Original Articles

Immunohistochemical bcl-2 expression, p53 overexpression, PR and ER status in endometrial carcinoma and survival outcomes

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Summary

Immunohistochemical expression of bcl-2, p53, PR and ER in cases with endometrial carcinomas arrayed on a tissue microarray (TMA) was tested and correlated with clinicopathologic features, overall survival (OS), cancer-related survival (CRS) and disease-free survival (DFS).

Seventy-seven patients with endometrial cancer were reviewed. Slides were evaluated by two pathologists blinded to patient clinical characteristics and survival data. Mean age of patients was 62.5 years (range 35- 80), median follow up 60 months (range 9-120).

Seventy-nine percent of patients were FIGO Stage I; 39% of the cases showed bcl-2 cytoplasmic staining and its expression was significantly correlated with low-grade tumor differentiation and age ≤ 60 years. Nuclear p53 overexpression was detected in 23.4% of the cases and was significantly correlated with advanced stages (IIB-IV), non-endometrioid histology, nodal metastasis and advanced age (> 60 years). PR and ER were positive in 63.6% and 30% of the cases, respectively. Analysis of p53 overexpression and bcl-2 expression in relationship with PR and ER status showed a direct correlation between bcl-2 expression and PR positivity ($p = 0.001$). In a multivariate analysis FIGO staging was the only clinicopathologic parameter independently correlated with DFS.

In conclusion p53 overexpression was directly associated with unfavorable clinicopathologic factors such as advanced stage, histologic subtype, advanced patient age and nodal metastasis. Bcl-2 expression was related with younger age, favorable grade and PR expression by tumor cells. Patient survival was not related to the tested biomarkers.

Key words: bcl-2; Endometrial adenocarcinoma; ER; p53; PR, TMA.

Introduction

Endometrial carcinoma (EC) is the most common malignancy of the female genital tract in developed countries, affecting about 2-3% of the female population [1]. The overall survival in patients with EC of all stages is 75% [2]. Traditional clinicopathologic features such as FIGO staging, tumor differentiation (grade), histological subtype, depth of myometrial invasion and extrauterine spread are considered as the strongest prognostic factors of patients with EC [3]. Recently various molecular markers have been investigated providing a more accurate profile of tumor behavior in patients with EC.

The bcl-2 (B-cell leukemia/lymphoma-2) gene is a well known proto-oncogene involved in the regulation of apoptosis. The gene is located on chromosome 18q21 and encodes an oncoprotein of 24 kDa which is involved in the regulation of programmed cell death and cell survival. Bcl-2 dependent mechanisms allow the accumulation of genetic mutations contributing to neoplasmatic development [4-6]. In normal endometrium bcl-2 protein is expressed in the proliferative phase of the menstrual cycle and down-regulated in the secretory phase [7-9]. The prominence of bcl-2 expression in the proliferative phase is apparently under the control of steroid sex hormones [8]. Bcl-2 expression is also present in endometrial hyperplasia and it is hypothesized that this expression is related to early-stage EC. A relationship between bcl-2 expression with grade, stage and progesterone receptors status was demonstrated by previous authors, however the role of bcl-2 protein in endometrial carcinogenesis is largely unknown [4, 10].

The tumor suppressor gene p53 is located in the short arm of chromosome 17 and its mutation is the most common alteration in human malignancies. Wild type p53 protein contributes to programmed cell death by arresting cell proliferation. Thus, an inverse interaction exists between p53 and bcl-2 proteins concerning apoptosis and programmed cell death [11, 12]. P53 overexpression has been found more commonly in advanced endometrial cancer as well as in non endometrioid histologic subtypes and high-grade tumors [13-15]. Recent research showed that p53 overexpression was not related to progesterone (PR) or estrogen receptors (ER) status [16] and, the prognostic impact of p53 protein overexpression in patients with EC is still controversial [14, 16, 17].

In the present study we investigated the immunohistochemical expression of bcl-2, p53, PR and ER on a tissue microarray (TMA) of 77 endometrial carcinomas. The immunohistochemical results were correlated with clinicopathologic features and survival data.

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Material and Methods

Seventy-seven patients with endometrial carcinoma were included in the study. This is part of a cohort of 126 patients with endometrial cancer all surgically treated at our department from 1996 to 2003. Patients with a prior or concomitant diagnosis of malignancy as well as patients not fulfilling the inclusion criteria (complete immunohistochemical data, paraffin-embedded tumor material and clinical follow-up) were not enrolled.

Patients were staged according to the 1998 FIGO staging system and the histologic grading evaluation was done according to the revised FIGO grading system for endometrial adenocarcinoma [18, 19]. The surgical and postoperative approach were reported elsewhere [20]. Briefly, patients at low-risk for lymph node metastasis (endometrioid endometrial adenocarcinoma (EEA), grade 1 with myometrial infiltration (MI) ≤ 1/2 or grade 2 with MI ≤ 1/3) were treated with total abdominal hysterectomy (TAH), bilateral salpingo-oophorectomy (BSO), peritoneal washing and palpation of the peritoneal cavity while biopsy was performed when considered necessary. High-risk patients (all non-endometrioid adenocarcinomas; grade 1 with MI > 1/2 or grade 2 with MI > 1/3, and all grade 3 tumors), were further treated either with staging lymphadenectomy or with postoperative irradiation (external-beam pelvic radiation (EBR), 45-50Gy at 1.8 Gy per fraction). Paraaortic node sampling was performed in case of palpable or suspicious pelvic nodes.

Tissue microarray (TMA) and immunohistochemistry (IHC)

Representative formalin-fixed and paraffin-embedded tumor blocks of EC cases were selected based on hematoxylin and eosin stained slides for TMA construction and IHC. Two TMA paraffin blocks were constructed with a manual arrayer (Beecher Instruments, Sun Prairie, WI, USA), including all 77 EC cases, five cores per case, 0.6 mm in diameter. Before the sampling of selected cases, five cores from a breast carcinoma case were arrayed to each recipient block for identification and alignment, as well as acting as immunohistochemical positive controls.

Serial sections of 2.5 μm thick were cut form TMA blocks and placed on superfrost plus slides (DakoCytomation, Glostrup, Denmark). After deparaffinization by overnight incubation at 60˚C and subsequent immersion in xylene and rehydration in descending ethanol baths, the slides were treated with 0.3% hydrogen peroxide in methanol for 20 min and then cleared in running water for 5 min. Antigen unmasking was performed by heating the slides in a sodium citrate solution (pH 6.0) in a microwave oven or steamer and left to cool at room temperature for 30 min. After 3 x 5 min washings with TBS the slides were incubated for one hour with protein block (Power Block™, BioGenex) and then overnight with: bcl-2 (mouse mAb, clone bcl-2/100/D5, 1:20; estrogen receptor (ER) mouse mAb, clone 6F11, 1:30; progesterone receptor (PR), mouse mAb, clone 1A6, 1:20; Novocastra, UK) and p53 (mouse mAb, clone DO-7, 1:50, DakoCytomation). Immunostaining was performed using the stepABC method (DakoCytomation) and the antigen–antibody complex was visualized using diaminobenzidine (BioGenex) as a chromogen. Slides were counterstained with Mayer's hematoxylin for 20 sec, washed in fresh water, dehydrated and mounted. All cases, with a medium of four cores, were presented in TMA sections after the immunohistochemical procedure.

Table 1. — *Clinicopathologic characteristics of the study patients*.

Characteristics	Patients (n = 77) no.	(%)
Age		
≤ 60 years	28	36.5
> 60 years	49	63.5
FIGO staging		
I	61	79
II	11	15
III	4	5
IV	1	1
FIGO Stage I *(n = 58)* (endometrioid subtype)		
IA	14	25
IB	30	50
IC	14	25
Histology subtype		
Endometrioid	74	96
Non endometrioid	3	4
Tumor diameter		
≤ 2 cm	39	50.5
> 2 cm	38	49.5
Uterine diameter		
≤ 8 cm	53	69
> 8 cm	24	31
Tumor differentiation *(grade)*		
1	32	41.5
2	37	48
3	8	10.5
Myometrial infiltration		
≤ 50%	53	69
> 50%	24	31
Adnexal involvement		
Negative	73	95
Positive	4	5
Cervical involvement		
Negative	65	84.5
Positive	12	15.5
Nodal status *(n = 28)*		
Negative	26	93
Positive	2	7
Risk group*		
Low	27	35
High	50	65

* Risk group for nodal metastasis.

Interpretation of staining

The stained slides were evaluated simultaneously by two pathologists. The observers were blinded to the patient's clinical characteristics and survival data. Bcl-2 cytoplasmic staining and p53 overexpression were scored as previously described [17, 21]. The evaluation of ER and PR staining pattern was performed according to the method described in Carcangiu's study [22].

Statistics

The SPSS version 11.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Mean values of the different variables with continuous outcomes were analyzed using chi-square and Fisher's exact test, while Student's *t*-test was used for the categorical variables. Survival and disease-free survival rates were estimated according to the Kaplan Meier method and the log-rank test was used to assess the prognostic significance [23]. Overall survival (OS) and cancer-related survival (CRS) were calculated from the date of the cancer diagnosis to the date

Table 2. — *Patient characteristics according to p53 overexpression, bcl-2, PR and ER expression.*

Factor	No. of patients	p53 overexpression		bcl-2 expression		PR positive		ER positive	
		no. (%)	p	no. (%)	p	no. (%)	p	no. (%)	p
All patients	77	18 (23.4)		30 (39)		49 (63.6)		23 (30)	
Age									
≤ 60 years	28	3 (10.7)		15 (53.6)		19 (68)		7 (25)	
> 60 years	49	15 (30.6)	0.045	15 (30.6)	0.045	30 (61)	0.60	16 (32.7)	0.60
FIGO staging									
I	61	13 (21.3)		22 (36)					
II	11	2 (18.2)		7 (63.6)					
III	4	2 (50)		1 (25)					
IV	1	1 (100)	0.16	0	0.26	0	0.25	0	0.60
FIGO staging									
Early$^{\gamma}$	72	15 (21)		29 (40.3)		45 (62.5)		22 (29)	
Advance^{f¶}	5	3 (60)	0.045	1 (20)	0.60	4 (80)	0.65	2 (40)	0.60
FIGO stage I *(n = 58)* (endometrioid subtype)									
IA	14	4 (26,7)		9 (60)		13 (86.7)		7 (46.7)	
IB	30	6 (20)		10 (33.3)		18 (60)		8 (26.7)	
IC	14	3 (19)	0.85	3 (19)	0.05	7 (44)	0.04	4 (25)	0.30
Histology subtype									
Endometrioid	74	15 (20.3)		28 (38)		48 (65)		22 (29.7)	
Non endometrioid	3	3 (100)	0.01	2 (66.7)	0.55	1 (33)	0.20	1 (33.3)	0.90
Tumor diameter									
≤ 2 cm	39	8 (20.5)		13 (33.3)		24 (61.5)		13 (33.3)	
> 2 cm	38	10 (26.3)	0.60	17 (44.7)	0.35	25 (66)	0.70	10 (23.3)	0.60
Uterine diameter									
≤ 8 cm	53	12 (22.6)		22 (41.5)		34 (64)		19 (36)	
> 8 cm	24	6 (25)	0.80	8 (33.3)	0.60	15 (62.5)	0.90	4 (16.7)	0.08
Tumor differentiation *(Grade)*									
1	32	7 (22)		15 (46.9)		23 (72)		12 (37.5)	
2	37	7 (19)		15 (40.5)		22 (59.5)		8 (21.6)	
3	8	4 (50)	0.16	0	0.05	4 (50)	0.40	3 (37.5)	0.30
Tumor differentiation *(Grade)*									
Low $^{\partial}$	69	14 (20.3)		30 (43.5)		45 (65)		20 (29)	
High$^{\forall}$	8	4 (50)	0.06	0	0.017	4 (50)	0.45	3 (37.5)	0.70
Adnexal involvement									
Negative	73	16 (22)		29 (39.7)		46 (63)		21 (29)	
Positive	4	2 (50)	0.20	1 (25)	0.50	3 (75)	0.60	2 (50)	0.60
Myometrial infiltration									
≤ 50%	53	11 (21)		24 (45.3)		36 (68)		15 (28.3)	
> 50%	24	7 (29)	0.50	6 (25)	0.09	13 (54)	0.30	8 (33.3)	0.80
Nodal status *(n = 28)*									
Negative	26	7 (27)		9 (34.6)		15 (57.7)		8 (31)	
Positive	2	2 (100)	0.035	0	0.30	2 (100)	0.50	0	0.20
Risk group*									
Low	27	7 (26)		14 (52)		21 (78)		9 (33.3)	
High	50	11 (22)	0.80	16 (32)	0.08	28 (56)	0.05	14 (28)	0.80

* Risk group for nodal metastasis; γ = Stage I-IIA; *f* = Stage IIB-IV; ∂ = grade 1 and 2 combined; $\forall$ = grade 3; PR = progesterone receptor, ER = estrogen receptor.

of death from any cause or death related to endometrial carcinoma, respectively, while disease-free survival (DFS) was estimated from the date of the diagnosis to the first appearance of the recurrence or death related to endometrial carcinoma (whichever occurred first). Any p value < 0.05 was considered statistically significant (all p values were two-sided). p53 nuclear overexpression, bcl-2 cytoplasmatic expression, PR and ER status were analyzed according to the different clinicopathologic characteristics of the patients. OS, CRS and DFS were estimated using univariate analysis of the conventional clinicopathologic and immunohistochemical parameters (p53, bcl-2, PR, ER) of the study. Cox-regression analysis of DFS according to the different clinicopathologic and immunohistochemical features of the study was also performed.

Results

The mean age of the patients was 62.5 years with a range from 35 to 80 years. Distribution of patient characteristics according to the different clinicopathologic features is listed in Table 1. Seventy-nine percent of the population was classified as Stage I. Histologic subtype of the tumors was mainly endometrioid (96%), while only three cases of serous papillary carcinoma were identified (4%).

Immunohistochemical analysis

Bcl-2 expression. Thirty-nine percent of the cases showed bcl-2 cytoplasmic staining (Figure 1A). Bcl-2 expression was significantly correlated with low-grade

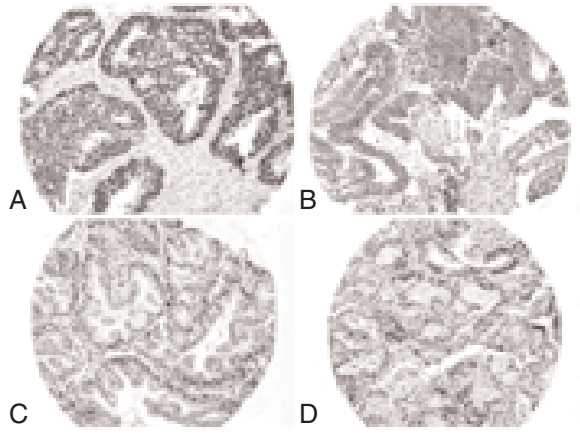

Figure 1. — Representative TMA cores of endometrial carcinoma cases stained positive for p53 (A), bcl-2 (B), estrogen (C) and progesterone receptors (D) (IHC x 200).

tumor differentiation (grade 1 and 2 combined) (p = 0.017) and younger age (≤ 60 years) (p = 0.045) (Table 2). Bcl-2 differed marginally (p = 0.05) when tumor differentiation (grade) was analyzed separately (grade 1 vs 2 vs 3). The same observation was made in a subgroup analysis of Stage I patients with only endometrioid histology subtype (Stage IA vs IB vs IC) (p = 0.05). Bcl-2 expression was mainly detected in cases with myometrial invasion less than half of the myometrial thickness and in low-risk patients but these differences were not statistically significant (p = 0.08 and p = 0.09, respectively).

P53 overexpression. Nuclear p53 overexpression was detected in 18 of 77 (23.4%) cases (Figure 1B). This overexpression was significantly correlated with advanced stage disease (IIB-IV), non endometrioid histology subtype, nodal metastasis and advanced patient age (> 60 years) (Table 2). Although there was a trend of p53 overexpression in high-grade tumor differentiation (grade 1 and 2 combined vs grade 3), it did not reach statistical significance (p = 0.06).

PR, ER status. 63.6% of patients were PR positive and 30% were ER positive (Figures 1C and 1D). PR expression differed marginally (p = 0.05) between the low- and high-risk groups of patients for nodal metastasis (78% vs 56%, respectively). A subgroup analysis of patients with Stage I of only endometrioid histology subtype, showed a significant relationship between PR expression and stage (Table 2). Furthermore, PR positivity was higher in endometrioid histology subtype compared with ER positivity (65% vs 33%, respectively) but did not reach a statistical significance (p = 0.20). ER positivity was higher in cases with uterine diameter ≤ 8 cm (p = 0.08). The same observation was made in cases with metastatic (nodal) disease.

Analysis of p53 overexpression and bcl-2 expression in relation to PR and ER status showed a direct correlation between bcl-2 expression and PR positivity (p = 0.001) (Table 3).

Table 3. — *Bcl-2 and p53 distribution according to ER and PR status*.

	No. of patients (n = 77)	bcl-2 expression (n = 30)	p value	p53 overexpression (n = 18)	p value
PR					
Negative	28	4 (13.3)		6 (33.3)	
Positive	49	26 (86.7)	0.001	12 (66.7)	0.80
ER					
Negative	54	19 (63.3)		11 (61)	
Positive	23	11 (36.7)	0.30	7 (40)	0.35

PR = progesterone receptor, ER = estrogen receptor.

Table 4. — *Patient prognosis according to the different clinico-pathologic and immunohistochemical characteristics*.

Characteristics	OS (%)	P	CRS (%)	P	DFS (%)	P
Age						
≥ 60 years	93		93		93	
> 60 years	75	0.03	88	0.25	85	0.25
FIGO staging						
I	87		93		92	
II	91		91		9	
III	25		50		50	
IV	0	< 0.0001	0	< 0.0001	0	< 0.0001
FIGO staging						
Early γ	87.5		93		92	
Advanced *f*	25	< 0.001	40	< 0.0001	40	< 0.0001
FIGO stage I *(n = 58)*						
IA	86.5		93		93	
IB	90		93		90	
IC	81	0.70	94	0.90	94	0.90
Histology subtype						
Endometrioid	84		90		89	
Non endometrioid	66.5	0.30	66	0.08	66	0.09
Tumor diameter						
≤ 2 cm	82		85		82	
> 2 cm	84	0.70	94	0.15	95	0.08
Uterine diameter						
≤ 8 cm	83		87		87	
> 8 cm	83.5	0.60	92	0.40	92	0.40
Adnexal involvement						
Negative	86		93		92	
Positive	25	0.0001	25	< 0.0001	25	< 0.0001
Tumor differentiation *(Grade)*						
1	85		94		94	
2	84		89		86	
3	75	0.50	75	0.10	75	0.20
Tumor differentiation *(Grade)*						
Low ∂	84		91		90	
High ∀	75	0.25	75	0.04	75	0.10
Myometrial infiltration						
≤ 50%	89		94		92	
> 50%	75	0.03	79	0.01	79	0.02
Nodal status *(n = 28)*						
Negative	92		96		92	
Positive	0	< 0.0001	50	0.0004	50	0.0004
Risk group*						
Low	85		93		93	
High	82	0.90	88	0.60	86	0.35
bcl-2 expression						
Negative	81		89		87	
Positive	87	0.20	90	0.60	90	0.60
p53 overexpression						
Negative	85		90		88	
Positive	75	0.35	88	0.70	89	0.70
PR						
Negative	86		89		86	
Positive	82	0.90	90	0.70	89	0.50
ER						
Negative	81		87		87	
Positive	87	0.70	95	0.30	91	0.70

* Risk group for nodal metastasis; γ = stage I-IIA; *f* = stage IIB-IV; ∂ = grade 1 and 2 combined; ∀ = grade 3; PR, ER = progesterone, estrogen receptor; OS = overall survival; CRS = cancer-related survival; DFS = disease-free survival.

Survival analysis

The median follow-up was 60 months (range 9-120 months). Univariate analysis of OS, CRS and DFS are presented in Table 4. OS of patients was significantly higher in the patients age ≤ 60 years, and there was a direct correlation between the OS and FIGO staging. There was a positive relationship between poor OS and ovarian metastasis, depth of myometrial invasion (> 50%) and lymph node metastasis. The same was also true concerning CRS and DFS. Furthermore, unfavorable tumor differentiation (grade 3) was significantly correlated with CRS. There were no differences between the studied immunhistochemical parameters and survival distribution. FIGO staging was the only clinicopathologic parameter independently correlated with DFS, when FIGO staging, grade, tumor diameter, myometrial infiltration, patient age and immunohistochemical features were included in a multivariate Cox regression analysis (Table 5).

Table 5. — *Multivariate Cox regression analysis of DFS for the different clinico-pathologic and immunohistochemical features of patients with uterine adenocarcinoma*.

Feature	RR	95% CI	p value
FIGO staging*	48	2.7-844	.008
Tumor diameter (≤ 2 cm)	0.36	0.06-2.0	NS
Tumor differentiation$^{\gamma}$	0.86	0.06-9.7	NS
Myometrial infiltration (≤ 50%)	1.45	0.25-8.3	NS
Age (≤ 60 yrs)	3.10	0.40-24	NS
bcl-2 expression^{f}	1.47	0.22-9.7	NS
p53 overexpression^{f}	0.56	0.05-6.3	NS
PR^{f}	0.54	0.09-3.1	NS
ER^{f}	0.18	0.01-2.9	NS

RR = risk ratio, CI = confidence interval, PR = progesterone receptor, ER = estrogen receptor; * Stage I-IIA vs IIB-IV; *f* Negative vs positive; γ Grade 1and 2 vs 3.

Discussion

In the current study bcl-2 cytoplasmatic expression, p53 nuclear overexpression and PR, ER status were estimated using immunohistochemistry on paraffin-embedded endometrial adenocarcinoma tissue specimens. P53 and bcl-2 proteins have been investigated in various human cancers, including those of the female genital tract. They have been studied immunohistochemically on frozen tissue or on paraffin-embedded blocks [14, 16, 17, 24-27]. Previous studies showed a positive correlation of p53 overexpression with advanced FIGO staging, non endometrioid histology subtype and lymph node metastasis [17]. These results are similar to the present study. Ohkouchi *et al*. demonstrated a significant correlation of p53 overexpression with unfavorable tumor differentiation and myometrial depth (> 50%). However, in our study the difference of p53 overexpression between low and high tumor grade was of borderline significance, in favor of high-grade (p = 0.06), while no significant difference was noted in relation to myometrial invasion. Kounelis et al., using a cutoff value of 10% for nuclear staining of p53 protein, showed a direct association of p53 with non endometrioid histology, high-grade tumor (grade 3) and advanced stages (Stage IIA-IV) [28]. Similar results have been shown by others [14, 25, 29]. A recent study on p53 protein overexpression also demonstrated a positive relationship between protein overexpression and recurrent disease [24]. From the previous and the present study it seems that p53 overexpression is correlated with advanced stage, unfavorable grade and non endometrioid histology subtype, suggesting the essential role of p53 overexpression in carcinogenesis of patients with endometrial adenocarcinoma.

The correlation between p53 protein overexpression and gene mutations is not absolute in human malignancy with a reported concordance of 75% for endometrial cancer [30]. Although p53 overexpression has been widely used as an indication for p53 mutation, skepticism is needed for interpretation of the results. P53 immunoreactivity does not necessarily predict gene mutation, nor does negative p53 protein staining exclude the possibility of p53 exon mutations [13, 31]. A recent study on patients with recurrent endometrial cancer using PCR for the mutation analysis of p53 exons 5-8 and 11 did not demonstrate a correlation between p53 protein overexpression and gene mutation [24]. However, in another study protein overexpression and missense mutation of the p53 gene was correlated to patients with early-stage disease [32]. The concordance between gene mutation and p53 overexpression was not the object of the present trial.

Data concerning the relationship between bcl-2 expression and the clinicopathologic characteristics of patients with endometrial carcinoma is less clear. One study showed a positive correlation between bcl-2 expression and negative lymphovascular space involvement, negative lymph nodes and superficial myometrial invasion (≤ 1/2) [17], while another showed a significant correlation between bcl-2 cytoplasmatic expression and myometrial depth [33]. We showed higher bcl-2 expression of patients with superficial myometrial infiltration (≤ 50%) but with no statistically significant value (p = 0.09). Furthermore previous reports have shown a significant association of bcl-2 with all favorable clinicopathologic features of patients with endometrial cancer including early stage, favorable tumor grade, superficial myometrial infiltration, negative lymphovascular space involvement and endometrioid histology subtype [34, 35], while Taskin *et al*., described a direct relationship of bcl-2 only with early stage and favorable grade tumors [4].

ER and PR positivity separately or in addition to other molecular markers has been assessed by previous authors. Previous reports using immunohistochemical techniques demonstrated a direct correlation between ER and PR positive levels and early stage, favorable tumor differentiation (grade 1 and 2 combined) and endometrioid subtype [16, 28, 36]. Furthermore, Yamauchi *et al*. demonstrated that bcl-2 expression was ER-related, while p53 overexpression was not [5]. This was also true in our study concerning p53 overexpression, while bcl-2 expression was PR-related. Similar results have been shown by others [4, 16]. It seems that a common mechanism exists between bcl-2 protein and sex steroid status that stimu-

lates carcinogenesis in EC, while p53 overexpression is probably unrelated to the former mechanism.

Survival analysis of the present study (OS, CRS and DFS) showed favorable prognosis of EC patients with early stage, no adnexal metastasis, superficial myometrial invasion and negative nodal status. Previous reports are in accordance with our findings [17, 21, 27, 37]. We detected better CRS and DFS in patients with endometrioid histology subtype but these differences were not statistically significant. Immunohistochemical marker expression in our study was not correlated significantly (univariate analysis) with patient prognosis, in accordance with a recent report [16]. In a multivariate analysis FIGO staging was the only parameter independently correlated with DFS.

Data related to the prognostic impact of p53, bcl-2 and hormonal receptor status reported in the literature are conflicting. Lukes and collaborators showed in a multivariate model that p53 overexpression and sex steroid status were not independently correlated with FIGO stage, grade, myometrial invasion and DNA ploidy [27]. A univariate analysis by Oreskovic *et al*. showed that p53 expression (< 15% positive cells), ER and PR status of patients with EC, correlated significantly with patient survival, while in a multivariate analysis only p53 expression remained independently correlated with patient survival [21]. Erdem *et al*., in a multivariate model, demonstrated independent correlation of FIGO stage and bcl-2 expression with prognosis [37]. Others, using a similar model, showed p53 overexpression and lymph node metastasis were independently correlated with prognosis [14, 17, 38, 39]. However, another report failed to show an independent correlation between recurrence and p53 overexpression [40]. Geisler *et al*., including bcl-2 cytoplasmatic persistence in a multivariate analysis, showed an independent association with patient prognosis [34], while Sakuragi *et al*. failed to demonstrate these results [33].

Deficiencies of the current study were the retrospective nature and the small number of patients, as well as the short median follow-up of 60 months. However, as previously shown, events on recurrence are mostly noted in the first 36 months after the diagnosis of malignancy in two-thirds of patients with endometrial cancer [41, 42].

Conclusion

Our findings show that FIGO staging was the only clinicopathologic parameter independently associated with patient prognosis, while the immunohistochemical markers used in the present study (p53, bcl-2, ER and PR) were not. We found that p53 overexpression was directly associated with unfavorable clinicopathologic factors of patients with endometrial adenocarcinoma such as advanced stage, non endometrioid histology subtypes, advanced patient age and lymph node positive status, while bcl-2 expression was related with younger age and favorable tumor differentiation. Furthermore, a significant relationship was noted between PR tumor positivity and bcl-2 expression.

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