The relationship between mutant p53 gene, DNA contents and conventional clinicopathological prognostic variables in cases with endometrial carcinoma

M.A. Osmanağaoğlu[1], *M.D., Assist. Prof.*; **S. Kadıoğlu**[1], *M.D., Specialist*; **S. Osmanağaoğlu**[2], *M.D., Resident*; **H. Bozkaya**[1], *M.D., Prof.*; **A. Reis**[3], *M.D., Prof.*; **Y. Tekelioğlu**[4], *Associate Prof.*

[1]*Department of Obstetrics and Gynecology,* [2]*Department of Anesthesiology and Reanimation,* [3]*Department of Pathology,* [4]*Department of Histology and Embryology, Trabzon (Turkey)*

Summary

Purpose of investigation: To determine whether p53 expression and DNA ploidy are related to traditional prognostic indicators in patients with endometrial cancer.

Methods: Tumor material (n = 136) was analyzed regarding flow cytometric DNA ploidy and immunohistochemical p53 expression. Pearson's correlation, Fisher's exact test, Cox's regression analysis and the Kaplan-Meier survival test were used, as appropriate.

Results: P53 overexpression and DNA ploidy were higher in patients with nonendometrioid histology, FIGO advanced stage, poor grade, positive peritoneal cytology, lymphovascular space invasion (LVSI) and lymph node involvement (LNI). Histologic subtype, stage, grade, LVSI, LNI, tumor recurrence and overall survival rate correlated with p53 and DNA ploidy. No association of depth of myometrial invasion and age with p53 and DNA ploidy was observed. P53 was related to DNA ploidy. Of the factors analyzed, histologic subtype and myometrial invasion were found to be most important independent determinants of recurrence. Utilizing survival as the endpoint for multivariate analysis, when considering p53 and DNA ploidy together, histologic subtype, stage, peritoneal cytology, LNI and DNA ploidy were independent prognostic indicators.

Conclusion: p53 expression and DNA ploidy were related to histologic subtype, FIGO stage, grade, LVSI, LNI, peritoneal cytology, tumor recurrence and overall 5-year survival. As compared to p53, DNA ploidy was the stronger independent predictor factor for survival. Neither p53 nor DNA ploidy were significant independent factors for tumor recurrence when submitted to multivariate analysis in this study. However, since p53 or DNA ploidy were found to be significant factors in univariate analysis and were correlated with tumor recurrence, they could be useful factors in making prognoses.

Key words: Endometrial cancer; p53; DNA ploidy; Prognostic factors.

Introduction

Endometrial carcinoma is the most common gynecologic malignancy in developed countries. The number of new cases of endometrial cancer and cancer deaths in the USA estimated for 2003 is 40,100 and 6,800, respectively [1].

A number of clinical and pathologic risk factors including the Federation of Gynecology and Obstetrics (FIGO) stage [2], tumor grade [2], histologic type [3], depth of myometrial invasion [2, 3], lymphovascular invasion [3], cervical involvement [3] and the presence and extent of extrauterine disease [2], and patient age have been evaluated as prognostic variables serving as selection criteria for adjuvant therapy.

The p53 gene which is located on chromosome 17p13.1 [4] is one of the most common tumor suppressor genes and is involved in multiple central cellular processes, including transcription, DNA repair, genomic stability, senescence, cell cycle control, and apoptosis. Wild-type p53 in normal tissue has a short half-life and is not detectable by immunohistochemical methods. Mutant p53 protein resists degradation and accumulates in the nucleus, where it can be demonstrated by immunohistochemistry [5]; p53 expression has been correlated with recurrence and survival in genital cancers including endometrial carcinoma [4-17].

DNA flow cytometric analyses, measuring DNA content and proliferative activity, has proven to have prognostic significance in regard to overall patient survival and risk of recurrent disease in endometrial cancer [18]. With regard to endometrial carcinoma, most studies suggest that ploidy status has an independent prognostic value on patient survival and risk of recurrence [18].

The purpose of this study was to determine whether p53 expression and DNA ploidy are related to clinical and pathological prognostic factors (FIGO surgical stages (1988), histological tumor grade, myometrial invasion, lymph node metastasis and peritoneal cytology as well as recurrence of disease, survival and patient age in patients with endometrial carcinoma.

Materials and Methods

Between January 1992 and December 1999, 136 patients with endometrial carcinoma, who were managed at the Department of Obstetrics and Gynecology in Karadeniz Technical

Revised manuscript accepted for publication July 28, 2004

University Hospital, were recruited into this retrospective study. Clinical data was obtained from the patient medical records and the tumors were staged retrospectively according to the International Federation of Gynecology and Obstetrics (FIGO) staging system for endometrial carcinoma, but without subdividing stage into IA (4 patients), IB (52 patients) or IC (36 patients). All patients underwent surgery as the primary treatment. The standard procedure was total extrafascial hysterectomy and bilateral salpingo-oophorectomy. Pelvic and/or paraaortic lymphadenectomy were performed at the surgeon's discretion, but not systematically. Criteria for lymph node dissection included poor grade of differentiation, macroscopic evidence of deep myometrial invasion, or suspicion of extrauterine tumor spread. Omentectomy was performed when clear peritoneal or adnexal involvement was found. Overall, 124 patients underwent lymphadenectomy (91%) and 32 patients underwent omentectomy (24%). Lymphadenectomies were not performed in the eight Stage IV patients and in the four women who had FIGO Stage Ia disease. If dilatation and curettage prior to surgery revealed cervical involvement, the total abdominal hysterectomy was replaced with a modified radical hysterectomy. Lymphatic vascular space invasion was defined as the presence of tumor in endothelial-lined spaces in the myometrium. Poor grade of differentiation, deep myometrial invasion (> 50%), and cervical infiltration were the main criteria for adjuvant treatment. Thirty-seven patients (54%) received postoperative irradiation (56 gray) to the whole pelvis. Nine patients (7%) with more advanced disease (FIGO Stages III-IV) were treated by radiotherapy with concomitant chemotherapy.

The surgical specimens were fixed in a l0% buffered formalin (pH 7.4) and embedded in paraffin blocks according to routine laboratory procedures. A single 5 μm section from paraffin-embedded specimens was routinely deparaffined and incubated in methanol with 0.3% H_2O_2 to block endogenous peroxidase activity. The specimens were embedded in buffer-citrate 0 0 1 H and incubated in a microwave at 750 W for 7 min. After being cooled and washed in PBS (pH 7.6), the first antibody was used. We used two antibodies: the monoclonal antibody DO7 (Novocastra Laboratory, Newcastle, UK), which recognizes an epitope in human p53 at optimal dilution in Tris-albumin (1/50); and the monoclonal antibody anti-c-erbB-2 (Medac) that recognizes the intracellular domain of p185 protein at optimal dilution in Tris-albumin (1/40). Both antibodies were incubated overnight in a humidified chamber at 2-8°C. After treatment with biotinated anti-mouse inmunoglobulin for 30 min at room temperature, the sections were incubated with peroxidase-conjugated streptavidin for 30 min at room temperature. A final wash was followed by samples developed with 3 mg of 3,3'-diaminobencidine and 15μl of hydrogen peroxide in Tris–HCl buffered at pH 7.6. The slides were then rinsed for 10 min in tap water, stained with Mayers' hematoxylin and coverslips were mounted. Positive and negative control antibodies were used to ensure tissue viability and exclude the possibility of nonspecific staining with laryngeal carcinoma sections. Staining reaction was confined to the nucleus. Nuclear staining of more than 10% of the tumor cells was interpreted as positive.

Nuclear suspensions were prepared from paraffin-embedded tissue blocks by the technique of Hedley *et al.* [19] after histologic documentation of tumor adequacy. Five 50 μm sections were cut from suitable tumor blocs, deparaffinized in xylene and rehydrated in phosphate-buffered saline through graded alcohols. Nuclear DNA was stained with propidium iodide by standard methods. Nuclear content was measured with a Coulter Epics Elite ESP flow cytometer using multicycle computer software with an argon laser set at a wavelength of 488 nm for fluorescence excitation. Histograms of 10,000 nuclei were recorded for each specimen at a maximal scanning flow rate of 1,000 nuclei per second. Cell cycle evaluation of the DNA histograms derived from flow cytometry was performed with the data acquisition triggered on red fluorescence, and the cell cycle distribution was determined by analyzing ungated data from 10,000 nuclei in a rectangular S-phase computer model. Tumors with only one G0/G1 peak were designated as "diploid" (2n), and those with histograms suggesting more than one G_0/G_1 population were categorized as "aneuploid". Tumor samples with ≥ 9% of nuclei associated with the 4*n* peak were considered tetraploid. Specimens demonstrating three or more G_0/G_1 peaks were classified as "multiploid" but, for statistical purposes, were analyzed as aneuploid tumors. The DNA index was calculated as the ratio of the mean channel of the aneuploid G_0/G_1 population compared with the diploid G_0/G_1 channel. Therefore, diploid tumors possessed a DNA index of 1.0 and aneuploid > 1.0, with the most prominent population in multiploid tumors used to assign a DNA index. "Proliferative index" (PI) was defined as the sum of the percentage of cells in the S phase plus the percentage of cells in the G_2/metaphase. Cases were not considered suitable for the analysis if the endometrial sample did not have enough cells, if no tumor was detected in the section, if the histogram was not interpretable or if the coefficient of variation was > 10.

Fisher's exact test, Cox's regression analysis and the Kaplan-Meier survival test were used to analyze the association between p53 accumulation, DNA ploidy and clinicopathological prognostic features of endometrial carcinomas. The relationship between p53 accumulation, DNA ploidy and clinicopathological variables was assessed by Pearson's correlation test. A p value less than 0.05 was considered significant.

Results

The median and mean age of the patients were 60 and 61 years, respectively (range 38-80). The stage distribution of the 136 cases, according to the FIGO staging system were Stage I (92 cases) 68%, Stage II (12 cases) 9%, Stage III (24 cases) 18% and Stage IV (8 cases) 6%. Histologically, 104 (76%) patients had endometrioid type carcinomas, 20 (15%) serous papillary, four (3%) squamous and eight (6%) clear cell. The overall recurrence rate was 39% (53/136). Of the 53 patients with recurrence, 20 had local and 25 had distant metastases. In the remaining four patients, both local and pelvic metastases were determined. Four have been diagnosed with progression of persistent abdominal disease following initial surgery. A significantly higher proportion of the patients with nonendometrioid histology relapsed (84%, $p < 0.01$, OR: 0.049 (CI: 0.01-0.14). The mean and median time of survival was 61 and 63 (3-112) months, respectively. The overall survival rate was 68% at five years.

p53 overexpression was present in 40 of 136 cases (29%) and was observed only in tumor cells as nuclear staining. Patients with positive p53 overexpression had a higher prevalance of nonendometrioid histology, advanced stage, higher grade (poor) histology, lymphovascular space invasion, lymph nodal involvement and peritoneal cytology (Table 1). Moreover, the mean p53 expression of the patients tumors with recurrence was

Table 1. — *Clinical and pathologic findings according to p53 overexpression and DNA ploidy.*

	Overexpression p53		DNA ploidy		Fisher's exact test	
	Negative (n = 96)	Positive (n = 40)	Diploid (n = 100)	Aneuploid (n = 36)	p^a, OR (95% CI)	p^b, OR (95% CI)
Age at diagnosis					> 0.05, 1.333 (0.62-2.85)	> 0.05, 0.8889 (0.39-1.98)
< 60 years (n = 48)	32 (67%)	16 (33%)	36 (75%)	12 (25%)		
≥ 60 years (n = 88)	64 (73%)	24 (27%)	64 (73%)	24 (27%)		
FIGO stage					< 0.01, 0.060 (0.02-0.15)	< 0.01, 0.1091 (0.04-0.26)
I and II (n = 104)	88 (85%)	16 (15%)	88 (85%)	16 (15%)		
III and IV (n = 32)	8 (25%)	24 (75%)	12 (37%)	20 (63%)		
Histologic subtype					< 0.01, 0.018 (0.00-0.06)	< 0.01, 0.0434 (0.01-0.11)
Endometrioid (n = 104)	92 (88%)	12 (12%)	92 (88%)	12 (12%)		
Non endometrioid (n = 32)	4 (12%)	28 (88%)	8 (25%)	24 (75%)		
Myometrial invasion					> 0.05, 0.4762 (0.16-1.37)	> 0.05, 0.6286 (0.29-1.35)
≤ 50% (n = 72)	56 (78%)	16 (22%)	56 (78%)	16 (22%)		
> 50% (n = 64)	40 (62%)	24 (38%)	44 (69%)	20 (31%)		
Grade					< 0.01, 27.000 (8.70-83.73)[e/d]	< 0.01, 20.571 (6.66-63.53)[e/d]
Well (n = 76)[c]	72 (95%)	4 (5%)	72 (95%)	4 (5%)	< 0.05, 0.3333 (0.13-0.82)[c/e]	< 0.01, 0.2727 (0.10-0.68)[c/e]
Moderate (n = 24)[d]	12 (50%)	12 (50%)	12 (50%)	12 (50%)	< 0.01, 0.0952 (0.03-0.22)[d/e]	< 0.01, 0.1524 (0.06-0.35)[d/e]
Poor (n = 36)[e]	12 (33%)	24 (67%)	16 (44%)	20 (56%)		
Peritoneal cytology					< 0.01, 25.667 (9.53-69.12)	< 0.01, 14.667 (5.85-36.76)
Positive (n = 18)	8 (22%)	28 (78%)	12 (33%)	24 (67%)		
Negative (n = 50)	88 (88%)	12 (12%)	88 (88%)	12 (12%)		
Lymphovascular space invasion						
No (n = 116)	88 (76%)	28 (24%)	92 (79%)	24 (21%)	< 0.01, 0.2121 (0.01-0.57)	< 0.05, 0.1739 (0.06-0.47)
Yes (n = 20)	8 (40%)	12 (60%)	8 (40%)	12 (60%)		
Lymph node involvement					< 0.05, 0.3714 (0.15-0.86)	< 0.01, 0.1364 (0.05-0.33)
No (n = 106)	80 (75%)	26 (25%)	88 (83%)	18 (17%)		
Yes (n = 30)	16 (53%)	14 (47%)	12 (40%)	18 (60%)		

p^a: Comparison of traditional prognostic indicators to p53 overexpression; OR: odds ratio; CI: confidence interval; p^b: Comparison of traditional prognostic indicators to DNA ploidy

[c/d]: grade 1 versus grade 2; [c/e]: grade 1 versus grade 3; [d/e]: grade 2 versus grade 3.

65%, while the p53 overexpression of the patients tumors without recurrence was 9% ($p < 0.01$, OR: 0.0538, 95% CI: 0.02-0.13). Patients whose tumors overexpressed p53 had a significantly shorter tumor recurrence time and overall 5-year survival than those whose tumors did not (5 ± 11.50 vs 33 ± 18.56, $p < 0.01$ and 69 ± 16.48 vs 41 ± 20.54, $p < 0.01$, respectively). There was a significant association between p53 expression and histologic subtype, FIGO stage, grade, lymphovascular space invasion, lymph nodal involvement, peritoneal cytology and tumor recurrence. A negative correlation between p53 overexpression and overall 5-year survival was found but no correlation between p53 expression and age or myometrial invasion was found (Table 2).

Of the total analyzed material, DNA was diploid in 100 (74%) patients and aneuploid in 36 (26%). Patients with aneuploid tumors had a higher prevalance of nonendometrioid histology, advanced stage, higher grade (poor) histology, lymphovascular space invasion, lymph nodal involvement and peritoneal cytology. The DNA flow of the tumors with recurrence was 33% while of those without recurrence it was 3% ($p < 0.01$, OR: 0.0736, 95% CI: 0.02-0.26). Patients with aneuploid tumors had a significantly shorter tumor recurrence time and overall 5-year survival than those with diploid tumors (4 ± 10.50 vs 38 ± 14.04, $p < 0.01$ and 70 ± 15.17 vs 37 ± 19.82, $p < 0.01$, respectively). There was a significant association between aneuploidy and histologic subtype, FIGO stage, grade, lymphovascular space invasion, lymph nodal involvement, peritoneal cytology and tumor recurrence. A negative correlation between aneuploidy and overall 5-year survival was found but no correlation between p53 expression and age or myometrial invasion was found (Table 2). Moreover, there was a positive correlation between p53 and aneuploidy ($r = 0.783$, $p < 0.01$).

A multivariate analysis based on Cox's regression model showeld that when p53 without DNA ploidy was used separately the independent variables, histologic subtype, stage, grade, myometrial invasion, peritoneal cytology, lymphovascular space invasion, lymph node

Table 2. — *Relationship between p53 and DNA ploidy and traditional prognostic factors in endometrial carcinoma.*

	Overexpression p53		DNA ploidy	
	r	p	r	p
Age at diagnosis	– 0.113	> 0.05	– 0.168	> 0.05
FIGO stage	0.555	< 0.01	0.453	< 0.01
Histologic subtype	0.656	< 0.01	0.543	< 0.01
Myometrial invasion	0.167	> 0.05	0.102	> 0.05
Grade	0.596	< 0.01	0.541	< 0.01
Peritoneal cytology	0.637	< 0.01	0.547	< 0.01
Lymphovascular space invasion	0.279	< 0.01	0.316	< 0.01
Lymph node involvement	0.201	< 0.05	0.404	< 0.01
Relapse	0.681	< 0.01	0.795	< 0.01
Overall 5-year survival	– 0.588	< 0.01	– 0.659	< 0.01

Table 3. — *Cox regression analysis with regard to the overall 5-year survival.*

	Statistical significance			RH (95% CI)		
	P^a	P^b	P^c	RH^a	RH^b	RH^c
Histologic subtype (endometrioid/ nonendometrioid)	p = 0.0006*	p = 0.0000*	p = 0.0005*	0.21 (0.08 - 0.51)	0.12 (0.05 - 0.28)	0.16 (0.05 - 0.45)
Stage (I-II/III-IV)	p = 0.0330*	p = 0.0099*	p = 0.0107*	0.29 (0.09 - 0.90)	0.22 (0.07 - 0.69)	0.22 (0.07 - 0.70)
Grade (G1-G2/G3)	p = 0.1775	p = 0.7843	p = 0.7901	0.74 (0.49 - 1.14)	0.93 (0.59 - 1.47)	0.93 (0.59 - 1.49)
Myometrial invasion (yes/no)	p = 0.9301	p = 0.2288	–	0.98 (0.66 - 1.44)	0.78 (0.52 - 1.16)	–
Peritoneal cytology (positive/negative)	p = 0.0833	p = 0.0144*	p = 0.0267*	3.04 (0.86 - 10.75)	5.59 (1.40 - 22.21)	4.62 (1.19 - 17.88)
Lymphovascular space invasion (yes/no)	p = 0.9093	p = 0.9452	p = 0.6878	1.04 (0.49 - 2.18)	0.97 (0.45 - 2.07)	1.15 (0.57 - 2.34)
Lymph node involvement (yes/no)	p = 0.0000*	p = 0.0003*	p = 0.0001*	0.29 (0.16 - 0.51)	0.35 (0.20 - 0.61)	0.31 (0.17 - 0.55)
p53 (overexpression/no overexpression)	p = 0.0006*	–	p = 0.3060	0.19 (0.07 - 0.49)	–	0.52 (0.15 - 1.78)
DNA ploidy (diploid/aneuploid)	–	p = 0.0000*	p = 0.0024*	–	0.10 (0.04 - 0.26)	0.18 (0.06 - 0.54)

Results of the regression analysis expressed as statistical significance and relative hazard (RH) with 95% confidence interval (CI); P^a: P53 without DNA ploidy; P^b: DNA ploidy without p53; P^c: By combined p53 with DNA ploidy; *: significant.

involvement and p53 overexpression demonstrated a significant independent prognostic influence on the overall 5-year survival for histologic subtype [relative hazard (RH) 0.23], FIGO stage (RH 0.29), lymph node involvement (RH 0.29) and p53 (RR 0.19). When DNA ploidy without p53 was used for the same independent variables, a significant independent prognostic influence on overall 5-year survival was found for histologic subtype (RH 0.12), FIGO stage (RH 0.22), peritoneal cytology (RH 5.59), lymph node involvement (RH 0.35) and DNA ploidy (RH 0.10). Finally, when p53 was combined with DNA by excluding myometrial invasion from the analysis with the same independent variables, a significant independent prognostic influence on overall 5-year survival was found for histologic subtype (RH 0.16), FIGO stage (RH 0.22), peritoneal cytology (RH 4.62), lymph node involvement (RH 0.31) and DNA ploidy (RH 0.18) (Table 3, Figures 1 and 2). After multivariate analysis including histologic subtype, stage, grade, myometrial invasion, lymphovascular space invasion, lymph node involvement, p53 overexpression and DNA ploidy as independent factors for tumor recurrence, only histologic subtype and myometrial invasion had independent significance (Table 4).

Discussion

p53 overexpression is associated with high malignant potential, including aggressive histologic types, advanced surgical stage, extensive myometrial invasion, high histological grade, lymph node metastases and poor survival in several studies [6-8, 20, 21]. Studies of p53 in endometrial cancer are in disagreement regarding the independent prognostic impact of this marker. Some authors find p53 to be a significant independent marker [6, 8, 21] while others do not [9, 20]. Using different models of multivariate analysis: 1) using only p53 with the other independent variables, histologic subtype, grade, lymph node involvement and p53 had an independent impact on overall survival; 2) when considering DNA ploidy alone without p53, histologic subtype, lymph node involvement and DNA ploidy had an independent impact on overall survival; and, 3) when combining p53 with DNA ploidy

g. 1

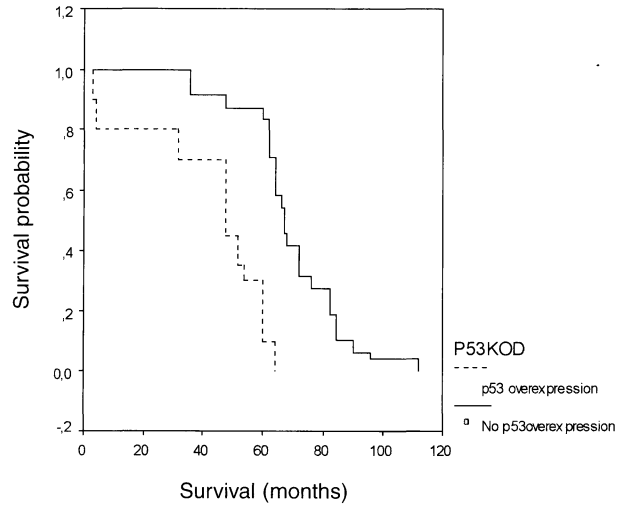

Fig. 2

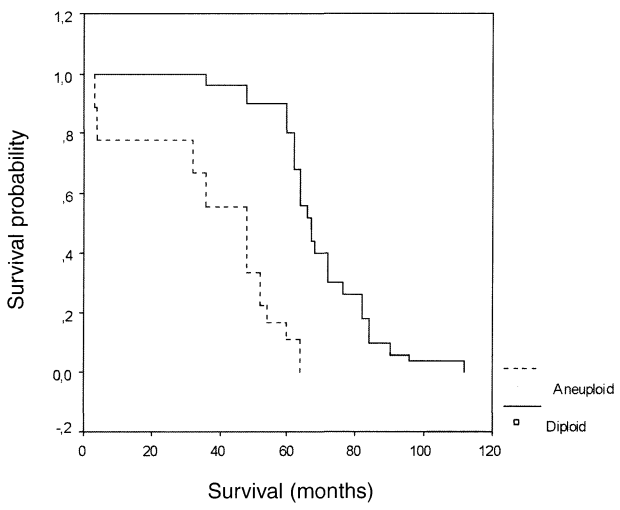

Figure 1. — Kaplan-Meier survival curve according to p53 overexpression in 136 patients with endometrial cancer.
Figure 2. — Kaplan-Meier survival curve according to DNA ploidy in 136 patients with endometrial cancer.

Table 4. — *Cox regression univariate analysis with regard to the tumor recurrence.*

	Statistical significance			RH (95% CI)		
	P^a	P^b	P^c	RH^a	RH^b	RH^c
Histologic subtype (endometrioid/ nonendometrioid)	p = 0.0081*	p = 0.0070*	p = 0.0067*	9.71 (1.80 - 52.29)	7.38 (1.72 - 31.62)	10.77 (1.93 - 60.13)
Stage (I-II/III-IV)	p = 0.0896	p = 0.0901	p = 0.0776	0.33 (0.09 - 1.18)	0.34 (0.09 - 1.18)	0.31 (0.08 - 1.13)
Grade (G1-G2/G3)	p = 0.1063	p = 0.1727	p = 0.1410	2.86 (0.79 - 10.28)	2.22 (0.70 - 7.03)	2.58 (0.73 - 9.12)
Myometrial invasion (yes/no)	p = 0.0359*	p = 0.0133*	p = 0.0376*	2.32 (1.05 - 5.11)	2.53 (1.21 - 5.28)	2.29 (1.04 - 5.04)
Peritoneal cytology (positive/negative)	p = 0.5221	p = 0.6529	p = 0.6950	0.67 (0.20 - 2.26)	0.74 (0.21 - 2.64)	0.77 (0.22 - 2.72)
Lymphovascular space invasion (yes/no)	p = 0.1367	p = 0.2076	p = 0.3172	2.05 (0.79 - 5.33)	1.93 (0.69 - 5.42)	1.71 (0.59 - 4.93)
Lymph node involvement (yes/no)	p = 0.6613	–	p = 0.4431	0.69 (0.13 - 3.48)	–	0.46 (0.06 - 3.34)
p53 (overexpression/no overexpression)	–	p = 0.7845	p = 0.5019	–	1.17 (0.36 - 3.84)	1.69 (0.36 - 7.97)

Results of the regression analysis expressed as statistical significance and relative hazard (RH) with 95% confidence interval (CI); P^a: P53 without DNA ploidy; P^b: DNA ploidy without p53; P^c: By combined p53 with DNA ploidy; *: significant.

by excluding myometrial invasion, histologic subtype, lymph node involvement and DNA ploidy had an independent impact on overall survival. We suggest that these different results may be due to distinct primary antibodies and various staining procedures [7], selecting materials in different ways, such as Stage I [22], Stage I-II [23, 24], and advanced stages [8]. In addition, Geisler and Hamel used image analysis [8, 12] while most others, as well as us, scored visually [9, 20, 21]. In addition, since the presence of lymph node metastases was found to be independent predictive factor on survival rate, lymphadenectomy would appear to be indicated as a routine part ot the staging procedure in endometrial cancer.

The frequency of p53 protein overexpression differs from 17% to 52% [10]. In the present study this rate correlated with the literature and was found to be 29%. In addition, the frequency of p53 protein overexpression differs among the histologic subtypes of endometrial carcinoma, from 10 to 48% in endometrioid carcinoma but from 45 to 86% in papillary serous carcinoma [8, 10]. We found that 12% of endometrioid carcinoma and 87% of nonendometrioid carcinoma overexpress p53 and that the difference in p53 overexpression between these histologic subtypes is statistically significant. Additionally, according to the previously reported data [11-14], expression of p53 is significantly associated with histologic subtype. The frequency of aneuploidy differs from 18 to 43% in endometrial cancer [23-25] and our result of 36/136 (26%) is consistent with the literature.

According to previously reported data [6, 11, 13, 26), we showed that p53 expression was 15% in early stage (Stage I and II) and 75% in advanced stage (Stage III and IV) of endometrial carcinoma suggesting that p53 is associated with stage and is more frequent in advanced stage. It seems reasonable to consider that p53 alteration may be an early event in the development of uterine nonendometrioid carcinomas and early detection of p53 nuclear accumulation may help to identify precursor lesions of nonendometrioid carcinomas. In disagreement with some studies [15] but in agreement with other previous studies on p53 and grade [12-14, 20, 26], we found 5% p53 expression in grade 1, 33% in grade 2 and 78% in grade 3 and increasing histologic grade correlated with increased p53 expression. On the other hand, Newbury *et al.* [25] analyzed retrospectively 233 cases of endometrial carcinoma for DNA content using flow cytometry of cell nuclei extracted from paraffin blocks and they found an association between aneuploidy and adverse histologic type, high grade, and depth of invasion in the uterus independent of stage or tumor grade. The rate of aneuploidy varies with the difference in histologic type. For clear cell carcinoma and papillary serous carcinoma, the frequency of aneuploidy increased compared to endometrioid carcinoma. Similar to previously reported studies [24, 25, 27], the aneuploidy rate was found to be 12% in the endometrioid group and 75% in the nonendometrioid group, and a significant association between histologic type and aneuploidy was found in the present study. Moreover, as suggested by Larson *et al.* [28], we found a significant relationship with aneuploidy rate and tumor stage (18% in early stage vs 43% in advanced stage). Furthermore, we determined that the aneuploidy rate was 5% in grade 1, 17% in grade 2 and 78% in grade 3 suggesting increasing histologic grade correlates with an increasing rate of aneuploidy.

Focusing on traditional histopathologic variables, Hamel and *et al.* [12] demonstrated that intense expression of p53 was significantly associated with depth of myometrial invasion. However in aggrement with Geisler *et al.* [8], no direct correlation between increasing p53 expression and depth of myometrial invasion was found in the present study. In addition, in disagreement with the previous studies [23, 25] but in agreement with the study of Iversen [27], no correlation between aneuploid frequency and deep myometrial invasion was found. Further studies are needed to confirm this different finding.

When compared to patients with diploid tumors, a significantly increased p53 expression was found in patients with aneuploid tumors (8% vs 89%, respectively). On the other hand, a positive correlation between lymphovascular space invasion and p53 and DNA ploidy and between lymph node involvement and p53 and DNA ploidy was found in this study. Patients with positive p53 overexpression, although it was not statistically significant, had a 2-times higher prevalence of pelvic lymph node metastases ($p > 0.05$, OR: 0.4651, 95% CI: 0.16-1.28). In contrast, they had a 5-times higher prevalence of para-aortic lymph node metastases ($p < 0.01$, OR: 0.1739, 95% CI: 0.04-0.61). On the other hand, patients with aneuploid

tumors had a 5.5-times higher prevalence of pelvic lymph node metastases ($p < 0.01$, OR: 0.1277, 95% CI: 0.04-0.37) and a 5.5-times higher prevalence of para-aortic lymph node metastases ($p < 0.05$, OR: 0.1458, 95%CI: 0.04-0.52) suggesting that patients with aneuploid tumors are at higher risk for lymph node metastases. Therefore, preoperative tumor ploidy determination could be an effective evaluation especially for para-aortic lymphadenectomy. Thus, an important proportion of patients with clinical tumors limited to the uterus has been shown to have extrauterine spread after surgical-pathologic staging and in 30% of patients with para-aortic nodal metastases, pelvic nodes were found to be tumor-free. On the other hand, DNA ploidy was the strongest predictor of persistent or recurrent disease [9, 18]. Although individualization or combination of DNA ploidy and p53 overexpression failed to show their independent prognostic significance on tumor recurrence when submitted to multivariate analysis (Table 4), p53 overexpression and DNA ploidy were found to be strong significant factors with regard to tumor recurrence in univariate analysis. In addition, an association of tumor recurrence with p53 and DNA ploidy was found (Table 2). Therefore, in agreement with Susini *et al.*, we suggest again that preoperative tumor ploidy determination could be accurate and the standard evaluation in selecting patients at risk for occult extrauterine tumor diffusion and recurrence [29]. However, we believe that further studies are needed to determine whether p53 and DNA ploidy are independent factors for prediction of lymph node metastasis and tumor recurrence.

As we found, Ito K. *et al.* [6], Reinartz *et al.* [13], and Erdem *et al.* [14] reported a significant correlation between decreased 5-year survival and p53 expression. Patients with aneuploid tumors had a significantly shorter tumor recurrence time and overall 5-year survival than those with diploid tumors suggesting that aneuploidy confers a risk for endometrial cancer death. Thus these patients should be candidates for clinical trials evaluating treatment following surgery [28].

In conclusion, p53 expression and DNA ploidy were directly related to histologic subtype, FIGO stage, grade, lymphovascular space invasion, lymph nodal involvement, peritoneal cytology and tumor recurrence, and negatively related to the overall 5-year survival. As compared to p53, DNA ploidy was the stronger independent predictor factor for survival. Neither p53 nor DNA ploidy were significant independent factors for tumor recurrence when submitted to multivariate analysis in this study. However, since p53 and DNA ploidy were found to be significant factors in univariate analysis and were correlated with tumor recurrence, they can be useful factors in making the prognosis.

References

[1] Jemal A., Murray T., Samuels A., Ghafoor A., Ward E., Thun M.J.: "Cancer statistics". *Cancer J. Clin.*, 2003, *53,* 5.

[2] Lanciano R.M., Corn B.W., Schultz D.J., Kramer C.A., Rosenblum N., Hogan W.M.: "The justification for a surgical staging system in endometrial carcinoma". *Radiother. Oncol.,* 1993, *28,* 189.

[3] Mayr N.A., Wen B.C., Benda J.A., Sorosky J.I., Davis C.S., Fuller R.W. *et al.*: "Postoperative radiation therapy in clinical stage I endometrial cancer: corpus, cervical, and lower uterine segment involvement-patterns of failure". *Radiology*, 1995, *196,* 323.

[4] el-Deiry W.S., Tokino T., Velculescu V.E., Levy D.B., Parsons R., Trent J.M. *et al.*: "WAF1, a potential mediator of p53 tumor suppression". *Cell.,* 1993, *75,* 817.

[5] Harris C.C.: "Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies". *J. Natl. Cancer Inst.,* 1996, *88,* 1442.

[6] Ito K., Watanabe K., Nasim S., Sasano H., Sato S., Yajima A. *et al.*: "Prognostic significance of p53 overexpression in endometrial cancer". *Cancer Res.,* 1994, *54*, 4667.

[7] Kohlberger P., Gitsch G., Loesch A., Tempfer C., Kaider A., Reinthaller A. *et al.*: "p53 protein overexpression in early stage endometrial cancer". *Gynecol. Oncol.*, 1996, *62*, 213.

[8] Geisler J.P., Wiemann M.C., Zhou Z., Miller G.A., Geisler H.E.: "p53 as a prognostic indicator in endometrial cancer". *Gynecol. Oncol.,* 1996, *61,* 245.

[9] Lukes A.S., Kohler M.F., Pieper C.F., Kerns B.J., Bentley R., Rodriguez G.C. *et al.*: "Multivariable analysis of DNA ploidy, p53, and HER-2/neu as prognostic factors in endometrial cancer". *Cancer,* 1994, *73*, 2380.

[10] Ambros R.A., Sheehan C.E., Kallakury B.V., Ross J.S., Malfetano J., Paunovich E. *et al.*: "MDM2 and p53 protein expression in the histologic subtypes of endometrial carcinoma". *Mod. Pathol.*, 1996, *9,* 1165.

[11] Kohler M.F., Berchuck A., Davidoff A.M., Humphrey P.A., Dodge R.K., Iglehart J.D. *et al.*: "Overexpression and mutation of p53 in endometrial carcinoma". *Cancer Res.,* 1992, *52*, 1622.

[12] Hamel N.W., Sebo T.J., Wilson T.O., Keeney G.L., Roche P.C., Suman V.J. *et al.*: "Prognostic value of p53 and proliferating cell nuclear antigen expression in endometrial carcinoma". *Gynecol. Oncol.,* 1996, *62,* 192.

[13] Reinartz J.J., George E., Lindgren B.R., Niehans G.A.: "Expression of p53, transforming growth factor alpha, epidermal growth factor receptor, and c-erbB-2 in endometrial carcinoma and correlation with survival and known predictors of survival". *Hum. Pathol.,* 1994, *25,* 1075.

[14] Erdem O., Erdem M., Dursun A., Akyol G., Erdem A.: "Angiogenesis, p53, and bcl-2 expression as prognostic indicators in endometrial cancer: comparison with traditional clinicopathologic variables". *Int. J. Gynecol. Pathol.*, 2003, *22*, 254.

[15] Nielsen A.L., Nyholm H.C.: "p53 protein and c-erbB-2 protein (p185) expression in endometrial adenocarcinoma of endometrioid type. An immunohistochemical examination on paraffin sections". *Am. J. Clin. Pathol.*, 1994, *102,* 76.

[16] Lundgren C., Auer G., Frankendal B., Moberger B., Nilsson B., Nordstrom B.: "Nuclear DNA content, proliferative activity, and p53 expression related to clinical and histopathologic features in endometrial carcinoma". *Int. J. Gynecol. Cancer,* 2002, *12,* 110.

[17] Nordstrom B., Strang P., Lindgren A., Bergstrom R., Tribukait B.: "Endometrial carcinoma: the prognostic impact of papillary serous carcinoma (UPSC) in relation to nuclear grade, DNA ploidy and p53 expression". *Anticancer Res.,* 1996, *16*, 899.

[18] Britton L.C., Wilson T.O., Gaffey T.A., Cha S.S., Wieand H.S., Podratz K.C.: "DNA ploidy in endometrial carcinoma: major objective prognostic factor". *Mayo Clin. Proc.,* 1990, *65*, 643.

[19] Hedley D.W., Friedlander M.L., Taylor I.W., Rugg C.A., Musgrove E.A.: "Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry". *J. Histochem. Cytochem.*, 1983, *31*, 1333.

[20] Strang P., Nordstom B., Nilsson S., Bergstrom R., Tribukait B.: "Mutant p53 protein as a predictor of survival in endometrial carcinoma". *Eur. J. Cancer,* 1996, *32,* 598.

[21] Nielsen A.L., Nyholm H.C.: "The combination of p53 and age predict cancer specific death in advanced stage (FIGO Ic-IV) of endometrial carcinoma of endometrioid type. An immunohistochemical examination of growth fraction: Ki-67, MIB- 1 and PC10; suppressor oncogene protein: p53; oncogene protein: p185 and age, hormone treatment, stage, and histologic grade". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 1996, *70*, 79.

[22] Pfisterer J., Kommoss F., Sauerbrei W., Renz H., du Bois A., Kiechle-Schwarz M. *et al.*: "Prognostic value of DNA ploidy and S-phase fraction in Stage I endometrial carcinoma". *Gynecol. Oncol.,* 1995, *58*, 149.

[23] Lindahl B., Alm P., Ferno M., Killander D., Langstrom E., Norgren A. *et al.*: "Prognostic value of steroid receptor concentration and flow cytometrical DNA measurements in Stage I-II endometrial carcinoma". *Acta Oncol.,* 1989, *28*, 595.

[24] Rosenberg P., Wingren S., Simonsen E., Stal O., Risberg B., Nordenskjold B.: "Flow cytometric measurements of DNA index and S-phase on paraffin-embedded early stage endometrial cancer: an important prognostic indicator". *Gynecol. Oncol.,* 1989, *35,* 50.

[25] Newbury R., Schuerch C., Goodspeed N., Fanning J., Glidewell O., Evans M.: "DNA content as a prognostic factor in endometrial carcinoma". *Obstet. Gynecol.*, 1990, *76*, 251.

[26] Riethdorf L., Begemann C., Riethdorf S., Milde-Langosch K., Loning T.: "Comparison of benign and malignant endometrial lesions for their p53 state, using immunohistochemistry and temperature-gradient gel electrophoresis". *Virchows Arch.,* 1996, *428,* 47.

[27] Iversen O.E.: "Flow cytometric deoxyribonucleic acid index: a prognostic factor in endometrial carcinoma". *Am. J. Obstet. Gynecol.*, 1986, *155,* 770.

[28] Larson D.M., Berg R., Shaw G., Krawisz B.R.: "Prognostic significance of DNA ploidy in endometrial cancer". *Gynecol. Oncol.*, 1999, *74,* 356.

[29] Susini T., Rapi S., Massi D., Savino L., Amunni G., Taddei G.L. *et al.*: "Preoperative evaluation of tumor ploidy in endometrial carcinoma: An accurate tool to identify patients at risk for extrauterine disease and recurrence". *Cancer,* 1999, *86,* 1005.

Address reprint requests to:
M.A. OSMANAĞAOĞLU, M.D.
Karadeniz Technical University
Faculty of Medicine
Department Obstetrics
and Gynecology
61080 Trabzon (Turkey)