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Urokinase plasminogen activator and its inhibitor, PAI-1, in association with progression-free survival in early stage endometrial cancer

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Abstract

Components of the urokinase plasminogen activator (u-PA) system are involved in the metastatic process, and have accordingly been associated with clinical outcome in a variety of malignant tumours. We investigated the prognostic importance of u-PA and plasminogen activator inhibitor type 1 (PAI-1) in endometrial cancer, analysed with luminometric immunoassay (LIA) and enzyme-linked immunosorbent assay (ELISA), respectively. Two different cut-off levels were used: the median and the 80th percentile—the latter because of the low progression rate for patients with early stage (I-II) endometrial cancer. After a median follow-up time of 6.8 years, univariate analysis of patients with stage I–II disease ($n=188$) showed that high u-PA and high PAI-1 content was associated with a shorter progression-free survival (PFS), but at different cut-off levels, uPA at the median ($P=0.003$), and PAI-1 at the 80th percentile ($P<0.001$). Among the other factors, DNA ploidy status was most strongly correlated to PFS, followed by age (continuous), International Federation of Gynaecology and Obstetrics (FIGO) grade of differentiation, S-phase fraction and progesterone receptor (PgR) status. Bivariate analyses, including ploidy and one of the factors u-PA or PAI-1, showed that both add significant prognostic information. We conclude that u-PA and PAI-1 are promising prognostic factors in early stage endometrial cancer.

Keywords: Endometrial cancer; Urokinase plasminogen activator (u-PA); Plasminogen activator inhibitor type 1 (PAI-1); DNA ploidy status; Prognosis

1. Introduction

Endometrial carcinoma is one of the most common female malignancies. It is often diagnosed at an early stage, which probably is the reason why it is also one of the most curable gynaecological malignancies. Approximately 85% of all endometrial cancers are diagnosed in early surgical stages, with a 5-year survival of approximately 90% for patients in surgical stage I, and 70–80% for patients in stage II [1].

Decisions about adjuvant therapy are generally based on prognostic factors such as tumour stage, histopathological subtype, myometrial invasion, and degree of tumour differentiation. Other factors, e.g. DNA ploidy status, nuclear grade, oestrogen (ER) and progesterone receptors (PgR), S-phase fraction, HER-2/neu, and p53, as well as age at diagnosis have also been reported to be predictors of outcome (for review, see Refs. [2,3]). There is, however, no consensus about the use of prognostic factors or agreement about if or when to give adjuvant treatment to patients with surgical stage I–II disease [4].

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Another putative prognostic factor is the urokinase plasminogen activator (u-PA), first described in cultures of ovarian tumour tissue [5]. u-PA converts plasminogen to plasmin, which subsequently activates a cascade of proteolytic enzymes and results in degradation of fibronectin, laminin and collagen in basement membranes and extracellular matrix. These events are a prerequisite for tumour cell invasion and metastasis. The activity of u-PA is regulated by specific plasminogen activator inhibitors, PAI-1 and PAI-2 (for review, see Ref. [6]. Components of the u-PA system have prognostic importance in several malignancies (for review, see Ref. [7]), most notably breast cancer [8–12], but also gastric cancer [13,14], squamous cell lung cancer [15,16], renal cancer [17] and cancer of the uterine cervix [18]. In endometrial cancer, there are only a few reports regarding the u-PA system. The concentrations of u-PA and its inhibitors, PAI-1 and PAI-2, were higher in cancer tissue than in normal endometrial tissue [19–23]. Furthermore, increased plasma levels of u-PA as well as other components of the fibrinolytic system were found in patients with endometrial cancer when compared with an age-matched healthy control group [24]. To our knowledge, the prognostic importance of u-PA and PAI-1 has only been investigated in one small study, concluding that elevated u-PA and PAI-1 levels appear to correlate with unfavourable prognosis [25].

The aim of this study was to evaluate the prognostic importance of the tumour tissue contents of u-PA and PAI-1 with special reference to early endometrial cancer, and to relate the results to other known prognostic factors.

2. Patients and methods

2.1. Patients and samples collection

Between January 1988 and September 1990, tumour tissue samples were collected from 293 patients (age 38–92 years, median 66 years) with endometrial cancer referred to the Department of Gynaecological Oncology, University Hospital, Lund, for preoperative clinical staging and a second curettage 2–4 weeks after the diagnostic curettage. This number represents approximately two-thirds of all patients diagnosed with endometrial cancer in the Southern Health Care Region of Sweden (1.5 million inhabitants) during this time period. The tissue from the second curettage was mixed and divided into two parts. One part was used for the histopathological diagnosis, and the other part was frozen for additional analyses (see below).

Verification of cancer cells in the frozen samples was performed on imprints, prior to cytosol preparation, or on drops from the nuclear suspensions used for flow cytometric DNA analysis (see below). Cancer cells/nuclei were found in 274 of the samples (imprints or drops). The remaining 19 cases were excluded from further analyses.

Concerning associations between u-PA, PAI-1 and other prognostic factors, all patients in the study were included ($n=274$). However, when studying the prognostic importance of different factors, only patients in surgical stage I–II ($n=112$), and patients without cancer left after preoperative radiation ($n=76$) were included. The reason, for studying patients in stage I–II only, is that the presence of extrauterine disease itself is a strong adverse prognostic factor. Furthermore, if the prognostic factors are to be used to select patients for adjuvant therapy, this will be restricted to patients with stage I-II disease. 86 patients were thus excluded for the following reasons: not operated upon ($n=23$), surgical stage III-IV ($n=29$), not surgically staged ($n=2$), prior or concomitant additional carcinomas ($n=39$), or no follow-up data ($n=3$). 10 patients were excluded for more than one of these reasons.

2.2. Staging and histopathology

A preoperative clinical staging and a retrospective surgical staging (no lymph node exploration was done), based on review of patient records, were performed according to International Federation of Gynaecology and Obstetrics (FIGO) [26,27]. Histopathological grading according to the Word Health Organization (WHO) [28] and FIGO [27], and histological subtype, according to WHO [29], were reviewed by our pathologist.

2.3. Treatment

For patients with clinical stage I-II, the most commonly used treatment was two intra-uterine Heymann packings with radium separated by a 3-week interval with the aim of delivering 30 Gy at a distance of 1.5 cm from the mucosal surface, and three vaginal treatments with after-loaded cobolt sources (Selectron), delivering 6.33 Gy at a distance of 5 mm from the surface of the vaginal cylinder, followed by operation (total hysterectomy and bilateral oophorectomy) 6–8 weeks later. Lymph nodes were not routinely explored. Patients with myometrial invasion, exceeding the inner half of the myometrium or poorly differentiated tumours, or advanced stages received adjuvant chemotherapy once weekly (vinblastine (V) 3 mg, epirubicin (E) 30 mg, and cyclophosphamide (C) 300 mg—VEC) for 9 weeks.

In April 1989, a new treatment programme started. Patients in clinical stage I with low risk factors (well or moderately differentiated endometrioid cancer) were primarily operated upon. If the pathological examination showed tumour invasion exceeding 50% of the myometrium, the patients received adjuvant pelvic external radiation (40 Gy, mid-pelvic dose, two-field technique), two vaginal treatments as described above,

and 12 weekly doses with VEC as described above. Patients in clinical stage I with risk factors indicating an aggressive tumour type (poorly differentiated endometrioid cancer and all grades of seropapillary, clear cell, adenosquamous and undifferentiated cancer), and all patients in clinical stage II, were randomised to preoperative intrauterine Heymann packings as above (during 1989, the radium sources were changed to three afterloading cesium sources administered weekly) versus preoperative chemotherapy with three weekly cycles of VEC. Both randomisation groups then received adjuvant treatment with 12 weekly cycles of postoperative VEC. The results of this programme have not yet been published. Patients with stage III-IV were treated with individualised therapy.

2.4. Analytical methods

The concentrations of u-PA and PAI-1 were analysed in cytosol samples. In 121 of the cases (Part A), the cytosols were prepared in 1988–1989 for ER and PgR analyses, whereafter the remaining cytosols were kept frozen at −80 °C. The samples were homogenised with an all-glass Potter-Elvehjem homogeniser in a buffer (10 mM Tris, 1.5 mM EDTA, 5.0 mM Na_2MoO_4, 1 mM monothioglycerol final concentrations, pH 7.4) and centrifuged at 20 000*g*. The centrifugation force was changed to 105 000*g* after the first 54 tissue samples. The proportion of cases classified as high and low in the subgroups based on the centrifugation force was not significantly different for u-PA ($P = 0.25$) and PAI-1 ($P = 0.40$). For 152 of the remaining 153 cases ((Part B) for one of the samples, there was only enough tissue for the flow cytometric (FCM) DNA analysis), the analyses were performed in newly prepared cytosols from frozen tissue samples from our tumour bank. For these 152 samples, the homogenisation technique was changed to a microdismembrator and the *g* value was 105 000.

- *ER and PgR* were analysed with an enzyme immunoassay according to the kit instructions (Abbott Laboratories, Diagnostic Division, Chicago, IL, USA).
- *u-PA* measurements were performed with a luminometric immunoassay, with monoclonal antibodies (both catching and detecting) according to the kit instructions (LIA-mat® u-PA test, AB Sangtec Medical, Bromma, Sweden). This assay detects u-PA in different forms: the proenzyme (pro-u-PA), the active form (u-PA), u-PA bound to its receptor (u-PAR), and in a complex with one of its inhibitors (PAI-1). The detection limit was approximately 5 pg u-PA/ml cytosol. The between-run coefficient of variation (CV) was 7%.
- *PAI-1* was measured with a sandwich enzyme-linked immunosorbent assay (ELISA) with a monoclonal catching and a polyclonal detecting antibody according to the kit instructions (Monozyme®, Copenhagen, Denmark). PAI-1 was detected both in its active and latent form, and also in complex with the tissue plasminogen activator (t-PA) and u-PA. The detection limit was approximately 25 pg PAI-1/ml cytosol. The intra- and interassay was below 11% [11].
- *Flow cytometric (FCM) DNA analysis* was performed as previously described in Refs. [30,31] using an Ortho Cytofluorograph 50H system (Ortho Instruments, Westwood, MA, USA), equipped with a 4W argon ion laser (Model 95, Lexel Coro, Palo Alto, CA, USA) after staining with propidium iodide. Tumours with one DNA stemline were designated as diploid and those with two or more DNA stemlines as non-diploid [32]. The fraction of nuclei in the S-phase was calculated according to Baisch and co-workers [33].
- *Cut-off values:* Based on a large number of studies on the prognostic relevance of u-PA and PAI-1 in non-endometrial cancers, our hypothesis was that high levels of u-PA and PAI-1 were related to short survival, or equivalently that u-PA and PAI-1 might be useful for selection of patients at high risk of progression. The prognostic importance of u-PA and PAI-1 was evaluated at two different cut-offs: the median and 80th percentile. The latter was chosen because of the low progression rate for patients with endometrial cancer stage I-II. Due to methodological changes (see above), we had to use different cut-off values for both u-PA and PAI-1 in the two parts of the study (parts A and B). The concentrations of ER, PgR, u-PA and PAI-1 were expressed per mg cytosol protein [34]. ER and PgR were routinely analysed in part A with a cut-off of 25 fmol/mg protein [35]. These analyses were not performed in part B. For the S-phase fraction, both median (7.7%) and 80th percentile (12%) cut-off were evaluated. All cut-offs were calculated in the subgroup of patients with stage I-II disease ($n = 188$).

2.5. Follow-up

The follow-up consisted of clinical examination every 3 months for the first half-year, then once or twice every year for the following 5 years. After that, the follow-up was individualised. A clinically evident progression was confirmed by biopsy and/or other relevant diagnostic procedures and was defined as the appearance of new lesions of endometrial cancer in patients with no previous clinical evidence of disease after the primary treatment. The median follow-up time for the 142 patients with stage I–II alive and progression-free at the last visit was 6.8 years (range: 0.7–9.9 years). Only 2

patients had a follow-up time of less than 2 years. Time to progression was chosen as endpoint. 4 patients were registered as dead of endometrial cancer without information about the date of progression. These patients were considered to have had progression at the time of death.

2.6. Statistics

The software package Stata Version 6.0 [36] was used to perform all the statistical analyses. Associations between categorical factors were evaluated using the Fisher Exact test, or where appropriate Chi-squared tests for trend. Spearman's rho was used as a measure of correlation between continuous factors. Univariate analysis of progression-free survival (PFS) was performed using the Kaplan–Meier method, the log rank test and the Cox proportional hazard models. The latter model was also used for multivariate analysis. Due to the low number of events (23 progressions), we decided not to fit Cox models with more than two covariates [37]. Kaplan–Meier curves were truncated when less than 5 patients remained in the corresponding risk set. Proportional hazards assumptions were checked graphically and by means of Schoenfelds test [38]. All tests were two-sided and the significance level was set to 5%, corresponding to 95% confidence intervals (CI).

3. Results

3.1. Tumour tissue content of u-PA and PAI-1

The median (and ranges) concentrations of u-PA in the two parts of the study were 0.29 (0.02–5.21) and 0.14 (0.00–1.45) ng/mg protein, respectively. The corresponding figures for PAI-1 were 0.76 (0.09–26.7) and 0.47 (0.09–7.94) ng/mg protein. The Spearman rank correlations between u-PA and PAI-1 in the two parts were 0.19 (Part A) and 0.08 (Part B), respectively. A high u-PA content was significantly associated with a high PAI-1 when the 80th percentiles were used as the cut-off level ($P=0.009$; Table 1), but not when using the median cut-offs ($P=0.33$).

3.2. Associations between u-PA/PAI-1, and other clinical, histopathological and cell biological factors

With the 80th percentiles as cut-offs, u-PA was not associated with the other factors, whereas a high PAI-1 content was associated with higher clinical and surgical stages, a lower degree of differentiation (WHO and FIGO), ER and PgR negativity, and a high S-phase fraction. No association was found between PAI-1 and DNA ploidy status (Table 1). When instead the median values were used as cut-off levels, similar results were obtained, with one exception: u-PA was now significantly associated with ER status.

3.3. Association between progression-free survival (PFS) and u-PA, PAI-1 and other variables

3.3.1. Univariate analyses

At the last follow-up, 23 of the 188 patients (12%) with endometrial cancer stage I–II had documented progression (4 vaginal, 15 regional or distant, and 4 unknown (died with endometrial cancer without a date of progression)). Progression rates for the different histological subtypes were 20/164 (endometrioid), 1/10 (clear cell), 1/5 (serous), 0/8 (adenosquamous) and 1/1 (undifferentiated).

In order to investigate the importance of different cut-off levels (median and 80th percentile), the samples were categorised in three subgroups: <50th, 50–79th and $\geqslant 80$th percentiles (Fig. 1a and b). The PFS curves in Fig. 1a indicate that for u-PA, the two subgroups of patients with values above the median value had a similar prognosis, which was worse than that for patients with u-PA values below the median. In addition, univariate analysis revealed that patients with high u-PA concentrations in their tumours, using the median value as a cut-off point, had a significantly worse PFS compared with patients with low u-PA tumours (hazard ratio (HR) = 6.4 (95% CI: 1.9–22), $P=0.003$, Table 2). The estimated PFS at 5 years for the two groups was 84% (95% CI: 75–90%) versus 96% (95% CI: 89–99%). When instead the 80th percentile was used as cut-off level, the prognostic strength of u-PA was weaker ($P=0.14$).

For PAI-1, a different pattern was found. Fig. 1b indicates that the 80th percentile rather than the median discriminates patients with worse prognosis, since the two subgroups with the PAI-1 values <80th percentile had a similar and considerably better prognosis than patients with PAI-1 values $\geqslant 80$th percentile. Univariate analyses (Table 2) supported this conclusion, since the prognostic value of PAI-1 was significant using the 80th percentile as a cut-off (HR = 5.0 (95% CI: 2.2–11), $P<0.001$), but not when using the median as a cut-off ($P=0.10$). The 5-years PFS for the two subgroups based on the 80th percentile cut-off were 73% (95% CI: 55–84%) and 94% (95% CI: 88–97%), respectively.

Furthermore, it should be noted that no progressions were found among the 35 cases (45%) with the lowest u-PA levels in Part A ($n=78$) and the corresponding 42 cases (41%) with the lowest u-PA levels in Part B ($n=103$). For PAI-1, the corresponding numbers of progression-free cases were 11 in Part A and 20 in Part B.

Among the other factors, DNA ploidy status was most strongly correlated with PFS, followed by age (continuous), FIGO grade of differentiation (3 versus 1), S-phase fraction (80th percentile), and PgR (Table 2).

S-phase fraction was not associated with PFS at the median cut-off.

In this study, 87% of the patients had common endometrioid adenocarcinoma. The univariate prognostic value for u-PA in this subgroup (80th percentile cut-off) was somewhat stronger than when all histopathological subgroups were included ($P=0.04$ versus $P=0.14$). The other univariate analyses (u-PA and PAI-1, median values as cut-offs, and PAI-1, 80th percentile as cut-off) for this subgroup showed results similar to those when all histopathological subgroups were included.

3.3.2. Dependency on the duration of follow-up

Hazard ratios for u-PA and PAI-1 were estimated, under the assumption of proportional hazards, using

Table 1
Associations between u-PA/PAI-1 and other prognostic factors in endometrial cancer

		u-PA		PAI-1	
Category	*n* (%)	% high[a]	*P* value	% high[a]	*P* value
Age (years)					
<55	42 (15)	15		19	
55–69	128 (47)	18		19	
>69	104 (38)	23	0.22[b]	30	0.07[b]
Clinical stage					
I–II	254 (93)	19		21	
III–IV	18 (7)	22	0.76	56	0.002
Surgical stage (FIGO)					
No tumour after irradiation	76 (28)	21		24	
I–II	144 (53)	20		16	
III–IV	29 (11)	17		45	
Not operated	23 (8)	13	0.89	39	0.002
Histopathological subtypes (WHO)					
Adenocarcinoma	238 (87)	19		22	
Clear cell carcinoma	14 (5)	29		36	
Squamous cell carcinoma	0 (0)	–		–	
Adenosquamous carcinoma	12 (4)	17		17	
Serous carcinoma (UPSC)	9 (3)	11		44	
Undifferentiated carcinoma	1 (0)	–	–	–	–
Degree of differeniation (WHO					
Well	65 (24)	16		11	
Moderately	109 (40)	19		16	
Pooly	100 (36)	21	0.40[b]	39	<0.001[b]
Grade of differentiation (FIGO)					
1	175 (64)	18		14	
2	45 (16)	14		29	
3	53 (19)	28	0.16[b]	49	<0.001[b]
ER					
High	78 (64)	21		14	
Low	43 (36)	17	0.63	37	0.006
PgR					
High	76 (63)	19		15	
Low	45 (37)	20	1.00	36	0.012
DNA ploidy					
Diploid	205 (76)	19		21	
Non-diploid	64 (24)	19	1.00	29	0.23
S-phase fraction					
Low	195 (75)	18		15	
High	64 (25)	22	0.46	45	<0.001
u-PA					
Low	216 (81)	–		20	
High[a]	51 (19)	–	–	37	0.009
PAI-1					
Low	209 (77)	16		–	
High[a]	63 (23)	31	0.009	–	–

WHO, World Health Organization; FIGO, International Federation of Gynaecology and Obstetrics; ER, oestrogen receptor; PgR, progesterone receptor; u-PA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor type 1.

[a] 80th percentile in the subsample used for analysis of progression-free survival.

[b] Test for linear trend.

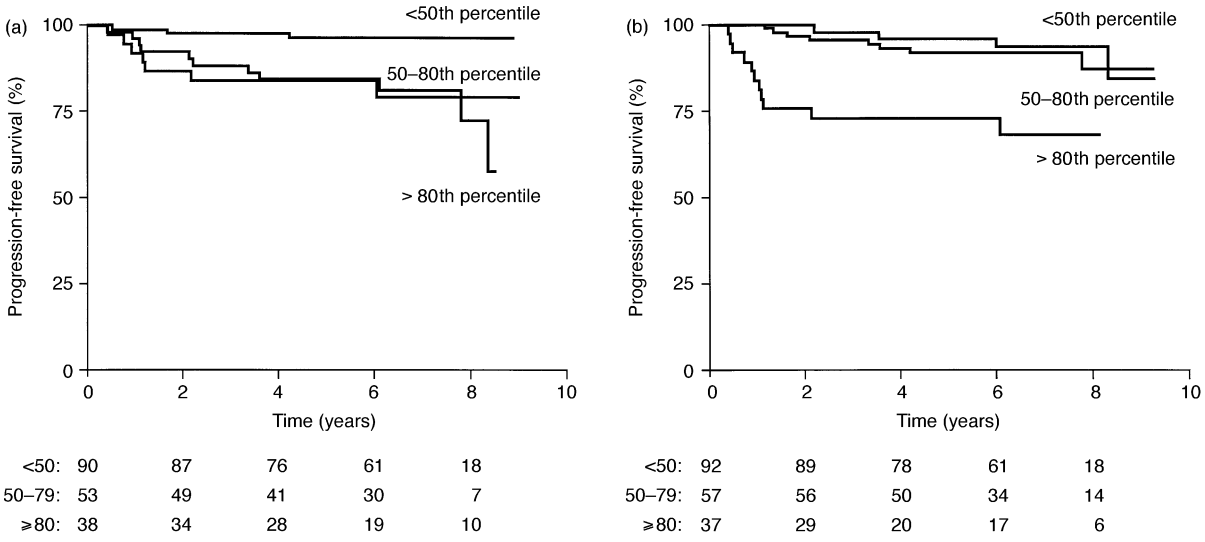

Fig. 1. Progression-free survival of 188 patients with endometrial cancer, stage I–II, in relation to (a) u-PA (urokinase plasminogen activator) and (b) PAI-1 (plasminogen activator inhibitor type 1). The number of patients at risk in each subgroup is given below each graph.

Cox models. Thus, we assume that the hazard ratio for, e.g. high versus low levels, is constant independent of time. However, a graphical check indicates that the prognostic value of some of the factors is restricted to the first few years after surgery. This observation was verified by the Schoenfelds test [37], which revealed marginally significant departures from proportional hazards for PAI-1 ($P=0.045$, cut-off: 80th percentile) and continuous age ($P=0.045$), but not for u-PA (median ($P=0.61$)) and ploidy status ($P=0.85$). The time-dependent hazard ratio for PAI-1 is fairly obvious in Fig. 1b, but it is even more obvious if we divide the time axis at 2 years and study the estimated effects before and after the break point. The hazard ratio for the first time interval (12 events) is highly significant ($P<0.001$), whereas that for the second interval (11 events) is far from significant ($P=0.66$, Table 3). DNA ploidy status and u-PA, however, showed significant prognostic value during both time periods (Table 3).

3.3.3. Bivariate analyses

The univariate analyses revealed that PAI-1 (80th percentile), DNA ploidy status, and u-PA (median) were the strongest prognostic factors. The importance of these factors was therefore further studied in multivariate analyses. Due to the low number of progressions ($n=23$), we choose to include only two factors in each analysis. Bivariate Cox regression showed that u-PA was a strong prognostic factor for PFS also after adjustment for ploidy status (Table 4). Fifty percent of the patients in the group with high u-PA and non-diploid tumours ($n=20$) had a documented progression, which should be compared with the lower progression rates in the other subgroups: 3% (low u-PA/diploid, $n=71$), 6% (low u-PA/non-diploid, $n=18$), and 11% (high u-PA/diploid, $n=70$). In addition, PAI-1 was found to be a significant prognostic factor after adjustment for ploidy status (Table 4). The progression rate among patients with low PAI-1 and DNA diploid tumours ($n=119$) was 7% compared with 78% for those 9 patients with high PAI-1 and DNA non-diploid tumours. The progression rates for the other two groups were 14% (low PAI-1 and DNA non-diploid, $n=29$) and 15% (high PAI-1 and DNA diploid, $n=26$). A combination of u-PA and PAI-1 identified a group (high u-PA and high PAI-1) with a progression rate of 50% (10/20). The progression rates for the other three groups were: 2/74 (low/low), 1/15 (low/high), and 8/71 (high/low). In addition, u-PA and PAI-1 were found to be independent factors in a third bivariate analysis (Table 4).

Bivariate analyses for the histopathological subgroup endometrioid adenocarcinoma showed similar results as for all the subgroups together.

4. Discussion

Our investigation shows that the u-PA and PAI-1 content of tumour tissue extracts, as determined by LIA and ELISA, respectively, are associated with the PFS of patients with early-stage endometrial carcinoma (surgical stage I–II). These techniques have previously been validated in cytosols from breast cancer for the analysis of u-PA and PAI-1 [11,12]. The associations between PFS and u-PA and PAI-1, respectively, were comparable to that of the DNA ploidy status, which has been previously shown to be a strong prognostic factor. Our data on DNA ploidy status thus confirms previous reports from our group [39,40] and others [41–44]. Due to the prognostic independence of u-PA and PAI-1 from DNA ploidy status, combinations of each of them with

Table 2
Univariate analysis of prognostic factors for progression-free survival of patients with endometrial cancer stage I + II

Category	*n*	% Progressions	Hazard ratio (95% CI)	*P* value
Age	188	–	1.06 (1.02–1.11)	0.007
Age (years)				
< 55	33	6	1.0	
55–69	96	10	1.8 (0.4–8.2)	0.4
> 69	59	19	4.5 (1.0–21)	0.05
Degree of differentiation (WHO)				
Well	47	9	1.0	
Moderately	85	12	1.5 (0.5–4.7)	0.5
Poorly	56	16	2.3 (0.7–7.5)	0.2
Grade of differentiation (FIGO)				
1	130	9	1.0	
2	22	9	1.0 (0.2–4.7)	1.0
3	35	23	3.1 (1.2–7.5)	0.01
DNA ploidy				
Diploid	146	8	1.0	
Non-diploid	39	28	4.3 (1.9–9.9)	0.001
S-phase fraction				
Low	143	10	1.0	
High	39	23	2.6 (1.1–6.0)	0.03
ER				
High	59	15	[a]	
Low	23	0		0.06[b]
PgR				
High	56	16	[a]	
Low	26	0		0.04[b]
u-PA[c]				
Low	90	3	1.0	
High	91	20	6.4 (1.9–22)	0.003
PAI-1[d]				
Low	149	8	1.0	
High	37	30	5.0 (2.2–11)	< 0.001

CI, confidence interval; WHO, World Health Organization; FIGO, International Federation of Gynaecology and Obstetrics; ER, oestrogen receptor; PgR, progesterone receptor; u-PA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor type 1.

[a] The hazard ratio is not defined when the number of progressions is zero in one of the groups.

[b] *P* value corresponding to a log-rank test.

[c] Median value.

[d] 80th percentile value.

ploidy status in a prognostic index might result in a more refined prognostic instrument for the individualised treatment of patients having different risks of progression.

The prognostic importance of u-PA and PAI-1 was dependent on the cut-off level. When using the median value as cut-off, u-PA was a strong prognostic factor for PFS in univariate analyses and an independent prognostic factor for PFS after correction for ploidy status. In a corresponding analysis including PAI-1 and ploidy status, PAI-1 was not an independent prognostic marker. When instead using the 80th percentile as a cut-off, PAI-1 was a stronger prognostic factor than u-PA for PFS, and PAI-1 was also an independent prognostic factor for PFS in a bivariate analysis adjusting for ploidy status. To our knowledge only one small unpublished study ($n = 92$, including all stages) has previously shown that elevated levels of u-PA and PAI-1 were associated with an unfavourable prognosis in endometrial cancer [25]. In contrast to the results obtained in our study, they found a correlation between u-PA and histology, grade or PgR. Differences in the definition of histology, analytical methods used for PgR, and statistical methods, and also the small number of cases may explain these contradictory findings. Additional studies are needed in order to confirm the prognostic importance of u-PA and PAI-1 in endometrial cancer, and to clarify the optimal cut-off levels for these factors. Such studies should also consider myometrial invasion, which is a generally accepted prognostic factor in endometrial cancer [45–47], but could not be evaluated in this study since a considerable portion of the patients was treated with preoperative radiation. In our planned confirmative retrospective study, all patients have been primarily treated with surgery, and myometrial invasion has been examined. A majority of the patients (133/188) included in this series received preoperative therapy (radiation or

cytotoxic), and the analysis of the prognostic importance of u-PA and PAI-1 was, therefore, not meaningful in the small ($n = 55$) subset of patients treated with surgery only.

We found fewer tumours with well and moderate differentiation (WHO) compared with Nordström and colleagues [48]. This may be explained by the fact that approximately one-third of all patients, operated upon in this region, were not referred to our hospital and thus not included in this study. The reason for not referring them to us may be that they represent less aggressive tumours (well and moderately differentiated). Heterogeneity within the tumour, together with the fact that the grading to a certain degree is subjective, could also explain the discrepancy and emphasises the need for more objective prognostic markers. A striking observation was that with the exception of DNA ploidy status, PAI-1 (median cut-off) was strongly associated to all the other factors, whereas u-PA only was associated to ER. These results were principally the same when using the 80th percentile as a cut-off, with two exceptions; u-PA was now associated with PAI-1, but not to ER.

Table 3
The time-dependency of prognostic strength of DNA ploidy status[a] u-PA[b] and PAI-1[c] for progression-free survival

Factor	Time interval		
	0–2 year HR (95% CI)	2–10 years HR (95% CI)	0–10 years HR (95% CI)
DNA ploidy	4.0 (1.3–12)	4.7 (1.4–16)	4.3 (1.9–9.9)
u-PA	4.6 (1.0–21)	10 (1.3–81)	6.4 (1.9–22)
PAI-1	14 (3.8–52)	1.4 (0.3–6.5)	5.0 (2.2–11)
Number of progressions	12	11	23

HR, hazard ratio; CI, confidence interval; WHO, World Health Organization; FIGO, International Federation of Gynaecology and Obstetrics; ER, oestrogen receptor; PgR, progesterone receptor; u-PA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor type 1.

[a] Non-diploid versus diploid.
[b] Median cut-off.
[c] 80th percentile cut-off.

Table 4
Bivariate analyses of progression-free survival based on combinations of DNA ploidy status[a], u-PA[b] and PAI-1[c]

Variable	Hazard ratio (95% CI)	*P* value
DNA ploidy	5.1 (2.2–12)	<0.001
u-PA	6.5 (1.9–22)	0.003
DNA ploidy	4.7 (2.0–11)	<0.001
PAI-1	5.5 (2.4–13)	<0.001
u-PA	6.8 (2.0–23)	0.002
PAI-1	6.7 (2.8–16)	<0.001

CI, confidence interval; u-PA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor type 1.

[a] Non-diploid versus diploid.
[b] Median cut-off point.
[c] 80th percentile cut-off.

The time dependency of the prognostic factors has previously been demonstrated in breast cancer, usually with a decrease in the prognostic importance over time [49]. The present study found that the PAI-1 (cut-off at the 80th percentile) was a significant prognostic factor only during the first 2 years of follow-up. However, DNA ploidy status and u-PA were significant prognostic factors also after 2 years of follow-up.

There is no accepted standard treatment for early endometrial cancer [50,51]. Even though surgery is universally accepted, the addition of adjuvant treatment is controversial. One of the main purposes for investigations of prognostic factors is to provide support for decisions regarding adjuvant therapy (pre- or post-operative radiotherapy, systemic hormonal therapy and/or chemotherapy). Adjuvant therapies for endometrial cancer sometimes have pronounced side-effects and it is important not to overtreat patients and to be aware of the risks and benefits of adjuvant therapy [4]. Although not supported by randomised studies, the most commonly used adjuvant treatment is radiotherapy, and the complications resulting are not negligible. Despite this, many treatment programmes recommend adjuvant external-beam irradiation in up to more than 50% of patients with stage I endometrial cancer. This figure should be compared with the rate of locoregional recurrences (<25%) in this subgroup. The importance of u-PA and PAI-1 for selecting a group for local treatment could not be investigated in this study since only 4 patients developed local recurrences. It should also be mentioned that a localised vaginal metastasis in a previously not irradiated patient is highly treatable by radiation. If a group with a high risk to develop distant progressions could be identified, this would be a suitable group to evaluate the efficiency of adjuvant endocrine and/or cytotoxic treatment in clinical trials.

u-PA may also be useful in the identification of a real low-risk group, not in need of any adjuvant therapy, since none of the patients with the lowest u-PA values (about 40% of the patients in each of the two parts) had any progressions registered. In this study, the total contents of both u-PA and PAI-1 in the cytosol were analysed. Future studies should be designed in order to investigate whether the analyses of free and complex-bound u-PA and PAI-1, separately, in the cytosol or plasma will be associated with PFS. Indeed, a significant association between the concentration of u-PA:PAI-1 complexes in breast cancer tumour extracts and patient survival was recently published [52].

In conclusion, both PAI-1 and u-PA are promising prognostic factors in endometrial cancer and may, thus, be useful for this purpose, together with other known prognostic factors (e.g. grade, myometrial invasion and

DNA ploidy status). The prognostic importance of PAI-1 and u-PA needs, however, to be confirmed in additional investigations in which the optimal cut-off level should also be determined.

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