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Clinicopathologic and prognostic significance of cyclooxygenase-2 expression in endometrial carcinoma

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Summary. Backgroud: Endometrial carcinoma is the most common malignancy of the female genital tract in the Western world. COX-2 is highly expressed in endometrial carcinoma, but there is controversy regarding its clinical role and its possible prognostic role. COX-2 expression was determined by immunohistochemistry and was correlated to standard clinicopathologic variables in a series of primary untreated endometrial carcinoma patients. COX-2 as an accurate predictor of the disease was also analyzed. Methods: One-hundred and ten cases of primary untreated endometrial carcinoma hosts who were admitted to the Department of Obstetrics and Gynecology, University General Hospital of Alexandroupolis, were investigated. Immunohistochemistry was performed using rabbit polyclonal antiserum against human COX-2. Results: Twenty-eight patients (25.5%) were scored as COX-2 positive. A statistically significant association was found between COX-2 overexpression and FIGO stage (p=0.010). A positive correlation was also found with histological grade (p=0.019) and myometrial invasion (p=0.026). No significant association was found with histologic type of the tumor (p=0.164). COX-2 positive patients had a significant association with sort survival (p=0.028). Conclusions: COX-2 expression is an independent clinicopathologic factor and an independent prognostic factor in endometrial carcinoma. It could be used to plan treatment modalities for hosts.

Key words: COX-2, Endometrial cancer, Immunohistochemistry

Introduction

The cyclooxygenase (COX) is the key enzyme in the conversion of arachidonic acid to Prostaglandin G2 (PGG2) and the consequent reduction of PGG2 in PHG2. These reactions constitute the first steps in the production of a variety of prostanoids (Madaan et al., 2000; Jabbour et al., 2001). Two COX isoforms have been characterized (Chapple et al., 2000). COX-1, which is expressed constitutively in almost all tissues serves homeostatic functions, whereas COX-2, which is highly inducible by growth factors, prostaglandins, and tumor promoters, plays a key role in the inflammatory response (Singer et al., 1998).

In 1994, the expression of COX-2 was reported in carcinomas of the colon, and in recent years, increased levels of COX-2 were found in carcinomas of the stomach, breast, esophagus, and lung (Eberhart et al., 1994; Ristimaki et al., 1997; Hwang et al., 1998; Wilson et al., 1998). Importantly, overexpression of COX-2 in human carcinomas appears to be significant (Oshima et al., 1996; Fujiwaki et al., 2002). COX-2 overexpression in human cancer cells is associated with apoptosis inhibition, increased adhesion to the extracellular matrix, metastatic potential, and neoangiogenesis (Tsujii et al., 1998; Jabbour et al., 2001; Saukkonen et al., 2001). In fact, various studies are currently being conducted based on the potential effect of COX-2 inhibitors on cancer prevention and/or therapy (Hong and Sporn, 1997; Taketo, 1998; Li et al., 2002).

In the present study, we examined the expression of COX-2 and its association with clinicopathological features and clinical outcome, in settings of primary untreated endometrial carcinomas patients.

Materials and methods

One hundred and ten patient-cases of endometrial carcinoma documented by the Pathology Department, University General Hospital of Alexandroupolis were studied. Patients' age ranged from 40-88 years. For all patients diagnostic curettage and hysterectomy paraffin embbeded block sections were available. Slides were stained with conventional hematoxylin and eosin (H&E). Unstained slides were obtained for the detection of COX-2 rabbit polyclonal antigen (Assay Designs, Inc.).

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The clinicopathological parameters evaluated were age, FIGO stage, type of carcinoma, depth of myometrial invasion. Survival was also studied. The Regional Ethics Committee approved the study. Written informed consent was obtained from all patients and the procedures were in accordance with the institutional guidelines.

Immunohistochemistry

Immunohistochemistry was performed with COX-2 used on serial sections. Tissue specimens were fixed in formalin and embedded in paraffin according to standard procedures. Four-micron sections (4μm) of representative blocks from each case were deparaffinized, rehydrated, and treated with 0.3% H_2O_2 for 5 min in methalol to prevent endogenous peroxidase activity. Slides were then incubated for 75 min with the COX-2 rabbit polyclonal antibody at a 1:40 dilution. Control slides were incubated for the same period with nonimmunized rabbit serum (negative control). A positive control was always run in the assay. We used the "envision kit" (dextran free biotin one step, DAKO) according to the manufacturer's instructions. Finally, bound antibody complexes were stained for 10 min with 0.05% diaminobenzidine. Sections were then briefly counterstained with Mayer's haematoxylin, mounted, and examined under a Nikon (X200) microscope of X200 magnification. Scoring was assigned according to the proportion of cells stained. Cell count was performed by using 10 high power fields (X40) for each section. Sections with greater than 10% stained tumor cells were considered as being positive. COX-2 immunostaining was observed mainly in the cytoplasm of the tumor cells (Figs. 1, 2). Stromal lymphoid cells sometimes showed variable intensities of COX-2 immunoreaction.

Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS), version 10.0 (SPSS, Inc., Chicago, IL, USA). Categorical variables were expressed as frequencies (and percentages) and continuous variables were expressed as the mean ± standard deviation. The chi-square test was used to evaluate any potential association between COX-2 expression and the clinicopathological parameters, while odds ratios and their 95% confidence interval (CI) were calculated by means of simple logistic regression analysis. As an indicator of survival the disease-specific survival (including only death related to the disease as an event) was investigated. Survival rates were calculated with the Kaplan-Meier method and the statistical difference between survival curves was determined with the log-rank test. Multivariate logistic and Cox proportional hazards regression analysis, using a backward selection approach, were performed to explore the independent effect of variables on COX-2 expression and survival, respectively. All tests were two-tailed and statistical significance was considered for p values <0.05.

Results

Patients

One hundred and ten primary untreated endometrial cancer patients underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy at the Department of Obstetrics and Gynecology, University General Hospital of Alexandroupolis. Patient's age ranged from 40 to 88 years, with a mean age of 59.05 ± 8.57 years and 47 (43%) patients were over 60 years. Regarding the clinical stage, 89 (81%) carcinomas were FIGO stage I-II and 21 (19%) FIGO stages III-IV, while regarding histological type, 95 (86%) were endometrioid adenocarcinomas and 15 (14%) non-endometrioid carcinomas (Table 1). In particular, 2 (2.1%) of 95, endometrioid adenocarcinomas, showed focal squamous metaplasia and 8 (8.4%) papillary configuration. Four (26.7%) of 15 non-endometrioid carcinomas were adenosquamous, 4 (26.7%) serous-papillary, 5 (33.4%) clear cell carcinomas, 1 (6,6%) squamous and 1 (6.6%) undifferentiated. Seventy-eight (71%) were well differentiated (G1), 21 (19%) moderately (G2) and 11 (10%) poorly differentiated. Myometrial invasion did not exceed the inner half of the myometrial wall in 63 (57%) cases, while cancer infiltrated the outer half of the myometrium in 47 (43%) cases.

Association of COX-2 expression with clinicopathological parameters

Immunohistochemical staining showed COX-2 positivity of at least 10% of the tumor cells in 28 (25.5%) cases. COX-2 expression was analyzed in

Table 1. Patient and tumor characteristics of the 110 investigated endometrial carcinomas.

CHARACTERISTICS	NUMBER O PATIENTS	PERCENTAGE
Age		
≤ 60 years	63	57.3
>60 years	47	42.7
FIGO Stage		
I-II	89	80.9
III-IV	21	19.1
Histological type		
Endometrioid	95	86.4
Non- endometrioid	15	13.6
Histological grade		
G1	78	70.9
G2-G3	32	29.1
Myometrial invasion		
<1/2	63	57.3
>1/2	47	42.7

relation to the following parameters: patient's age, clinical stage, histological type, histological grade, and depth of myometrial invasion (Table 2). A statistically significant association was found between COX-2 overexpression and clinical stage, where COX-2 positive rate was significantly higher in stages III-IV compared

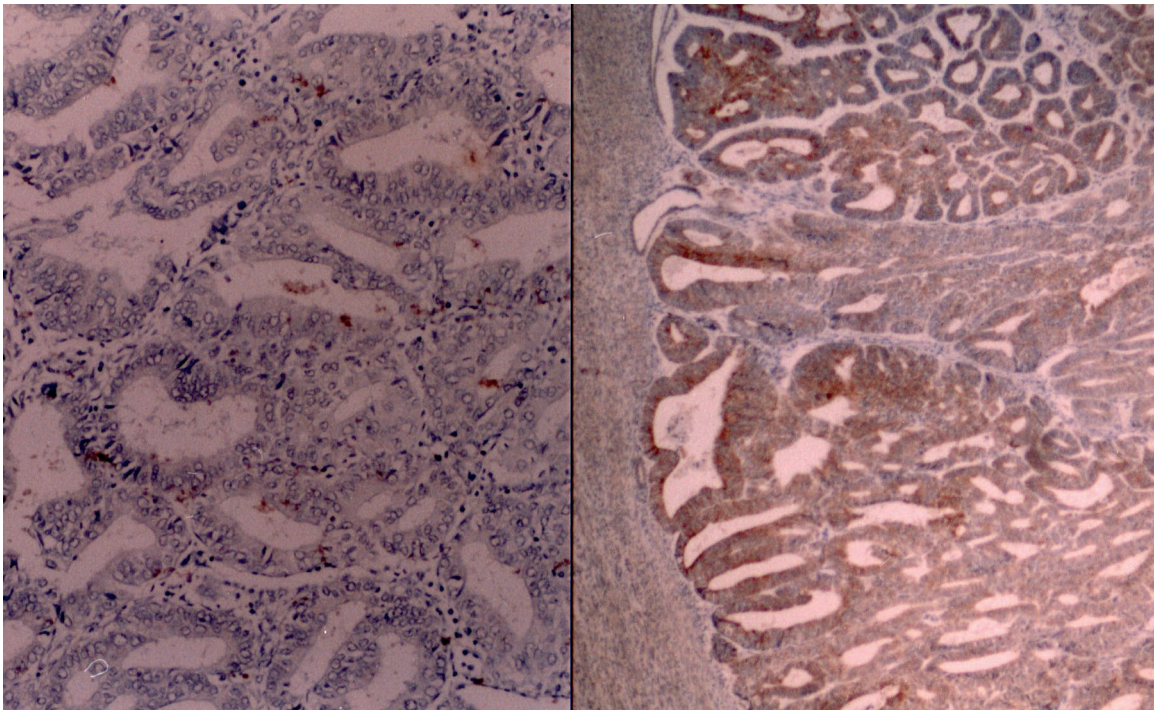

Fig. 1. Well-differentiated endometrial carcinoma, endometrioid type, negative (left figure) and positive (right figure) for COX-2 Immunostain. x 40

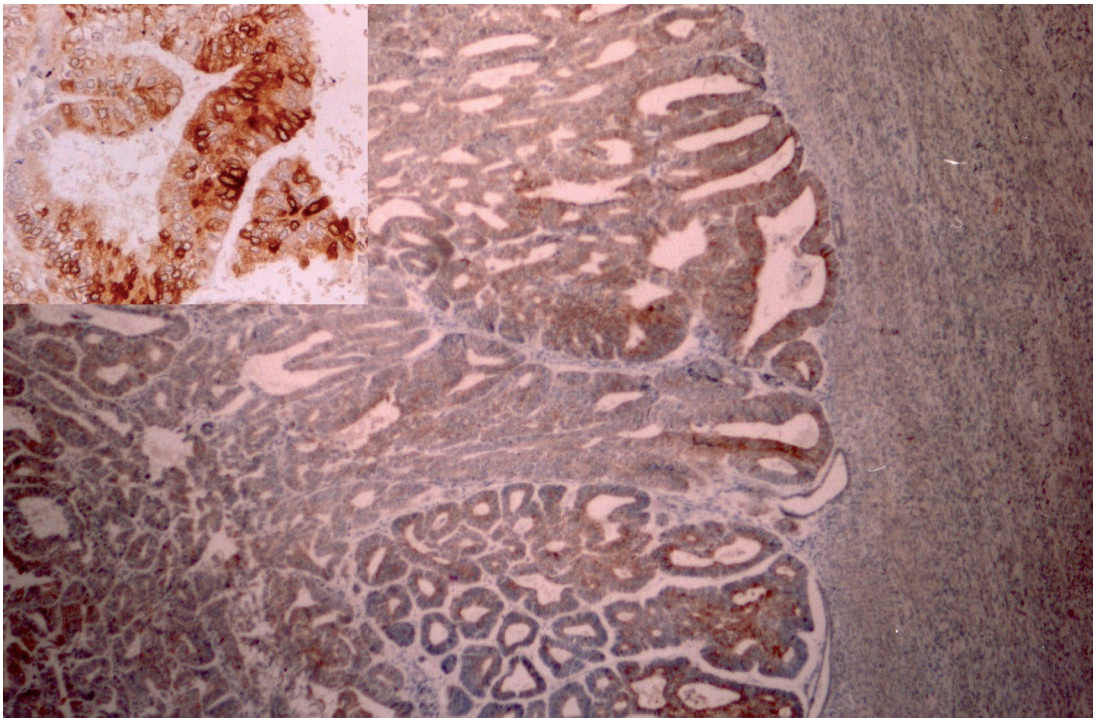

Fig. 2. Well-differentiated endometrial carcinoma, endometrioid type, COX-2 Immunostain. x 40 (inset x 200)

to stages I-II (47.6% [10 of 21 pts] vs 20.2% [18 of 89 pts], p=0.010). A positive correlation was also found between COX-2 overexpression and the histological grade of tumor. In this regard, moderately or poorly differentiated tumors were almost 3 times as likely to overexpress COX-2 as well-differentiated tumors (40.6% [13 of 32 G2-G3 tumors] vs 19.2% [15 of 78 G1 tumors], p=0.019). Elevated COX-2 expression was associated with deep myometrial invasion. In particular, COX-2 positive rate was significantly higher in cases with greater than 50% myometrial invasion compared to cases without or less than 50% myometrial invasion (36.2% [17 of 47 cases] vs 17.5% [11 of 63 cases], p=0.026). Regarding histological type, non-endometrioid tumors tend to overexpress COX-2 more frequently than endometrioid tumors, but this trend did not reach statistical significance (40.0% [6 of 15 non-endometrioid tumors] vs 23.2% [22 of 95 endometrioid tumors], p=0.164). No significant association was found between COX-2 overexpression and patient's age (p=0.987). Multivariate logistic regression analysis revealed that clinical stages III-IV (p=0.007) and moderately or poorly differentiated tumors (p=0.015) were independently associated with high COX-2 expression.

Table 2. COX-2 overexpression in endometrial carcinoma in association with clinicopathological parameters.

	COX-2 OVEREXPRESSION		
	No of pts (%)	Odds Ratio [95% CI]	p value
Age			0.987
≤ 60 years	16 (25.4%)	1	
>60 years	12 (25.5%)	1.01 [0,42-2.40]	
FIGO Stage			0.010
I-II	18 (20.2%)	1	
III-IV	10 (47.6%)	3.59 [1.32-9.75]	
Histological type			0.164
Endometrioid	22 (23.2%)	1	
Non- endometrioid	6 (40.0%)	2.21 [0.71-6.90]	
Histological grade			0.019
G1	15 (19.2%)	1	
G2-G3	13 (40.6%)	2.87 [1,18-6,95]	
Myometrial invasion			0.026
<1/2	11 (17.5%)	1	
>1/2	17 (36.2%)	2.68 [1.11-6.47]	

Survival analysis

Follow-up was available for 101 patients, since 9 patients (8.2%) were lost. Mean duration of follow-up was 76.58±42.57 months (range, 4 to 176 months), with a median follow-up time of 71 months. Twenty-five patients (24.8%) died during this time. The mean survival time was 136±7 months (95% CI= 123 to 150 months).

Patients divided into two groups according to COX-

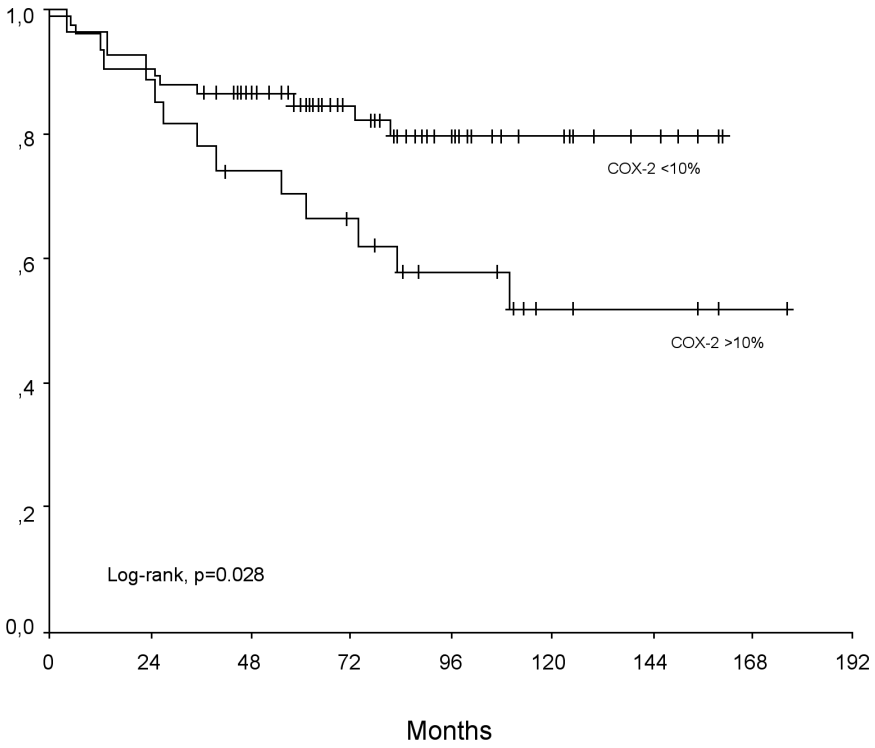

Fig. 3. Overall survival of patients with endometrial carcinoma, at a 71-month follow-up, according to COX-2 expression (≤ 10% vs >10%).

2 expression. Among patients with negative COX-2 expression (n=74), the Kaplan-Meier survival estimates of the 1-year, 5-year and 10-year survival rates were 93.24±2.92%, 84.65±4.29% and 79.42±5.40%, respectively, while the respective rates among patients with positive COX-2 expression (n=27) were 96.30±3.63%, 70.18±8.85% and 51.93±10.38%. The log-rank test revealed a statistically significant difference between survival rates over time (p=0.028), with patients overexpressing COX-2 having worse prognosis (Figure 3). Moreover, mean survival time was 135±7 months (95% CI=122 to 148 months) in patients with COX-2 negative expression and 115±13 months (95% CI=95 to 143 months) in patients with COX-2 positive expression. Mortality rate was significantly higher in patients with COX-2 positive expression compared to COX-2 negatives (44.4% vs 17.6%, p=0.006). COX-2 positives were 2.55 times more likely to die of cancer than COX-2 negatives (Hazard ratio=2.55, 95% CI=1.14 to 5.69, p=0.023). Further investigation with multivariate Cox proportional hazards regression analysis revealed that COX-2 overexpression was marginally independently associated with worse prognosis (Hazard ratio =1.87, 95% CI=0.92 to 4.41, adjusted for age, stage, type, grade and depth; p=0.082).

Discussion

COX -2 according to the literature is overexpressed in endometrial carcinoma cells compared to normal endometrial cells (Comerci et al., 2000; Einstein et al., 2000; Tong et al., 2000); yet preliminary data concerning COX-2 expression in endometrial hyperplasia are conflicting (Comerci et al., 2000; Einstein et al., 2000; Ferradina et al., 2002; Uotila et al., 2002). The progressive increase in COX-2 expression in normal epithelium through preneoplastic lesions to carcinoma has been observed in other epithelial tissues, suggesting that up-regulation of COX-2 expression may play a role in tumor onset and progression (Wilson et al., 1998; Chapple et al., 2000; Madaan et al., 2000; Saukkonen et al., 2001). Although the mechanism of COX-2 upregulation is unknown, recent studies suggest that it may result from the deregulation of key steps in the epidermal growth factor receptor signaling pathways, including the ras oncogene (Sheng et al., 2001) whose overexpression has been detected in endometrial tumors (Scambia et al., 1993). To our knowledge little has been reported about the correlation of COX-2 positivity with clinicopathological parameters and clinical outcome (Ferradina et al., 2002; Fujiwaki et al., 2002)

The overexpression of COX-2 was studied, however, in the intestinal and other tumors and it was related with clinicopathological factors concerning aggressiveness and prognosis (Chapple et al., 2000; Madaan et al., 2000; Tomozawa et al., 2000; Ohno et al., 2001; Saukkonen et al., 2001).

The mechanism of COX-2 increase is unknown; recent studies report that it can result from the release of EGFR (epithermal growth factor receptor) or in association with the ras oncogene (Tong et al., 2000; Sheng et al., 2001). Previous studies have suggested that COX-2 overexpression may enhance tumorigenic potential by promoting angiogenesis, increasing cellular adhesion, inhibiting apoptosis, and activating peroxisome proliferator-activated reporter δ (Daniel et al., 1999; Masferrer et al., 2000; Tong et al., 2000; Williams et al., 2000). Angiogenesis is induced by various factors, including vascular endothelial growth factor (VEGF) and thymidine phoshorylase (TP) produced by the tumor (Sivridis, 2000, 2001; Sivridis et al., 2002). In several epithelial cancers, including endometrial carcinomas, upregulation of COX-2 may be intimately involved in tumorigenesis starting in the early stages (Hao et al., 1999). Overexpression of COX-2 has been linked not only to tumorinogenesis, but also to increased metastatic potential with activation of matrix degrading metalloproteinase enzymes (Uefuji et al., 2000; Masunaga et al., 2000).

In our study we observed higher rate of COX-2 positivity in moderately and poorly differentiated carcinomas than in well-differentiated ones (p=0.019). These results confirm previous findings reported for endometrial (Einstein et al., 2000; Ferrandina et al., 2002) and prostate tumors (Madaan et al., 2000) and suggest that COX-2 overexpression could identify more aggressive endometrial carcinomas. COX-2 positive immunoreaction was observed in tumors FIGO stage III-IV (cervical or extrauterine involvement) with respect to tumors limited to the uterine corpus, indicating that COX-2 expression may be related to local tumor spread as confirmed by Ferradina et al, in cervical carcinoma (Ferrandina et al., 2004) and previous findings in other tumors (Wolff et al., 1998; Sheehan et al., 1999; Ryu et al., 2000; Ohno et al., 2001). The association between COX-2 overexpression and tumor spread could be supported by increased COX-2-mediated invasive ability. In particular, we reported a higher percentage of COX-2 positivity in endometrial carcinomas invading greater than >1/2 of myometrial thickness compared with tumors confined to less than <1/2 of the myometrium. We observed more intense staining in tumor margins and in regions with extended invasion. In this context, the observation that COX-2 overexpression in colorectal tumor cells is associated with an increase in invasive potential due to the ability of COX-2 to modulate adhesion molecule and protease expression (Tsujii and DuBois, 1995; Tsujii et al., 1997; Masunaga et al., 2000) is highly important.

A statistically significant positive correlation was observed between the expression of COX-2 and the FIGO stage; the rate of COX-2 expression was higher in patients with stages III-IV than patients with stage I-II (47.6% vs 20.2%, p=0.010). The possibility of positive expression in the patients with stage III-IV was 3.59 times (95% of CI=1.32 to 9.75) stronger than in patients with stage I-II. The study of Ferrandina et al. confirms the higher rate of COX-2 expression in the carcinomas

involving the cervix or pelvis in correlation with carcinomas arising in the endometrium exclusively (Ferradina et al., 2002). Thus, the expression of COX-2 is related with local spread of tumor, and this observation has been documented in other tumors (Okami et al., 1999; Ryu et al., 2000; Wulfing et al., 2003).

A higher rate of positive expression was also observed in tumors with depth of myometrial involvement >1/2 favoring tumors with ≤ 1/2 of invasion (36.2% vs 17.5%, p=0.026). The positive expression of COX -2 in tumors with depth of invasion >1/2 was 2.68 times (95% of CI=1.11 to 6.47) more likely than that in tumors with depth of invasion ≤ 1/2. Similar results are reported by Ferrandina et al. (2002).

The association between COX-2 overexpression and tumor spread could be supported by increased COX-2-mediated invasive capacity due to the ability of COX-2 to modulate adhesion molecule and protease expression (Tsujii and DuBois, 1995; Tsujii et al., 1997).

Conflicting data have been reported about the association between COX-2 expression and lymph node or distant metastases in solid tumors (Murata et al., 1999; Sheehan et al., 1999; Marrogi et al., 2000; Ryu et al., 2000). No lymph node status and COX-2 expression correlation was made due to inadequate material.

That COX -2 is a bad prognostic factor has been described in a lot of studies in the large intestine (Sheehan et al., 1999; Masunaga et al., 2000), stomach (Murata et al., 1999; Uefuji et al., 2000) and cervix (Gaffney et al., 2001; Landen et al., 2003; Ferrandina et al., 2004).

The correlation of survival of patients with the expression of COX -2, according to the Kaplan - Meier method showed a statistically important difference favoring positives that were related with bad outcomes. In the study of Ferrandina et al. patients with positive expression of COX -2 had a worse prognosis correlating with negatives not statistically important probably because of the small number of deaths (Ferrandina et al., 2002).

In our series of endometrial carcinomas we imply that COX-2 expression is an independent clinicopathologic variable strongly associated with differentiation, FIGO stage, and myometrial invasion. It is also an independent prognostic variable and this finding should draw special attention to plan treatment options to the patients' benefit.

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