive BMN was type 2 immunoreactivity (focal immunoreactivity) (Fig. 4a and b), while in HPV-positive BSPN, immunostaining was type 1 (strong immunoreactivity) (Table 3) (Fig. 5a and b).

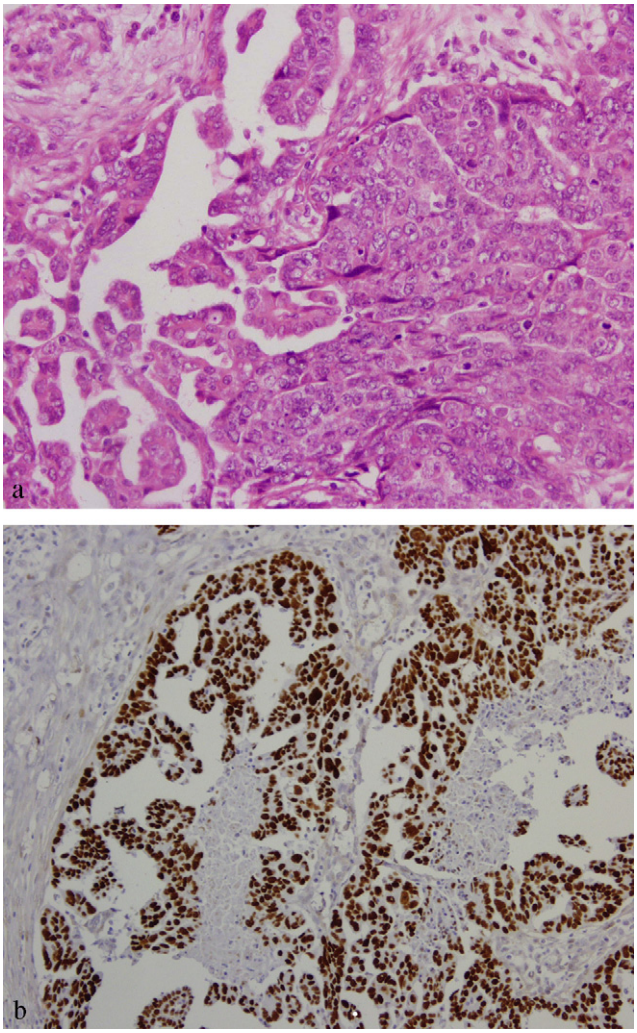

Fig. 2. Example of serous papillary ovarian carcinoma (G3) (a: hematoxylin–eosin × 200) showing p53 nuclear immunoreactivity in >50% of neoplastic cells (b: × 100).

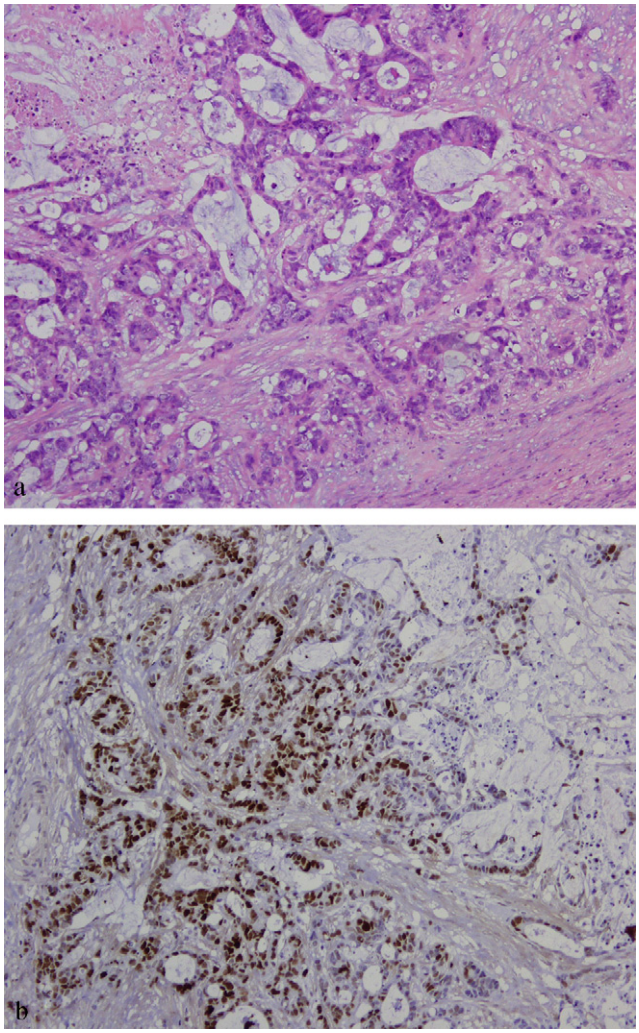

Fig. 3. Example of mucinous ovarian carcinoma (a: hematoxylin–eosin × 200) showing p53 nuclear immunoreactivity in >50% of neoplastic elements (b: × 100).

Instead, in these HPV-related neoplasms was p53 expression independent of the status of HPV. This marker was negative in both HPV-positive BSPN and HPV-positive BMN. Instead, in HPV-related SPC, p53 was positive (Fig. 4c).

Survival analysis of HPV status, p16 and p53 expression grading and staging

Follow-up (range 6 months–11 years) was available for 65 women, but not for 6 cases of malignant tumors (3 with SPC and 3 with MC) and 2 cases of borderline neoplasms (Table 4). Only 1 patient with borderline tumor died 2 years after diagnosis (4.76%), 16 patients were alive and well (70.19%), and 2 were alive with disease.

Eighteen patients with SPC died 1–11 years after diagnosis, while 6 were alive and well.

Seven patients with MC died 6 months–4 years after diagnosis (28%), while 15 were alive and well 6 years after diagnosis (60%) (Table 4).

The 73 patients with epithelial ovarian neoplasms were categorized along a Kaplan–Meier survival curve according to the presence of HPV DNA. In malignant epithelial neoplasms, survival curves were evaluated according to histological type (serous or mucinous), immunohistochemical expression levels of p16 and p53.

Since borderline tumors rarely lead to the death of patients, as observed in our series (only 1 dead patient) (Table 4) and in other studies reported in the literature, the relation between survival and p16 and p53 immunoreactivity was not considered.

No significant correlation was found between the presence of HPV DNA and survival of patients with ovarian tumors ($p = 0.6591$) (Fig. 6).

Table 1. p16 immunohistochemical staining in malignant epithelial neoplasms

Histological type	Grade	Total number	Type 1 immunoreactivity	Type 2 immunoreactivity	Negative
SPC (27 cases)	1	5	0	2	3
	2	9	4	4	0
	3	13	10	3[a]	0
MC (25 cases)	1	10	0	1	9
	2	10	1	4	5
	3	5	1	2	2

MC: mucinous carcinoma, SPC: serous papillary carcinoma.
[a]One case HPV positive on PCR analysis.

Table 2. p53 immunohistochemical staining in malignant epithelial neoplasms

Histological type	Grade	Total number	Positive cases	Negative cases
SPC (27 cases)	1	5	0	5
	2	9	0	9
	3	13	13[a]	0
MC (25 cases)	1	10	0	10
	2	10	0	10
	3	5	1	4

MC: mucinous carcinoma, SPC: serous papillary carcinoma.
[a]One case HPV positive on PCR analysis.

Regarding the subtype of malignant neoplasms, log–rank testing revealed poor prognosis for the serous subtype (Fig. 7) ($p<0.04$).

Moreover, in malignant epithelial neoplasms, log–rank testing revealed that p16 expression type 1 ($p<0.05$) (Fig. 8) and p53 overexpression ($p<0.02$) (Fig. 9) were significantly correlated with poor prognosis.

Instead, in multinomial logistic regression, used to evaluate the role of staging, grading, and p16 and p53 immunopositivity as predictor variables of unfavorable outcome of the disease, only p16 positivity was significantly related to the poor prognosis of the cancer ($p<0.003$).

Discussion

An accurate review of the literature reveals that there are no studies that have simultaneously analyzed HPV status, $p16^{INK4a}$, and p53 expression in malignant and borderline ovarian neoplasms.

This investigation is the first to evaluate simultaneously HPV status, $p16^{INK4a}$, and p53 immunoreactivity in these ovarian neoplasms. The results were analyzed and correlated with histological type, histological grade, and the survival of patients affected by these tumors.

Epithelial ovarian neoplasms considered in the current study are of the serous and mucinous subtypes.

PCR analysis, performed in our previous study, demonstrated that HPV does not seem to play a pathogenetic role in the development of these neoplasms. In fact, HPV DNA was detected as a weak signal in only 1 case out of 27 (SPC) (3.70%), in 1 out of 13 (BSPN) (7.69%), and in 1 out of 8 (BMN) (12.5%) [18].

The HPV DNA, detected as weak positivity on PCR analysis, could be a reflection of a latent infection in this series of ovarian neoplasms. This type of infection is characterized by few copies of HPV genome in the nuclei of infected cells, can be detected only by molecular methods, and cannot be considered to be responsible for ovarian neoplasms.

In fact, in this study, we demonstrated that the presence of HPV DNA does not seem to influence the survival of patients. In addition, we observed that two of three ovarian neoplasms failed to show the type of diffuse strong p16 staining that one would expect to see in HPV-positive tumors based on the observation of HPV-positive cervical neoplasms [24,40,47]. Moreover, we observed p16 immunoreactivity in many other HPV-negative cases of epithelial ovarian neoplasms. Therefore, this study confirms the hypothesis of Armes et al., [2] that p16 overexpression in the female genital tract is not related to HPV carcinogenesis.

Moreover, all our HPV-positive neoplasms (two serous subtypes and one mucinous borderline tumor) showed staining for p16, indicating that HPV and p16 positivity cannot always be considered proof of metastatic cervical neoplasm as suggested by other authors [11,55].

The results of our study revealed that p53 overexpression is also not related to an HPV infection. In fact, many cases of our study with p53 overexpression are HPV-negative. Moreover, in both HPV-positive BSPN and in HPV-positive BMN, this marker was negative. Instead, in the case of HPV-positive SPC, p53 was positive.

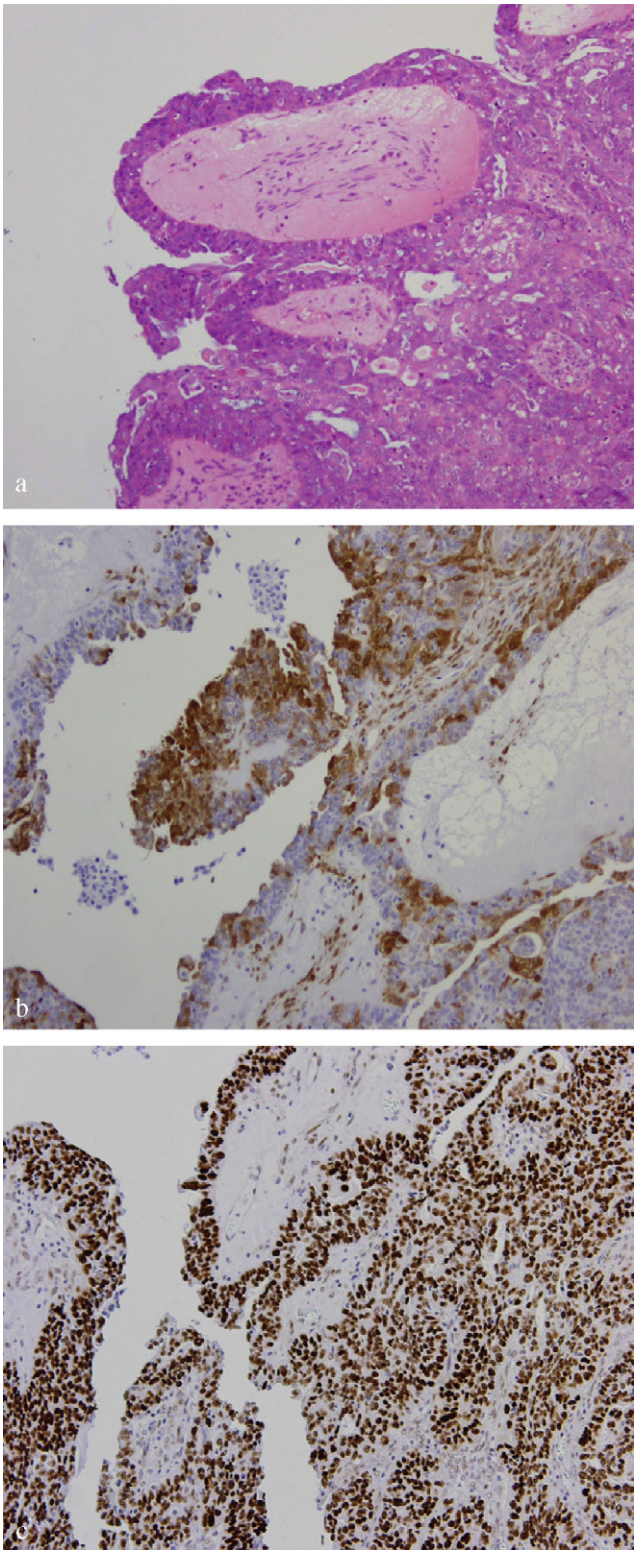

Fig. 4. Example of HPV-positive serous ovarian carcinoma (a: hematoxylin–eosin × 100) showing nuclear and cytoplasmic immunoreactivity type II (focal immunoreactivity) for p16 in 10–80% of neoplastic cells (b: × 100) and p53 nuclear positivity in >50% of neoplastic elements (c: × 100).

Table 3. p16 immunohistochemical staining in borderline neoplasms

Histological type	Total number	Type 1 immunoreactivity	Type 2 immunoreactivity	Negative cases
BSPN	13	2[a]	8	3
BMN	8	0	2[a]	6

BMN: borderline mucinous neoplasm, BSPN: borderline serous papillary neoplasm.
[a]One case HPV positive on PCR analysis.

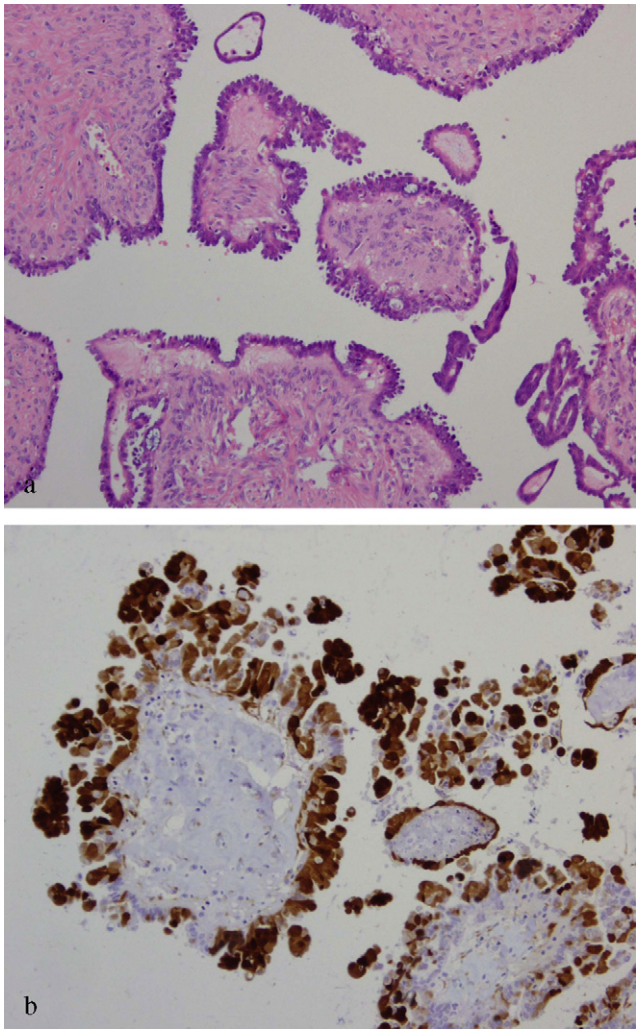

Fig. 5. HPV-positive serous borderline ovarian neoplasm (a: hematoxylin–eosin × 100) with type I nuclear and cytoplasmic immunoreactivity for p16 (strong immunoreactivity) in >80% of neoplastic cells (b: × 100).

Another important result of our investigation demonstrates that all serous papillary neoplasms, compared to mucinous neoplasms, showed significantly higher expression of p16 and p53 proteins. These results are in accordance with the study of Armes et al. [2].

Table 4. Survival data of patients with epithelial ovarian neoplasms

Histological type	Follow-up	Number of patients	Percentage (%)
Borderline tumors (21 cases)	Patients died (after 2 yr)	1	4.76
	Patients well and alive (range 1–9 yr)	16	70.19
	Patients alive with disease (after 4 yr)	2	9.52
	Lost	2	9.52
Serous papillary carcinomas (27 cases)	Patients died (range 1–11 yr)	18	66.1
	Patients well and alive (range 1–3 yr)	6	22.2
	Lost	3	11.1
Mucinous carcinomas (25 cases)	Patients died (range: 6 mo–4 yr)	7	28
	Patients well and alive (range 1–5 yr)	15	60
	Lost	3	12

Mo: months; Yr: years.

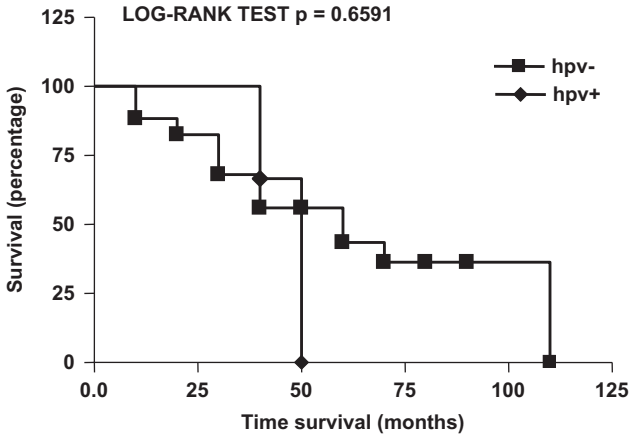

Fig. 6. Log–rank testing showed that no correlation was found between the presence of HPV DNA and survival in patients with epithelial ovarian in neoplasms.

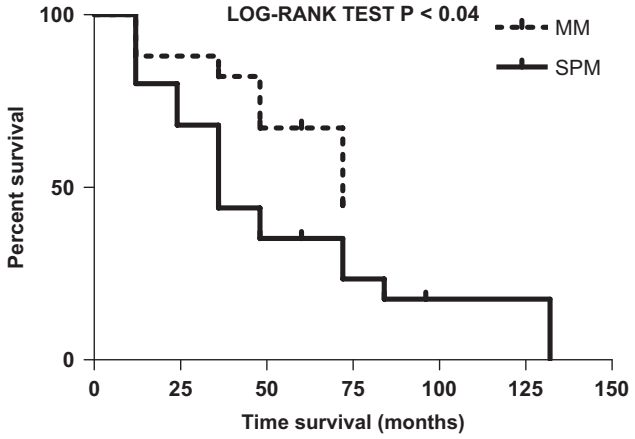

Fig. 7. Overall survival of patients with malignant neoplasms was related to histologic type, showing that the serous subtype was significantly correlated with poor prognosis.

However, these authors, examining a smaller number of cases of epithelial neoplasms, considered their results as preliminary data. In fact, they investigated p16 and p53 overexpression in only 10 SPC, in 3 BSPN, in only 5 BMN, and in no case of MC.

In our study, a significantly higher p53 expression rate observed in SPC than in MC and a higher p53 expression rate observed between borderline and malignant serous neoplasms can confirm a new model of ovarian carcinogenesis as suggested by Shih and Kurman [49]. According to this theory, high-grade serous carcinomas (type II tumors) develop from ovarian epithelial inclusion cysts with mutation of p53 and without a morphologically recognizable intermediate stage, while low-grade serous carcinomas, including atypical proliferative tumor, serous intraepithelial carcinomas, serous borderline neoplasms, and ovarian mucinous neoplasms (type I tumors), develop from mutation of KAS and BRAF genes [49].

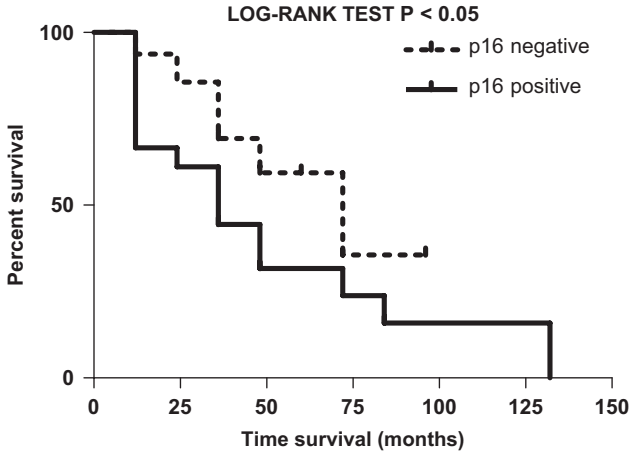

Fig. 8. Univariate analysis: overall survival of patients with malignant epithelial ovarian neoplasms in relation to type 1 p16 immunoreactivity (strong immunoreactivity), showing that p16 positivity was significantly correlated with poor prognosis.

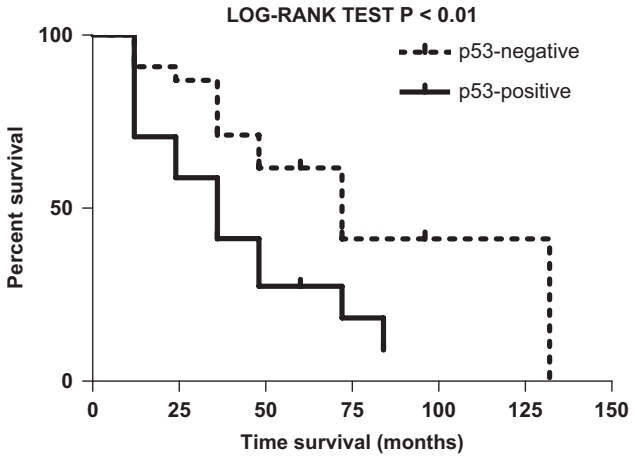

Fig. 9. Univariate analysis: overall survival patients with malignant epithelial ovarian neoplasms in relation to p53 positivity immunoreactivity, showing that p53 overexpression was significantly correlated with poor prognosis.

The KRAS gene encodes the human cellular homolog of a transforming gene isolated from the Kirsten rat sarcoma virus. The RAS proteins encoded by KRAS gene in humans play a vital role in normal tissue signaling, including proliferation, differentiation, and senescence. Mutated genes are potent oncogenes that are involved in many other human cancers [29,58].

BRAF gene is located on chromosome 7q34 and encodes a serine/threonine kinase that is involved in signal transduction pathway [60]. Recently, BRAF was found to be mutated in primary malignant melanomas and other neoplasms [6,8,62].

Regarding p16 immunoreactivity, when considering only type 1 positivity, serous papillary neoplasms showed a significantly higher expression of p16 than the mucinous subtype, and malignant serous neoplasms revealed strong positivity higher than borderline serous tumors. These findings are in accordance with the study of O'Neill et al. [42], who recently observed increased expression in high-grade serous carcinoma compared to low-grade serous borderline tumor.

When considering type 1 positivity, there was no difference in p16 expression between borderline and malignant mucinous tumors. In our view, these different patterns of p16 immunoreactivity also present between serous and mucinous malignant tumors, and between malignant and borderline serous neoplasms could confirm a dualistic model depicting the development of epithelial ovarian neoplasms.

Further studies of molecular biology could be useful to confirm this hypothesis. Another important finding of our study was the correlation between p16 expression and grading of neoplasms. In fact, we demonstrated that less differentiated carcinomas showed a more elevated expression of p16 protein. Thus, p16 expression could be exploited in ovarian carcinomas to increase the sensitivity for the detection of high-grade tumors.

Accurate estimation of prognosis of ovarian cancer is difficult. In this study, we investigated the question of whether the presence of HPV DNA, p53 and p16 expression, and staging of epithelial ovarian neoplasms could be considered as prognostic factors.

In our investigation, analysis of follow-up revealed that the survival of these tumors seems to be independent of HPV status, but was significantly correlated with p53 overexpression and staging of ovarian epithelial neoplasms at the diagnosis.

In malignant neoplasm, only type 1 p16 immunoreactivity (strong immunoreactivity) was significantly associated with poor prognosis.

Our results are in accordance with those of Dong et al. and Fujita et al. [10,14], who found that high expression of p16 was associated with poor prognosis in ovarian carcinomas.

With regard to the prognostic value of p53, this is controversial [5,7,12,20,27,35,46,53,56]. Although some authors have reported the prognostic value of p53 in ovarian cancer [5,7,20,35], independence of this prognostic value was often doubtful, and the positive relation between p53 overexpression and poor prognosis was thought to be secondary to the association of expression with tumor grade and aggression [12,20].

In our view, these discrepancies, observed by different researchers, could be due to different methodologies used and to different interpretation criteria considered to establish p53 overexpression, such as cutoff value of staining, as well as the types of antibodies used [53] and other technical aspects.

To summarize, the present study demonstrates that:

(1) HPV cannot be considered responsible for epithelial ovarian neoplasm.
(2) p16 overexpression in this series of ovarian neoplasms is not related to HPV carcinogenesis.
(3) Ovarian neoplasms with p16 immunoreactivity and the presence of HPV DNA are not always metastases from malignant cervical tumors [11,55].
(4) A higher p53 expression rate observed between borderline and malignant serous tumors and between serous and mucinous neoplasms can confirm a recent dualistic model of ovarian carcinogenesis. According to this theory, low-grade serous carcinomas (serous intraepithelial carcinomas, serous borderline neoplasm, and ovarian mucinous neoplasms) (type I tumors) develop from mutations of KAS and BRAF, while high-grade serous carcinomas (type II tumors) develop from mutation of p53.
(5) Borderline tumors rarely lead to the death of patients as we observed in our series (only 1 patient died).

(6) For univariate analysis, malignant neoplasms patient survival seems to be related to p53, strong and diffuse p16 overexpression, and the stage of development of neoplasms at diagnosis. Instead, in multinomial logistic regression, used to evaluate the role of staging, grading, and p16 and p53 immunopositivity as predictor variables of unfavorable outcome of disease, only p16 positivity was significantly related to the poor prognosis of the cancer.

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