Available online at www.sciencedirect.com
ScienceDirect
Gynecologic Oncology 108 (2008) 569–576
Gynecologic Oncology
www.elsevier.com/locate/ygyno

Role of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type 1 (PAI-1) for prognosis in endometrial cancer

E. Steiner [a,*], K. Pollow [a], D. Hasenclever [b], W. Schormann [c], M. Hermes [c], M. Schmidt [a], A. Puhl [a], M. Brulport [c], A. Bauer [c], I.B. Petry, H. Koelbl [a], J.G. Hengstler [c,*]

[a] *Department of Gynecology, University of Mainz, Mainz, Germany*
[b] *Institute of Medical Informatics, Statistics & Epidemiology (IMISE), University of Leipzig, Leipzig, Germany*
[c] *Leibniz Research Centre for Working Environment and Human Factors at the University of Dortmund (IfADo), Dortmund, Germany*

Received 18 June 2007
Available online 28 January 2008

Abstract

Background. Urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type 1 (PAI-1) contribute to the invasiveness of many carcinomas. Here, we studied a possible association between cytosolic uPA and PA-1 concentrations in tumor tissue with prognosis in patients with endometrial cancer.

Methods. Cytosolic concentrations of uPA and PAI-1 were determined in 69 primary endothelial adenocarcinomas using an enzyme-linked immunoassay (ELISA). A possible influence of uPA and PAI-1 was studied by multivariate Cox regression adjusting for the established clinical prognostic factors FIGO-stage, grading, depth of invasion, diabetes mellitus and age.

Results. Both uPA ($p=0.011$) and PAI-1 ($p=0.003$) were associated with relapse free time using the multivariate proportional hazards model. Association with overall survival was less pronounced with $p=0.021$ for uPA and $p=0.358$ for PAI-1. Concentrations of PAI-1 increased with FIGO stage ($p=0.003$) and with histological grading ($p=0.005$). Both uPA and PAI-1 concentrations were negatively correlated with estrogen and progesterone receptor levels.

Conclusion. The combination of high cytosolic concentrations of uPA (>5 ng/mg total protein) and high PAI-1 (>20 ng/mg total protein) may reveal a group of patients with increased risk of progression.

Keywords: Metastasis; Adhesion; Endometrial cancer; Time to relapse; Survival; uPA; PAI-1

Introduction

The urokinase (uPA) and plasminogen activator inhibitor system is involved in remodelling tissues during invasion and metastasis of tumor cells [1–3]. PAI-1 is a direct and fast acting inhibitor of urokinase (uPA) [4,5]. As a consequence conversion of plasminogen to plasmin is reduced. PAI-1 also binds to the extracellular matrix compound vitronectin, thereby limiting the binding of vitronectin to the integrin receptor αvβ3 [5,3,6]. Both mechanisms inhibit proteolysis, adhesion and migration. Such inhibition has been demonstrated in vascular smooth muscle cells [7,5], macrophages [8] and mouse embryonic fibroblasts [4]. However, in tumor cells the situation seems to be complex. It has been shown that PAI-1 can alternatively retard or enhance cell migration and adhesion [7,9,2,10]. Recently, it has been shown that also the concentration of PAI-1 is critical for tumor invasion in vivo [2]. At physiological concentrations host PAI-1 was reported to promote in vivo tumor invasion and angiogenesis. In contrast, supraphysiological PAI-1 concentrations inhibited tumor vascularization. This scenario suggests a "Dr. Jekyll and Mr. Hide" situation for PAI-1. Its influence on cell adhesion and migration seems to depend on the cell type, conformation and on the concentration.

* Corresponding authors. J.G. Hengstler is to be contacted at Leibniz Research Centre for Working Environment and Human Factors, University of Dortmund, Dortmund, Germany, Ardeystraße 67, D-44139 Dortmund, Germany. Fax: +49 0 231/1084 308. E. Steiner, Department of Gynecology, Langenbeck Straße 1, University of Mainz, Mainz, Germany.
E-mail address: jan.hengstler@medizin.uni-leipzig.de (J.G. Hengstler).

0090-8258/$ - see front matter © 2008 Published by Elsevier Inc.
doi:10.1016/j.ygyno.2007.11.025

Stimulated by these results we determined concentrations of uPA and PAI-1 in cytosolic fractions of endometrial carcinomas and studied a possible association with time to relapse. Both, uPA and PAI-1 were associated with time to relapse. Therefore, PAI-1 seems to act rather as "Mr Hyde" in the setting of endometrial cancer.

Materials and methods

Patients and tissue samples

Between 1985 and 2000 269 consecutive patients with histologically proven endometrial cancer have been treated at the Department of Obstetrics and Gynecology at the University Hospital in Mainz. Surgically obtained tissue was shock frozen and stored at −80 °C. Fresh frozen tissue specimens were available of only 69 of these consecutive patients for three reasons: (i) Aliquots of the frozen tissue specimens were additionally histologically controlled. If the fraction of tumor cells was smaller than 90%, the sample was not included into the present study. (ii) No tissue could be frozen from some patients with small tumors. (iii) For diagnostic reasons no fresh tissue was available from several patients. The fact that tissue was available from only 25% of the patients may lead to selection for more aggressive tumors in the analyzed cohort, since the tumor can be more easily seen at surgery allowing for adequate retrieval of fresh tissue for freezing. Therefore, we systematically compared patients of whom fresh frozen tissue was *versus* was not available. No significant differences have been observed concerning relapse-free survival, overall survival, FIGO-stage, grading and depth of invasion (data not shown).

Based on information from hospital records, including surgical notes and pathologic reports a database was generated. Histological tumor type and grade, weight, height and age of the patients, diabetes mellitus, FIGO stage, type of surgery and pathologic TNM classification have been included. The FIGO stage followed the surgical staging system for endometrial carcinoma of 1988 [11]. The body mass index (BMI) has been calculated using the formula BMI= [weight/(height)^2]. Relapse free time was calculated as the difference between the date of surgery and observation of metastasis. In our set of patients metastasis occurred in para-aortic-lymph nodes, pelvis, bone, lung, liver and vagina. Evaluation of all histological specimens has been performed by an experienced pathologist (Dr. M. Schmidt). All tumors have been classified according to the WHO/ISGPY classification [12]. The tumor grade has been determined as described by Kurman et al. including architectural and nuclear features [13]. The depth of invasion has been classified as described by Sevin and Angioli depending on infiltration of the inner, middle, and outer third of the myometrium [14]. For uPA and PAI-1 analysis only histologically controlled tumor specimens consisting of at least 95% tumor cells without non-neoplastic endometrium or myometrium have been included into the study. Standard surgical procedure was abdominal hysterectomy and bilateral salpingo-oophorectomy. Lymph node dissection was performed in cases where intraoperative frozen sections showed myometrial infiltration of the outer third of the myometrium and as far as possible also in cases with cervical involvement depending on factors of general morbidity of the patient. Patient's characteristics are summarized in Table 1. Kaplan–Meier plots illustrating the influence of the established clinical factors FIGO-stage, grading, depth of invasion, diabetes mellitus and age at surgery on survival time are shown in Supplemental Table 1.

Cytosolic concentrations of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type 1 (PAI-1)

Frozen tissue corresponding to 100–300 mg wet weight was pulverised using a stainless steel pestle and mortar on dry ice and suspended in 1.8 ml Tris buffered saline (TBS), pH 8.5. Subsequently, commercially available kits were applied (IMUBIND Tissue uPA ELISA Kit, product no. 894 and IMUBIND Tissue PAI-1 ELISA Kit, product no. 82, American Diagnostica Inc., Greenwich, USA) according to the manufacturer's instructions. The ELISA employs murine anti-human uPA or PAI-1 as the capture antibodies. Samples were incubated in precoated microtest wells. Detection was achieved with secondary biotinylated antibodies directed against the bound uPA or PAI-1 molecules.

Table 1
Characteristics of patients with endometrial cancer (n=69)

	Number evaluated (n=69)	%	Not evaluable
FIGO-stage			4
Stage I	40	58.0	
Stage II	10	14.5	
Stage III	13	18.8	
Stage IV	2	2.9	
Histologic grade			1
Grade I	21	39.9	
Grade II	30	44.1	
Grade III	17	25.0	
Histological type			0
Adenocarcinomas	69	100.0	
Others	0	0.0	
Depth of invasion [a]			0
Limited to endometrium	8	11.6	
Inner third of myometrium	22	31.9	
Mean third of myometrium	12	17.4	
Outer third of myometrium	27	39.1	
low [a]	30	43.5	
high [a]	39	56.5	
Metastasis [b]			7
No	50	72.5	
Yes	12	17.4	
Diabetes Mellitus			0
Yes	12	17.4	
No	57	82.6	
Age at surgery			
≥60 years	53	76.8	0
<60 years	16	23.2	
mean (years)±standard deviation	66.9±11.9		
Body size			0
(cm, mean±standard deviation)	162.9±6.0		
Weight			0
(kg, mean±standard deviation)	77.6±15.6		

[a] Depth of invasion was classified into low (infiltration maximally of the inner third of the myometrium) and high (infiltration of the middle or outer third of the myometrium).

[b] "Metastasis": if new tumor growth was observed at one of the following sites: para-aortic-lymph nodes, pelvis, bone, lung, liver or vagina.

Detection was performed by streptavidin conjugated horseradish peroxidase and addition of a perborate/3, 3′, 3, 5′-tetramethylbenzidine (TMB) substrate creating a blue colored solution as a consequence of reaction with HRP [15]. uPA and PAI-1 concentrations were measured by determination of absorbances at 450 nm and comparing the values with those of a standard curve. Protein concentrations of the tissue extracts were determined as described [16,17]. Division of the uPA or PAI-1 concentrations by the protein concentrations (mg/ml) of the tissues extracts allowed conversion of ng uPA or PAI-1/ml tissue extract to ng uPA or PAI-1/mg total protein. The latter was used for statistical analysis. The uPA ELISA technique detects single chain uPA and high molecular weight forms of uPA. Also receptor bound uPA and uPA complexed with PAI-1 or PAI-2 is recognized. The lower detection limit is 10 pg uPA/ml tissue homogenate. The PAI-1 ELISA detects inactive and active forms of PAI-1 as well as PAI-1

Table 2
Association of uPA, PAI-1 and clinical parameters with time to relapse in patients with primary endothelial adenocarcinoma using the univariate proportional hazards model (Cox-analysis)

Factor	Relative risk	95%-Confidence interval	*p*-value
uPA expression (ng/mg)	1.244	1.091–1.418	0.001
PAI-1 expression (ng/mg)	1.027	1.011–1.043	0.001
FIGO-stage (stages I, II vs. III, IV)	11.233	2.575–49.007	0.001
Grading (grade I vs. II vs. III)	3.901	0.487–31.246	0.200
Depth of invasion (low vs. high [a])	1.589	0.396–6.372	0.514
Diabetes mellitus (no vs. yes)	1.461	0.301–7.098	0.639
Age at surgery (<60 years vs. ≥60 years)	0.704	0.176–2.824	0.621
Body size (cm)	1.009	0.895–1.138	0.882
Weight (kg)	0.998	0.956–1.041	0.925

[a] Depth of invasion was classified into low (infiltration maximally of the inner third of the myometrium) and high (infiltration of the middle or outer third of the myometrium).

complexes. It does not recognize PAI-2. The lower detection limit is 50 pg PAI-1/ml tissue homogenate.

Determination of estrogen and progesterone receptor levels was performed as described by Steiner et al. [18].

Statistical analysis

The proportional hazards model was applied to examine whether uPA and PAI-1 are independent prognostic factors (univariable and multivariable analysis) similarly as described [19,20]. Analysis of interaction terms between factors was performed as described [21,22]. The proportional hazards assumption was tested by Cox-Snell residuals showing a good fit of all Cox analysis presented in Results. Kaplan–Meier curves were plotted to assess overall survival. To visualize interactions between two factors in the Kaplan–Meier plots products were calculated between these factors and the resulting values were subdivided into three groups with similar case numbers. Different survival curves were compared using the log-rank test. A difference between two independent

Table 3
Multivariate proportional hazards model (Cox-analysis) analyzing relapse free time in patients with primary endothelial adenocarcinoma

Factor	Relative risk	95%-Confidence interval	*p*-value
A. uPA expression (ng/mg)			
FIGO-stage (stages I, II vs. III, IV)	10.842	1.990–59.079	0.006
uPA expression (ng/mg)	1.301	1.061–1.596	0.011
Not regarded as explanatory:			
		p-value	
Grading (grade I vs. II vs. III)		0.587	
Depth of invasion (low vs. high [a])		0.183	
Diabetes mellitus (no vs. yes)		0.305	
Age at surgery (<60 years vs. ≥60 years)		0.181	
B. PAI-1 expression (ng/mg)			
FIGO-stage (stages I, II vs. III, IV)	11.600	2.061–65.294	0.005
PAI-1 expression (ng/mg)	1.048	1.016–1.081	0.003
Not regarded as explanatory:			
		p-value	
Grading (grade I vs. II vs. III)		0.382	
Depth of invasion (low vs. high [a])		0.128	
Diabetes mellitus (no vs. yes)		0.191	
Age at surgery (<60 years vs. ≥60 years)		0.067	

(A) uPA expression (ng/mg), (B) PAI-1 expression (ng/mg). Analysis was adjusted to FIGO-stage (stages I, II vs. III, IV), grading (grade I vs. II vs. III), depth of invasion (low vs. high), diabetes mellitus (no vs. yes) and age at surgery (<60 years vs. ≥60 years).

[a] Depth of invasion was classified into low (infiltration maximally of the inner third of the myometrium) and high (infiltration of the middle or outer third of the myometrium).

Table 4
Association of uPA, PAI-1 and clinical parameters with survival in 69 patients with primary endothelial adenocarcinoma using the univariate proportional hazards model (Cox-analysis)

Factor	Relative risk	95%-Confidence interval	*p*-value
uPA expression (ng/mg)	1.244	1.085–1.426	0.002
PAI-1 expression (ng/mg)	1.021	0.999–1.043	0.061
FIGO-stage (stages I, II vs. III, IV)	14.808	2.807–78.104	0.001
Grading (grade I vs. II vs. III)	36.379	0.044–30413	0.295
Depth of invasion (low vs. high [a])	4.547	0.547–37.777	0.161
Diabetes mellitus (no vs. yes)	0.886	0.107–7.366	0.911
Age at surgery (<60 years vs. ≥60 years)	31.890	0.023–43819	0.348
Body size (cm)	1.037	0.888–1.212	0.644
Weight (kg)	1.004	0.953–1.057	0.892

[a] Depth of invasion was classified into low (infiltration maximally of the inner third of the myometrium) and high (infiltration of the middle or outer third of the myometrium).

Table 5
Multivariate proportional hazards model (Cox-analysis) analyzing survival of 69 patients with primary endothelial adenocarcinoma

Factor	Relative risk 95%-Confidence interval	*p*-value	
A. uPA expression (ng/mg)			
FIGO-stage (stages I, II vs. III, IV)	12.105	1.729–84.766	0.012
uPA expression (ng/mg)	1.355	1.047–1.754	0.021
Not regarded as explanatory:			
		p-value	
Grading (grade I vs. II vs. III)		0.978	
Depth of invasion (low vs. high [a])		0.110	
Diabetes mellitus (no vs. yes)		0.785	
Age at surgery (<60 years vs. ≥60 years)		0.961	
B. PAI-1 expression (ng/mg)			
FIGO-stage (stages I, II vs. III, IV)	11.135	1.885–65.775	0.008
Not regarded as explanatory:			
		p-value	
PAI-1 expression (ng/mg)	1.015	0.983–1.048	0.358
Grading (grade I vs. II vs. III)		0.973	
Depth of invasion (low vs. high [a])		0.582	
Diabetes mellitus (no vs. yes)		0.838	
Age at surgery (<60 years vs. ≥60 years)		0.967	

(A) uPA expression (ng/mg), (B) PAI-1 expression (ng/mg). Analysis was adjusted to FIGO-stage (stages I, II vs. III, IV), grading (grade I vs. II vs. III), depth of invasion (low vs. high), diabetes mellitus (no vs. yes) and age at surgery (<60 years vs. ≥60 years).

[a] Depth of invasion was classified into low (infiltration maximally of the inner third of the myometrium) and high (infiltration of the middle or outer third of the myometrium).

groups was tested by the Mann–Whitney test. The Jonckheere Terpstra test was applied to test a possible difference between several independent groups. Statistical analysis was performed using SPSS 14.0 software.

Results

uPA and PAI-1 are associated with prognosis

In order to evaluate the clinical relevance of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type 1 (PAI-1) we determined uPA and PAI-1 concentrations in cytosolic extracts of 69 patients with endometrial cancer using enzyme-linked immunosorbent assays (ELISA). Median concentrations as well as 25 and 75% percentiles were 2.6 (1.6–4.1) ng/mg total protein for uPA and 7.0 (4.1–18.2) ng/mg total protein for PAI-1. Because the analyzed samples were collected throughout a period of 10 years, we analyzed a possible difference between older and younger tissue specimens. However, no correlation with storage time was found.

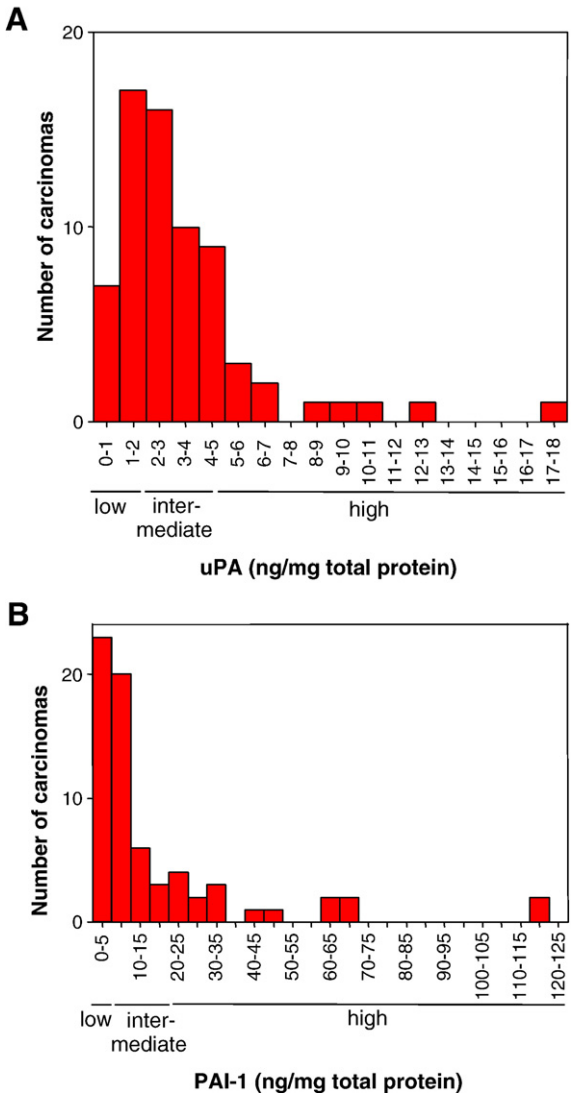

Fig. 1. Histogram of cytosolic concentrations of uPA (A) and PAI-1 (B). Patients were divided into three classes with similar case numbers: <2, 2–5 and >5 ng uPA/mg total protein and <5, 5–20 and >20 ng PAI-1/mg total protein.

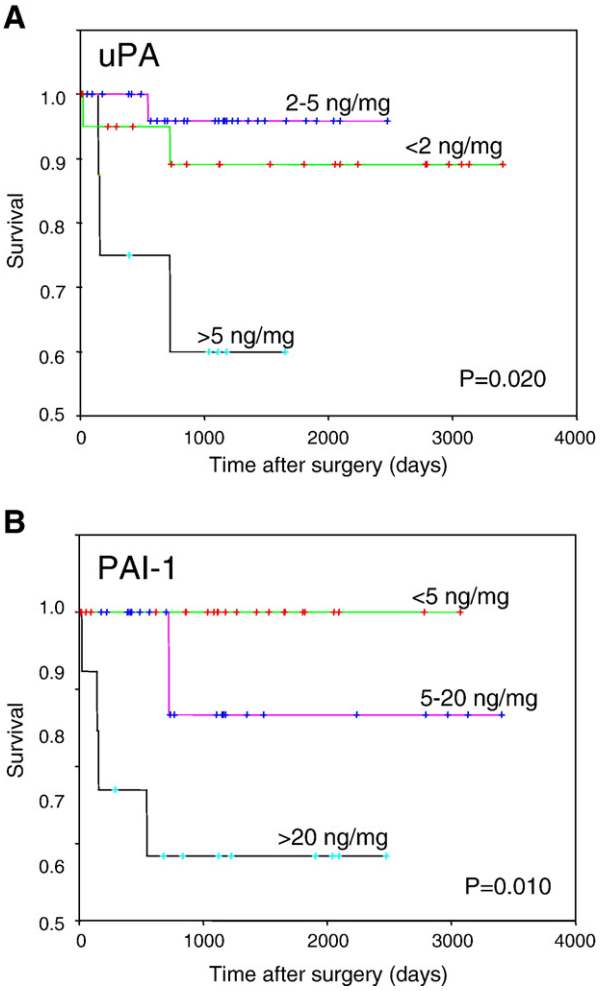

Fig. 2. Association of uPA and PAI-1 cytosolic concentrations with survival time in 69 patients with primary endometrial adenocarcinomas. (A) The differences between groups with <2, 2–5 and >5 ng uPA/mg total protein were significant in the log-rank test ($p=0.020$). (B) Similarly, significant differences were obtained between groups of patients with <5, 5–20 and >20 ng PAI-1/mg total protein ($p=0.010$).

To study the prognostic relevance of uPA and PAI-1 we first studied a possible association with time to relapse (Table 2). Both uPA ($p=0.001$) and PAI-1 ($p=0.001$) were associated with time to relapse. The relative risk for uPA and PAI-1 is relatively small (Table 2). However, it should be considered that uPA as well as PAI-1 were analyzed as continuous variables with ranges of 0.5–17.4 ng/mg and 0.7–118.4 ng/mg respectively. Next, we used the multivariate proportional hazard model to analyze the prognostic impact of uPA and PAI-1 adjusted for well-established clinical prognostic factors: FIGO-stage, histological grading, depth of invasion, diabetes mellitus and age at surgery. These clinical factors have previously been shown to be influential in a relatively large cohort of patients with endometrial cancer [23,18]. After stratification to these clinical factors uPA ($p=0.011$) and PAI-1 ($p=0.003$) were significantly associated with time to relapse (Table 3).

In a next step we studied a possible association of uPA and PAI-1 with survival (Tables 4 and 5). Using the univariate

proportional hazard model uPA ($p=0.002$) was associated with survival, whereas only a borderline significant association was obtained for PAI-1 ($p=0.061$) (Table 1). The multivariate proportional hazard model adjusted for FIGO-stage, grading, depth of invasion, diabetes mellitus and age at surgery showed a significant association of uPA with survival ($p=0.021$), whereas PAI-1 was not significant ($p=0.358$) (Table 5).

The influence of uPA and PAI-1 was also visualized by Kaplan–Meier plots (Fig. 2). For this purpose patients were divided into three groups of approximately equal size with <2, 2–5 and >5 ng/mg cytosolic uPA (Fig. 1A). A similar classification was performed for PAI-1 resulting in three groups of patients with <5, 5–20, and >20 ng/mg (Fig. 1B). The results visualize an association of both, uPA and PAI-1, with survival time (Fig. 2). Especially patients with the highest concentrations of >5 ng/mg uPA (Fig. 2A) and >20 ng/mg PAI-1 (Fig. 2B) survived shorter. The result prompted us to ask, whether high concentrations of both uPA and PAI-1 may be associated with an even worse prognosis. Unfortunately, the relatively low case number did not allow a reliable statistical analysis of uPA–PAI-1 interactions. In an explorative approach we multiplied concentrations of uPA and PAI-1 and divided into three groups corresponding to the products of concentration ranges shown in Fig. 2A and B. Obviously, patients with low (<10 ng^2/mg^2) or intermediate (10–100 ng^2/mg^2) uPA×PAI-1 products survived longer compared to patients with uPA×PAI-1 higher than 100 ng^2/mg^2 (Fig. 3A). The results suggest that high concentrations of both, uPA and PAI-1, resulting in products higher than 100 ng^2/mg^2 may be associated with a particularly bad prognosis. However, this observation requires confirmation in larger cohorts.

PAI-1 but not uPA is associated with tumor progression

Concentrations of PAI-1 increased with FIGO-stage (Fig. 4A). The median PAI-1 concentration was 6.2 (3.2–11.1) ng/mg (25th–75th percentiles) for FIGO stages I, II compared to 14.8 (11.1–41.6) ng/mg for FIGO stages III, IV ($p=0.003$). A similar correlation was observed with histological grading (Fig. 4B). PAI-1 concentrations were 5.2 (3.5–9.0), 6.6 (3.4–14.2) and 18.3 (6.9–30.4) in grade 1, 2 and 3 carcinomas, respectively ($p=0.005$). In contrast, uPA concentrations did not correlate with FIGO-stage and histologic grade (Fig. 4C, D). No correlations were observed between uPA as well as PAI-1 and diabetes mellitus as well as age (Supplemental Fig. 3).

PAI-1 and uPA negatively correlate with estrogen and progesterone receptor levels

We observed a strong decrease in biochemically determined progesterone receptor levels in tumors with high compared to intermediate and low cytosolic PAI-1 concentrations (Fig. 5A). Median progesterone receptor concentrations were 1012 (624–1890) ng/mg (25th–75th percentiles), 294 (147–597) ng/mg and 126 (0–674) ng/mg in tumors with <5, 5–20 and >20 ng PAI-1/mg total protein ($p<0.001$). Similarly, biochemically determined estrogen receptor concentrations were inversely associated with PAI-1 concentrations ($p=0.001$; Fig. 5B). In contrast,

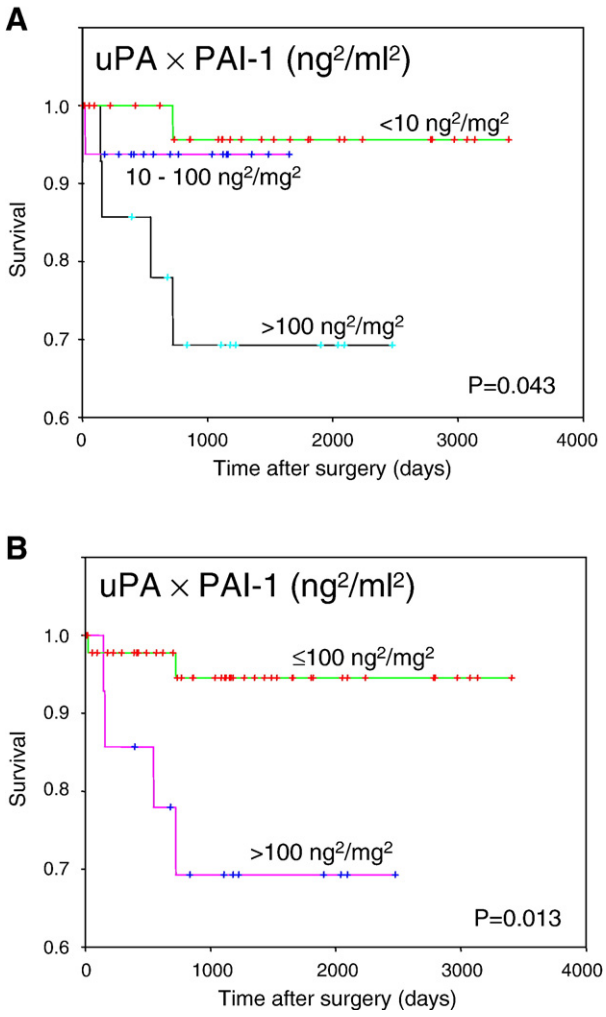

Fig. 3. Association of the product uPA × PAI-1 with survival time in 69 patients with primary endometrial adenocarcinomas. (A) The differences between groups with <10, 10–100 and >100 ng^2/mg^2 were significant in the log-rank test ($p=0.020$). Obviously, there was no relevant difference between tumors with <10 and 10–100 ng^2/mg^2. (B) Therefore, patients were additionally dichotomized using 100 ng^2/mg^2 as a cut-point ($p=0.013$).

uPA was neither associated with progesterone nor with estrogen receptor concentrations (Fig. 5C, D). In order to confirm the result by an independent technique we determined progesterone as well as estrogen receptor status by immunostaining. A very similar scenario was obtained (Supplemental Fig. 4). However, neither estrogen nor progesterone receptor concentrations were significantly associated with time to relapse or with survival.

Discussion

The prognostic relevance of the urokinase activation system has been intensively studied [1–3]. Especially in breast cancer high levels of uPA and PAI-1 have been shown to predict poor prognosis [24,6,25–29]. However, relatively little is known in endometrial cancer. Higher levels of uPA and PAI-1 were found in endometrial cancer tissue than in normal endometrium [30–32]. There are conflicting data regarding the prognostic role of uPA and PAI-1 in endometrial cancer. Endometrial

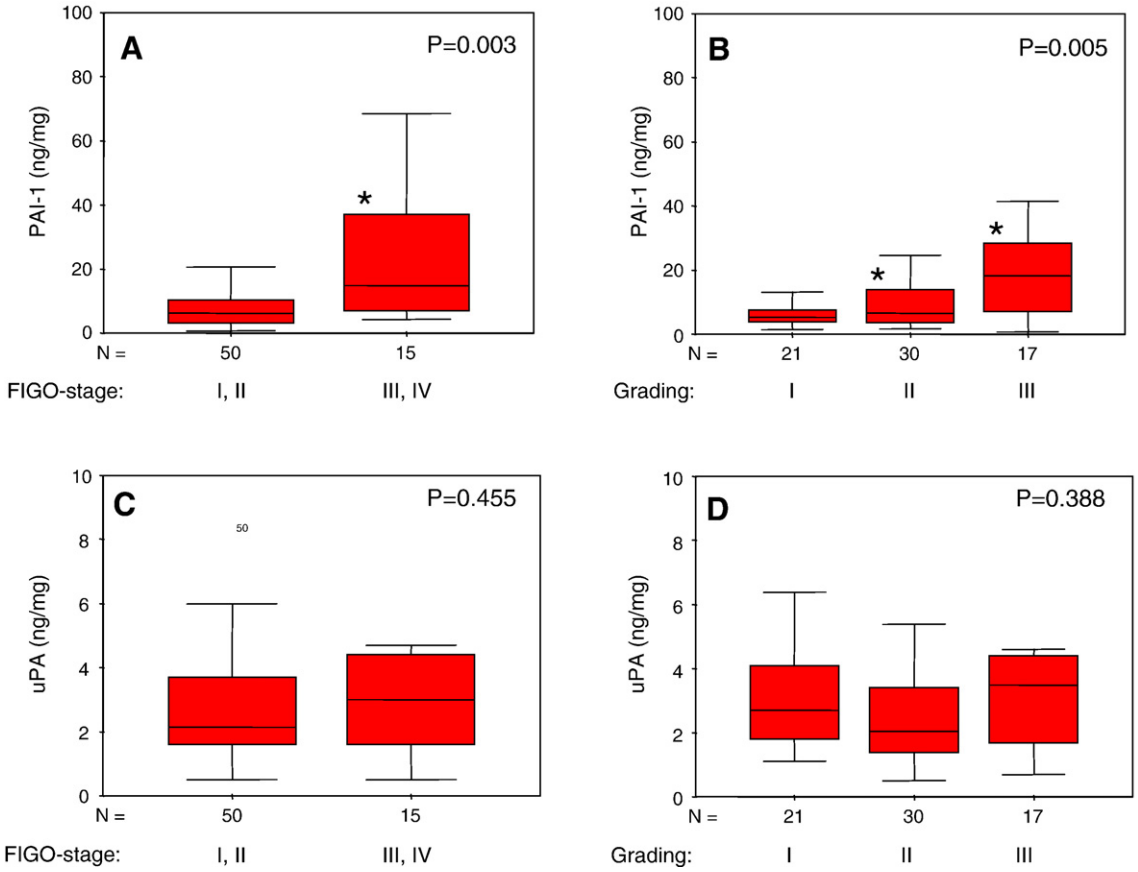

Fig. 4. Cytosolic concentrations of uPA and PAI-1 in relation to FIGO-stage (A) and histological grading (B). The difference in PAI-1 concentrations between FIGO stages I, II *versus* III, IV tumors was significant in the Mann–Whitney *U* test ($p=0.003$). Similarly, PAI-1 was higher in grade II, III compared to grade I tumors ($p=0.005$). In contrast, uPA was not significantly associated with FIGO-stage (C) and grading (D).

carcinomas with advanced stage and high grade were reported to have higher uPA and PAI-1 concentrations than tumors of less advanced stages [32,33]. However, a correlation with these standard prognostic parameters was not found in other studies [30,31]. Elevated expression of PAI-1 have been reported to be associated with shorter survival [33]. Similar results have been obtained in our study. Cytosolic concentrations of both, PAI-1 and uPA were clearly associated with relapse free survival, independent from FIGO-stage, grading, depth of invasion, diabetes mellitus and age at surgery. In contrast, association with overall survival was less pronounced. This is not surprising since overall survival is even the more multifactorial than time to relapse. Multivariate analysis resulted in a significant association for uPA but not for PAI-1.

In contrast to uPA, PAI-1 was associated with FIGO-stage and grading. We observed a 3.5-fold higher median PAI-1 concentration in grade III compared to grade I carcinomas. Similarly, PAI-1 concentrations were 1.8-fold higher in FIGO-stage III, IV compared to FIGO-stage I, II tumor samples. Therefore, grade III, FIGO III, IV carcinomas are more likely to exceed critical concentrations of PAI-1, which will probably contribute to the malignant phenotype of progressed endometrial carcinomas.

Great efforts have been made to identify factors that predict prognosis in gynaecological carcinomas [34–40]. Compared to ovarian cancer, prognosis of endometrial carcinomas is much better [41,42] . Nevertheless, it is of clinical relevance to identify patients at high risk for progression in early stages, such as FIGO-stages I and II. Based on the fact that maximally 20% of patients with FIGO-stage I and II endometrial carcinomas will experience progression, it is important to establish markers selecting patients for adjuvant therapy. Due to severe side effects overtreatment should be avoided and the selection process should be as specific as possible. In this respect uPA and PAI-1 may help to identify patients at risk of relapse. Since the proportional hazard model proved uPA and PAI-1 as independent from FIGO-stage they may be instrumental in identifying the relatively small fraction of high risk patients also in low FIGO-stages. Nevertheless, it should be considered that besides FIGO-stage uPA and PAI-1 are only moderate size prognostic factors.

Unfortunately, the case number in our study was not sufficient to perform a reliable statistical analysis of interactions between uPA and PAI-1. Our explorative analysis suggests that patients with both, high uPA and high PAI-1, resulting in a product of >100 ng^2/mg^2 may be associated with a particularly bad prognosis. However, this observation requires conformation in a larger cohort.

Recently, Nordengren et al. have demonstrated that PAI-2 is an independent marker for shorter progression-free survival in endometrial cancer [43]. Interestingly, the combination of high

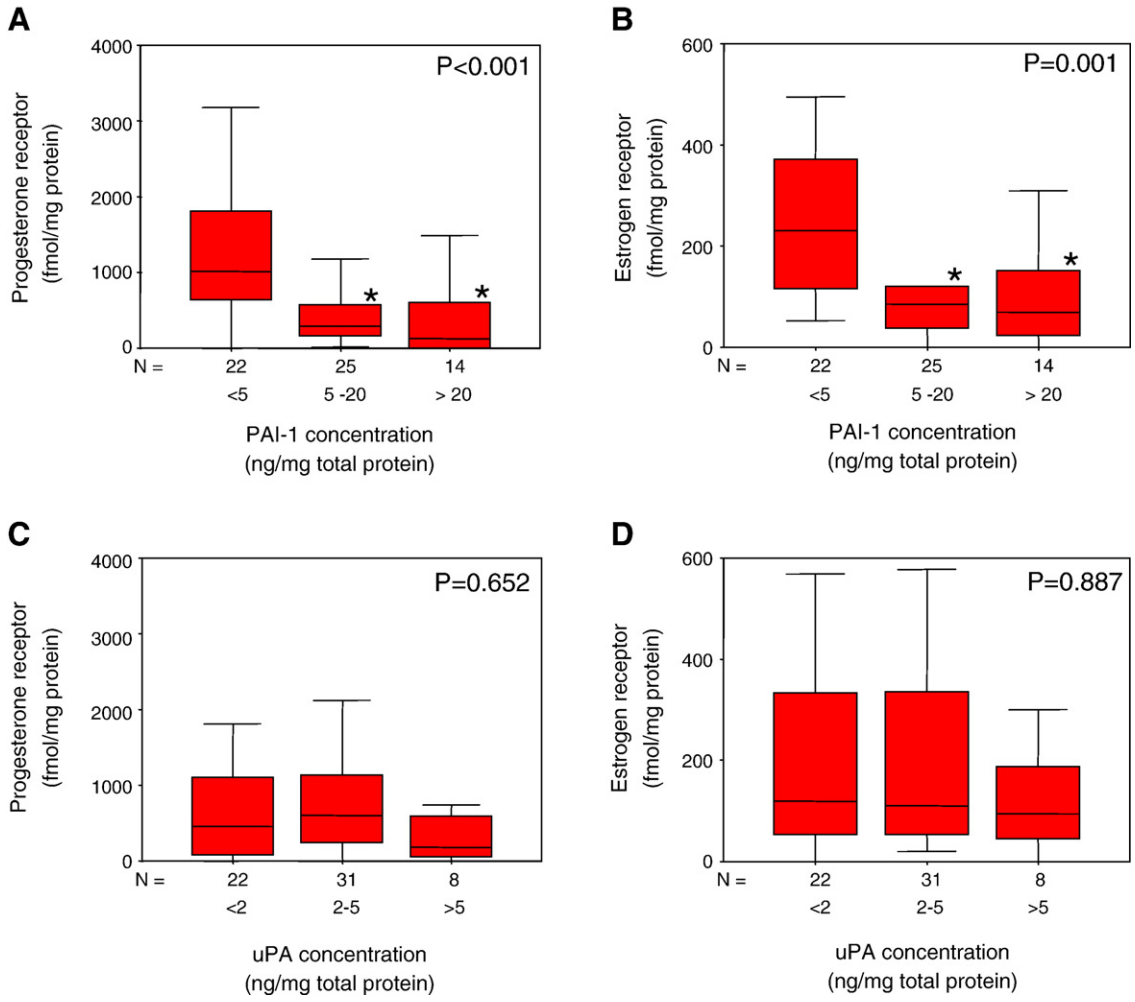

Fig. 5. Inverse association between cytosolic PAI-1 concentrations and biochemically determined progesterone (A) as well as estrogen (B) receptor concentrations ($p<0.001$ for the difference in progesterone receptor concentrations and $p=0.001$ for the difference in estrogen receptor concentrations for tumors with PAI-1 ≥ 5 ng/mg vs. PAI-1 < 5 ng/mg total protein). In contrast cytosolic uPA concentrations did not correlate with progesterone as well as estrogen receptor concentrations.

PAI-2 and high PAI-1 concentrations in tumors revealed a small group of FIGO-stage I and II patients with an increased risk of progression. It would be interesting to know, whether a triple-combination of PAI-1, PAI-2 and uPA allows an even better identification of high risk patients than only two of these factors. However, for statistical reasons we could not analyze interactions of three factors, due to the relatively small number of patients in our cohort.

In conclusion, we have identified uPA and PAI-1 as independent markers for shorter time to relapse in endometrial cancer. The combination of high cytosolic concentrations of uPA (>5 ng/mg total protein) and high PAI-1 (>20 ng/mg total protein) may reveal a group of patients with increased risk of progression.

Acknowledgments

The authors wish to thank Ms. Silke Hankinson for excellent organisation and management and Ms. Susanne Lindemann for excellent bibliographical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygyno.2007.11.025.

References

[1] Reuning U, Magdolen V, Hapke S, Schmitt M. Molecular and functional interdependence of the urokinase-type plasminogen activator system with integrins. Biol Chem 2003;384:1119–31.
[2] Bajou K, Maillard C, Jost M, et al. Host-derived plasminogen activator inhibitor-1 (PAI-1) concentration is critical for in vivo tumoral angiogenesis and growth. Oncogene 2004;23:6986–90.
[3] Han B, Nakamura M, Mori I, Nakamura Y, Kakudo K. Urokinase-type plasminogen activator system and breast cancer. Oncol Rep 2005;14:105–12 [Review].
[4] Buchwalter G, Gross C, Wasylyk B. The ternary complex factor Net regulates cell migration through inhibition of PAI-1 expression. Mol Cell Biol 2005;25:10853–62.
[5] Stefansson S, Lawrence DA. The serpin PAI-1 inhibits cell migration by blocking integrin alpha V beta 3 binding to vitronectin. Nature 1996;383:441–3.
[6] Harbeck N, Kates RE, Schmitt M, et al. Urokinase-type plasminogen

activator and its inhibitor type 1 predict disease outcome and therapy response in primary breast cancer. Clin Breast Cancer 2004;5:348–52.
[7] Vaughan DE. PAI-1 and cellular migration: dabbling in paradox. Arterioscler Thromb Vasc Biol 2002;22:1522–3.
[8] Cao C, Lawrence DA, Li Y, et al. Endocytic receptor LRP together with tPA and PAI-1 coordinates Mac-1-dependent macrophage migration. EMBO J 2006;25:1860–70.
[9] Chazaud B, Ricoux R, Christov C, Plonquet A, Gherardi RK, Barlovatz-Meimon G. Promigratory effect of plasminogen activator inhibitor-1 on invasive breast cancer cell populations. Am J Pathol 2002;160:237–46.
[10] Stahl A, Mueller BM. Melanoma cell migration on vitronectin: regulation by components of the plasminogen activation system. Int J Cancer 1997;71: 116–22.
[11] Creasman WT. Announcement. FIGO stages 1988 revisions. Gynecol Oncol 1989;1989:125–7.
[12] Scully RE, Poulson H, Sobin LH. International Classification and Histologic Typing of Female Genital Tract Tumours. New York: Springer; 1994 [p.].
[13] Kurman RJ, Zaino RJ, Norris HJ. Endometrial Carcinoma. In: Kurman RJ, editor. Blaustein's Pathology of the Female Genital Tract. New York: Springer; 1994. p. 439–86.
[14] Sevin B-U, Angioli R. Uterine Corpus: Multimodality Therapy in Gynecologic Oncology. New York: Thieme; 1996 [p.].
[15] Hausherr CK, Schiffer IB, Gebhard S, et al. Dephosphorylation of p-ERK1/2 in relation to tumor remission after HER-2 and Raf1 blocking therapy in a conditional mouse tumor model. Mol Carcinog 2006;45: 302–8.
[16] Carmo H, Hengstler JG, de Boer D, et al. Comparative metabolism of the designer drug 4-methylthioamphetamine by hepatocytes from man, monkey, dog, rabbit, rat and mouse. Naunyn Schmiedebergs Arch Pharmacol 2004;369:198–205.
[17] Carmo H, Brulport M, Hermes M, et al. Influence of CYP2D6 polymorphism on 3,4-methylenedioxymethamphetamine ('Ecstasy') cytotoxicity. Pharmacogenet Genomics 2006;16:789–99.
[18] Steiner E, Eicher O, Sagemuller J, et al. Multivariate independent prognostic factors in endometrial carcinoma: a clinicopathologic study in 181 patients: 10 years experience at the Department of Obstetrics and Gynecology of the Mainz University. Int J Gynecol Cancer 2003;13:197–203.
[19] Micke P, Hengstler JG, Ros R, et al. c-erbB-2 expression in small-cell lung cancer is associated with poor prognosis. Int J Cancer 2001;92:474–9.
[20] Micke P, Basrai M, Faldum A, et al. Characterization of c-kit expression in small cell lung cancer: prognostic and therapeutic implications. Clin Cancer Res 2003;9:188–94.
[21] Hengstler JG, Bolm-Audorff U, Faldum A, et al. Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. Carcinogenesis 2003;24:63–73.
[22] Jung D, Bolm-Audorff U, Faldum A, et al. Immunotoxicity of co-exposures to heavy metals: *In vitro* studies and results from occupational exposure to cadmium, cobalt and lead. EXCLI J 2003;2:31–44.
[23] Steiner E, Schmidt M, Weikel W, Koelbl H. Influence of diabetes mellitus and nodal distribution in endometrial cancer and correlation to clinicopathological prognostic factors. Eur J Gynaecol Oncol 2006;27: 477–80.
[24] Schneider J, Pollan M, Tejerina A, Sanchez J, Lucas AR. Accumulation of uPA–PAI-1 complexes inside the tumour cells is associated with axillary nodal invasion in progesterone-receptor-positive early breast cancer. Br J Cancer 2003;88:96–101.
[25] Duffy MJ, Maguire TM, McDermott EW, O'Higgins N. Urokinase plasminogen activator: a prognostic marker in multiple types of cancer. J Surg Oncol 1999;71:130–5.
[26] Ferno M, Bendahl PO, Borg A, et al. Urokinase plasminogen activator, a strong independent prognostic factor in breast cancer, analysed in steroid receptor cytosols with a luminometric immunoassay. Eur J Cancer 1996;32A: 793–801.
[27] Foekens JA, Buessecker F, Peters HA, et al. Plasminogen activator inhibitor-2: prognostic relevance in 1012 patients with primary breast cancer. Cancer Res 1995;55:1423–7.
[28] Grondahl-Hansen J, Christensen IJ, Rosenquist C, et al. High levels of urokinase-type plasminogen activator and its inhibitor PAI-1 in cytosolic extracts of breast carcinomas are associated with poor prognosis. Cancer Res 1993;53:2513–21.
[29] Janicke F, Schmitt M, Hafter R, et al. Urokinase-type plasminogen activator (u-PA) antigen is a predictor of early relapse in breast cancer. Fibrinolysis 1990;4:69–78.
[30] Taponeco F, Curcio C, Giuntini A, et al. Expression and prognostic significance of urokinase and plasminogen activator inhibitor type-1 in endometrial hyperplasia and cancer. J Exp Clin Cancer Res 2001;20:239–46.
[31] Osmak M, Babic D, Abramic M, Milicic D, Vrhovec I, Skrk J. Plasminogen activator inhibitor type 2: potential prognostic factor for endometrial carcinomas. Neoplasma 2001;48:462–7.
[32] Kohler U, Hiller K, Martin R, et al. Tumor-associated proteolytic factors uPA and PAI-1 in endometrial carcinoma. Gynecol Oncol 1997;66:268–74.
[33] Tecimer C, Doering DL, Goldsmith LJ, Meyer JS, Abdulhay G, Wittliff JL. Clinical relevance of urokinase-type plasminogen activator, its receptor and inhibitor type 1 in ovarian cancer. Int J Gynecol Cancer 2000;10:372–81.
[34] Hengstler JG, Lange J, Kett A, et al. Contribution of c-erbB-2 and topoisomerase IIalpha to chemoresistance in ovarian cancer. Cancer Res 1999;59: 3206–14.
[35] Tanner B, Grimme S, Schiffer I, et al. Nuclear expression of apurinic/apyrimidinic endonuclease increases with progression of ovarian carcinomas. Gynecol Oncol 2004;92:568–77.
[36] Tanner B, Hasenclever D, Stern K, et al. ErbB-3 predicts survival in ovarian cancer. J Clin Oncol 2006;24:4317–23.
[37] Spangenberg C, Lausch EU, Trost TM, et al. ERBB2-mediated transcriptional up-regulation of the alpha5beta1 integrin fibronectin receptor promotes tumor cell survival under adverse conditions. Cancer Res 2006;66: 3715–25.
[38] Trost TM, Lausch EU, Fees SA, et al. Premature senescence is a primary fail-safe mechanism of ERBB2-driven tumorigenesis in breast carcinoma cells. Cancer Res 2005;65:840–9.
[39] Schiffer IB, Gebhard S, Heimerdinger CK, et al. Switching off HER-2/neu in a tetracycline-controlled mouse tumor model leads to apoptosis and tumor-size-dependent remission. Cancer Res 2003;63:7221–31.
[40] Herrmann F, Lehr HA, Drexler I, et al. HER-2/neu-mediated regulation of components of the MHC class I antigen-processing pathway. Cancer Res 2004;64:215–20.
[41] Hengstler JG, Pilch H, Schmidt M, et al. Metallothionein expression in ovarian cancer in relation to histopathological parameters and molecular markers of prognosis. Int J Cancer 2001;95:121–7.
[42] Mohrmann G, Hengstler JG, Hofmann TG, et al. SPOC1, a novel PHD-finger protein: association with residual disease and survival in ovarian cancer. Int J Cancer 2005;116:547–54.
[43] Nordengren J, Fredstorp LM, Bendahl PO, et al. High tumor tissue concentration of plasminogen activator inhibitor 2 (PAI-2) is an independent marker for shorter progression-free survival in patients with early stage endometrial cancer. Int J Cancer 2002;97:379–85.