

# Association of Urokinase-Type Plasminogen Activator and Its Inhibitor with Disease Progression and Prognosis in Ovarian Cancer<sup>1</sup>

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## ABSTRACT

**Purpose:** Urokinase-type plasminogen activator (uPA) and its inhibitor, plasminogen activator inhibitor (PAI)-1, have been shown to be related to poor prognosis in a variety of malignant solid tumors. Studies on the prognostic relevance of uPA and PAI-1 in ovarian cancer, however, have been inconclusive. The current study tests the hypothesis that elevated expression of uPA and PAI-1 is associated with prognosis and disease progression.

**Experimental Design:** uPA and PAI-1 were prospectively measured by quantitative ELISA in tumor samples from 103 ovarian cancer patients (82 primary invasive epithelial carcinomas, 9 low malignant potential tumors, and 12 recurrent ovarian carcinomas).

**Results:** uPA but not PAI-1 levels were consistently associated with malignant progression, with levels increased from low malignant potential tumors to primary tumors (uPA,  $P = 0.04$ ; PAI-1,  $P = 0.019$ ), from early to advanced disease stages (uPA,  $P = 0.014$ ; PAI-1,  $P = 0.23$ ), and from primary to intra-abdominal metastatic tumors (uPA,  $P = 0.001$ ; PAI-1,  $P = 0.16$ ). High uPA and PAI-1 levels were associated with residual tumor volumes of  $>1$  cm ( $P = 0.001$  and  $P = 0.016$ , respectively). Among invasive International Federation of Gynecologists and Obstetrician stages I–IV tumors, elevated levels of uPA ( $>5.5$  ng/mg) and PAI-I

( $>18.8$  ng/ml) were associated with a shortened progression-free survival (uPA,  $P = 0.003$ ; PAI-1,  $P = 0.039$ ) and overall survival (uPA,  $P = 0.0002$ ; PAI-1,  $P = 0.007$ ). In multivariate analysis, uPA retained prognostic independence for progression-free survival ( $P = 0.037$ ) and overall survival ( $P = 0.006$ ).

**Conclusions:** These data suggest that the uPA/PAI-1 axis may play an important role in the intra-abdominal spread and reimplantation of ovarian cancer cells. The prognostic relevance of uPA and PAI-1 supports their possible role in the malignant progression of ovarian cancer.

## INTRODUCTION

Ovarian cancer is the leading cause of death from gynecological malignancies and the fourth leading cause of cancer deaths among American women. Little is known about the molecular biology underlying the metastatic process of intra-abdominal dissemination in ovarian cancer. Invasion and metastasis of solid tumors requires proteolytic enzymes that degrade the extracellular matrix and basement membranes (1). Among the proteases involved are the plasminogen activators, of which uPA<sup>4</sup> and/or its inhibitor, PAI-1, have been suggested to play a central role (2–4). Binding of uPA to the uPA receptor (CD87) activates the protease and catalyzes the conversion of plasminogen to plasmin, which subsequently activates type IV collagenase (3), or directly degrades extracellular matrix proteins such as fibrin, laminin, laminins, and proteoglycans (4). The enzymatic activity of uPA is regulated by the plasminogen activator inhibitors, PAI-1 and PAI-2 (5, 6). Both uPA and PAI-1 have been associated with disease outcome as statistically independent prognostic markers in breast (7–9), lung (10), colon (11), kidney (12), and gastrointestinal (13) cancers.

In ovarian cancer, significantly elevated uPA and PAI-1 levels have been described (14–16), however, studies analyzing the clinical impact of uPA and PAI-1 in ovarian cancer have reported inconclusive results, with studies either claiming prognostic importance of PAI-1 (17, 18) or uPA (19), or demonstrating no prognostic relevance for either uPA or PAI-1 (16). The studies with significant results were either performed with patient subsets of advanced disease stages only (17, 18), or without multivariate analysis (19). On the basis of these limitations, the current study was designed with the objective of analyzing the prognostic relevance of uPA and PAI-1 on PFS or OS in uni- and multivariate analyses among patients with all

Received 10/23/00; revised 1/16/01; accepted 3/13/01.

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<sup>1</sup> Supported in part by Ernst and Berta Grimmke-Stiftung, Düsseldorf, Germany, and by Department of Defense Grant DAMD17-00-1-9503.

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<sup>4</sup> The abbreviations used are: uPA, urokinase-type plasminogen activator; PAI, plasminogen activator inhibitor; FIGO, International Federation of Gynecology and Obstetrics; PFS, progression-free survival; OS, overall survival; LMP, low malignant potential.

Table 1 Patient and disease characteristics in invasive primary epithelial ovarian cancers ( $n = 82$ )

	N	Percentage
Age		
Median	56	
Range	34–82	
Stage		
FIGO I	12	14.6
FIGO II	6	7.3
FIGO III	50	61.0
FIGO IV	14	17.1
Histology		
Serous	53	64.6
Mucinous	10	12.2
Endometrioid	15	18.3
Undifferentiated	3	3.7
Clear cell	1	1.2
Grading		
G1	8	9.8
G2	28	34.1
G3	42	51.2
G4	4	4.9
Hormone receptor status ( $n = 70$ )		
ER-negative	27	38.6
ER-positive	43	61.4
PR-negative	41	57.1
PR-positive	29	42.9
uPA		
$\leq 5.5$	64	78.0
$> 5.5$	18	22.0
PAI-1		
$\leq 18.8$	41	50
$> 18.8$	41	50

disease stages as a cohort representative of primary ovarian cancer in general.

## PATIENTS AND METHODS

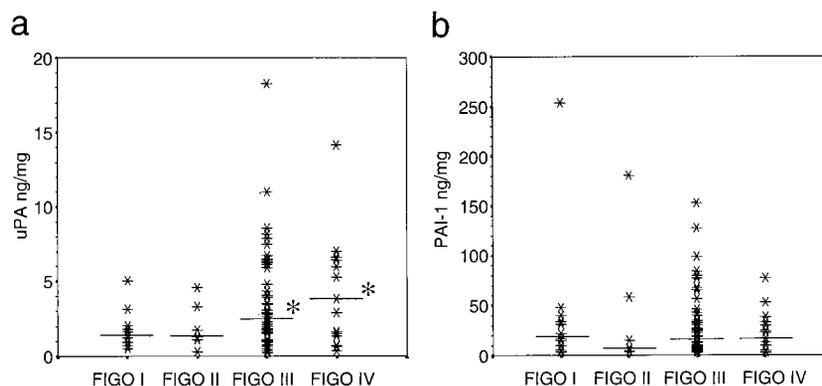
**Patients.** One hundred and three consecutive patients (1993–1997) who were treated for ovarian carcinoma at the Department of Obstetrics and Gynecology of the University of Munich, Klinikum Grosshadern, Munich, Germany were enrolled in this study. Complete surgical staging was followed by standard operative procedures, including a bilateral salpingo-oophorectomy, total abdominal hysterectomy, retroperitoneal pelvic and periaortic lymphadenectomy, and partial resection of the small or large intestine, diaphragmatic peritoneum, or upper abdominal surgery if indicated in advanced disease. Ovarian cancer disease was classified according to the FIGO staging system. Postoperative macroscopically visible tumor was the criterion for defining the presence or absence of residual tumor. The tumors studied included 82 primary invasive epithelial carcinomas, 9 LMP tumors, and 12 recurrent ovarian carcinomas. The patient and disease characteristics of the 82 primary invasive carcinomas are summarized in Table 1. Complete follow-up information was available for 80 of these patients.

Sixty-nine of 82 patients with primary ovarian cancer received platinum-based chemotherapy. Of the remaining 13 patients who received a single-drug therapy ( $n = 2$ ) or no adjuvant treatment ( $n = 11$ ), most had early stage grade 1 carcinomas ( $n = 8$ ) or unfavorable health conditions ( $n = 5$ ). During initiation of the study, standard chemotherapy for primary ovar-

ian cancer was carboplatin/cyclophosphamide with subsequent paclitaxel-containing regimens. A maximum of six cycles of chemotherapy was administered. Computed tomography scans of the abdomen were performed when disease progression was suspected on the basis of gynecological examination, vaginal ultrasound, patient symptoms, or increases in serum tumor markers. Disease progression was defined as a radiologically (computed tomography scan or nuclear magnetic resonance) proven disease recurrence or progression. Second-look procedures were not performed in this cohort. CA 125 and CA 72-4 levels were measured every three months by enzyme immunoassay (Abbott Laboratories, Chicago, IL) and RIA (Centocore, New York, NY), respectively. Median follow-up was 17 months (range, 1–55 months) for all patients. This study was performed after approval by the local Human Investigations Committee of the University of Munich, Munich, Germany. Informed consent was obtained from each patient or patient guardian.

**Methods.** Biopsies were obtained during surgery, and tissue sections were analyzed by histological assessment in all cases. The remainder of each sample was stored at  $-198^{\circ}\text{C}$  in liquid nitrogen until use. Subsequently, frozen specimens of 500 mg wet weight were pulverized with a microdismembrator (Braun-Melsungen, Melsungen, Germany), suspended in 2 ml of Tris-buffered saline containing 1% Triton X-100 detergent (Sigma Chemical Co., Munich, Germany), and incubated at  $4^{\circ}\text{C}$  for 12 h, with subsequent ultracentrifugation at  $100,000 \times g$  for 45 min. Quantitative levels of uPA and PAI-1 were prospectively measured in the supernants using ELISA kits, as described (Ref. 20; Imubind uPA and Imubind PAI-1; American Diagnostica, Greenwich, CT). Briefly, the uPA ELISA uses a murine monoclonal capture antibody directed to the uPA  $\beta$ -chain, thus detecting all forms of human uPA and uPA complexes with PAI-1. The detection system uses a biotinylated antibody of uPA  $\alpha$ -chain specificity. The PAI-1 ELISA uses a murine monoclonal capture antibody directed to active and inactive PAI-1 and PAI-1 complexes. The detection system uses a biotinylated antibody directed to an epitope that is noncompetitive with the above capture/binding site. Antigen concentrations for uPA and PAI-1 were measured in terms of ng/mg protein. Protein concentrations were measured using the protein assay reagent method (Pierce, Rockford, IL). Assays for estrogen and progesterone receptor content were performed with enzyme immunoassays (ER-EIA and PR-EIA, Abbott Laboratories, Chicago, IL), as described (21).

**Statistical Analysis.** Statistical analysis was performed using the SPSS statistical software program. Univariate and multivariate analyses were performed by the log-rank test and Cox's regression analysis, respectively. Two group comparisons assuming equal variance were performed using Student's  $t$  test (two-tailed). Nonparametric methods were used (Mann-Whitney  $U$  test) for non-normally distributed data.  $P$ s of  $<0.05$  were considered to be significant. The cutoff values of uPA and PAI-1 were calculated by log-rank statistic. A cutoff value for uPA and PAI-1 was identified that provided the maximum separation of patients with distinct prognosis with regards to PFS and OS. Survival curves were analyzed by the Kaplan-Meier method (22).



**Fig. 1** Comparison of uPA (A) and PAI-1 (B) levels for all patients with primary ovarian cancer according to FIGO stage. Patients with advanced disease stages had significantly higher uPA but not PAI-1 concentrations compared with patients with early disease stages (uPA ng/mg protein, mean values  $\pm$  SD; median): FIGO I,  $1.7 \pm 1.3$  (1.5); FIGO II,  $2.0 \pm 1.6$  (1.5); FIGO III,  $3.7 \pm 3.3$  (2.5); FIGO IV,  $4.2 \pm 3.8$  (3.4);  $P = 0.014$ ; (PAI-1, ng/mg protein, mean values  $\pm$  SD; median): FIGO I,  $40.7 \pm 68.7$  (19.4); FIGO II,  $44.7 \pm 69.9$  (10.7); FIGO III,  $30.1 \pm 33.3$  (19.0); FIGO IV,  $23.6 \pm 21.5$  (19.5);  $P = 0.23$ . \*,  $P = 0.014$  for FIGO stages III and IV compared with FIGO stages I and II. Lines, the median values.

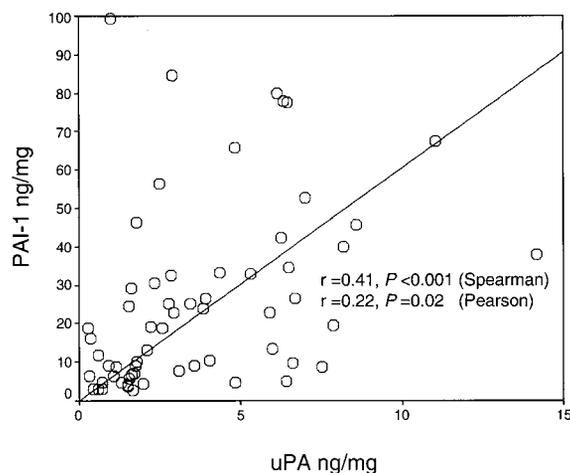
## RESULTS

uPA and PAI-1 concentrations were prospectively measured in tumor samples from 82 patients with primary ovarian cancer, 9 patients with LMP tumors, and 12 patients with recurrent ovarian carcinomas. Patient and disease characteristics of primary ovarian cancer patients are shown in Table 1. The expression of uPA in primary cancers was significantly associated with higher uPA concentrations among patients with higher FIGO stage disease [uPA ng/mg protein, mean values  $\pm$  SD (median): FIGO I,  $1.7 \pm 1.3$  (1.5); FIGO II,  $2.0 \pm 1.6$  (1.5); FIGO III,  $3.7 \pm 3.3$  (2.5); FIGO IV,  $4.2 \pm 3.8$  (3.4);  $P = 0.014$ ; Fig. 1A]. However, no significant association between PAI-1 concentrations and FIGO stage was found [PAI-1 ng/mg protein, mean values  $\pm$  SD (median): FIGO I,  $40.7 \pm 68.7$  (19.4); FIGO II,  $44.7 \pm 69.9$  (10.7); FIGO III,  $30.1 \pm 33.3$  (19.0); FIGO IV,  $23.6 \pm 21.5$  (19.5);  $P = 0.23$ ; Fig. 1B]. uPA and PAI-1 concentrations were significantly higher among primary ovarian cancers (FIGO I;  $n = 12$ ) compared with LMP tumors [ $n = 9$ ; uPA (mean  $\pm$  SD),  $1.7 \pm 1.2$  versus  $1.0 \pm 1.2$  ng/mg protein;  $P = 0.04$ ; PAI-1 (mean  $\pm$  SD),  $40.7 \pm 68.7$  versus  $9.0 \pm 3.0$  ng/mg protein;  $P = 0.019$ ]. uPA concentrations among primary ovarian cancer patients (FIGO I-IV) were comparable with those measured in recurrent ovarian cancers [uPA (mean  $\pm$  SD),  $3.4 \pm 3.1$  versus  $4.0 \pm 2.9$  ng/ml protein ( $P = 0.5$ )]; however, PAI-1 concentrations were significantly lower among recurrent ovarian cancers compared with primary ovarian cancers [PAI-1 (mean  $\pm$  SD),  $17.7 \pm 15.2$  versus  $31.6 \pm 41.5$  ng/mg protein ( $P = 0.035$ )]. Among patients with advanced disease stages (FIGO III and IV), uPA and PAI-1 concentrations were compared between primary tumor sites ( $n = 42$ ) and sites of metastases ( $n = 21$ ). Intra-abdominal metastasis demonstrated significantly higher uPA but not PAI-1 concentrations [uPA (mean  $\pm$  SD),  $5.5 \pm 3.8$  versus  $2.9 \pm 2.8$  ng/mg protein;  $P = 0.001$ ; PAI-1 (mean  $\pm$  SD),  $33.8 \pm 32.3$  versus  $24.8 \pm 29.8$  ng/mg protein;  $P = 0.16$ ]. The relationship of uPA to PAI-1 among all ovarian cancer patients ( $n = 103$ ) was analyzed and is shown in Table 2 and Fig. 2. The data demonstrate a significant positive association between uPA and PAI-1

**Table 2** Significant association between uPA and PAI-1 for primary ovarian cancer patients ( $n = 103$ ) using the defined cut-off value for uPA (5.5 ng/mg) and PAI-1 (18.8 ng/mg)<sup>a</sup>

	uPA-positive	uPA-negative	N
PAI-1-positive	15 (75%)	32 (39%)	47
PAI-1-negative	5 (25%)	51 (61%)	56
Total	20 (100%)	83 (100%)	100

<sup>a</sup> chi square;  $P = 0.003$ .



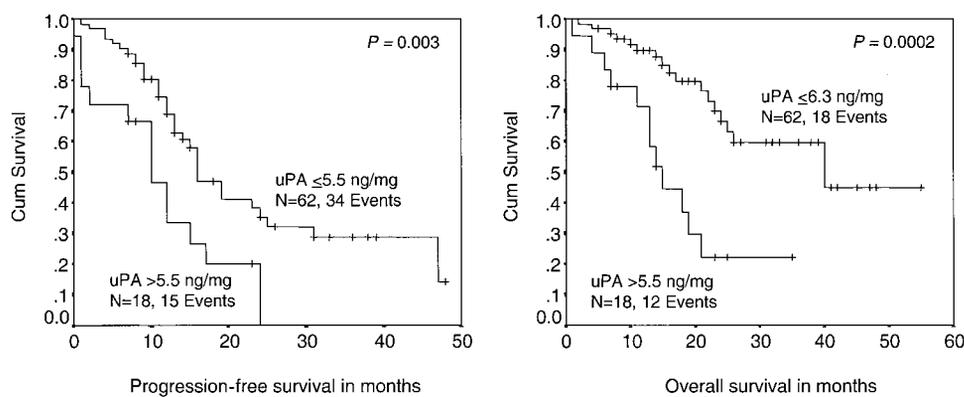
**Fig. 2** Association between uPA and PAI-1 concentrations as continuous variables among 103 ovarian cancer patients [ $r = 0.22$ ;  $P = 0.02$  (Pearson);  $r = 0.41$ ;  $P < 0.001$  (Spearman)].

as dichotomized variables or as continuous variables ( $\chi^2$  test,  $P = 0.015$ ; Pearson correlation,  $r = 0.22$ ;  $P = 0.02$ ; Spearman's correlation,  $r = 0.41$ ;  $P < 0.001$ ). Ovarian cancer patients (FIGO I-IV) with elevated uPA levels ( $>5.5$  ng/mg) also demonstrated significantly higher mean PAI-1 concentrations compared with those with lower uPA levels (PAI-1,  $44 \pm 32$  versus  $28 \pm 43$  ng/mg protein;  $P = 0.003$ ).

**Table 3** Uni- and multivariate analyses of prognostic factors for PFS and OS in 80 patients with ovarian cancer, FIGO stages I–IV

The following parameters were included in the analyses: tumor stage (FIGO Stages I and II versus Stages III and IV), residual tumor (presence or absence), age ( $\leq 56$  years versus  $> 56$  years), uPA ( $\leq 5.5$  versus  $> 5.5$  ng/mg protein), PAI-1 ( $\leq 18.8$  versus  $> 18.8$  ng/mg protein), and grading (G1, G2, G3, or G4).

	Ps for PFS		Ps for OS	
	Univariate	Multivariate	Univariate	Multivariate
FIGO stage	0.005	0.95	0.016	0.88
Residual tumor volume	$< 0.0001$	0.0005	0.0003	0.025
uPA	0.003	0.037	0.0002	0.006
PAI-1	0.039	0.31	0.007	0.582
Age	0.059	0.0008	0.15	0.018
Grading	0.0002	0.962	0.004	0.882



**Fig. 3** Kaplan-Meier plots for PFS and OS of ovarian cancer patients, FIGO stages I–IV. Patients with uPA levels below the cutoff value (5.5 ng/mg protein) had improved median PFS and OS compared with patients with elevated levels (PFS, 16 versus 10 months;  $P = 0.003$ ; OS, 40 versus 15 months;  $P = 0.0002$ ).

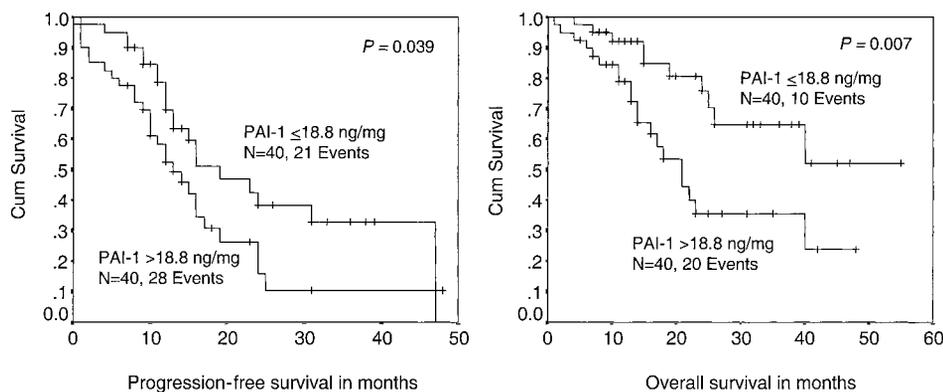
**Evaluation of uPA and PAI-1 on Prognosis (PFS and OS).** Among invasive cancers of all stages, residual tumor volume, FIGO stage, and grading were significant prognostic factors for both PFS and OS in univariate analyses (Table 3). Patients with early disease stages (FIGO I and II;  $n = 18$ ) had a significantly better prognosis than patients with advanced disease stages (FIGO III and IV;  $n = 62$ ; median PFS, 47 versus 13 months;  $P = 0.005$ ; median OS not reached for FIGO stages I and II patients versus 24 months for FIGO stages III and IV patients;  $P = 0.016$ ). Likewise, patients with no residual tumor volume ( $n = 28$ ) had a marked advantage in prognosis compared with patients with residual volume ( $n = 52$ ; median PFS, 47 versus 12 months;  $P < 0.0001$ ; median OS not reached for patients without residual disease versus 21 months for patients with residual disease;  $P = 0.0003$ ). Age ( $\leq 56$  or  $> 56$  years) demonstrated borderline significance for PFS ( $P = 0.059$ ) and was not significant for OS ( $P = 0.15$ ).

To evaluate the prognostic impact of uPA and PAI-1 levels on prognosis, we identified optimized cutoff values for separation of patients with distinct prognosis, using univariate analysis. A uPA value of 5.5 ng/mg provided the maximum separation of patients with regards to PFS and OS (log-rank test;  $P = 0.003$  and  $0.0002$ , respectively). A PAI-1 value of 18.8 ng/mg similarly provided the maximum separation of patients with regards to PFS and OS (log-rank test;  $P = 0.039$  and  $P = 0.007$ , respectively). Among invasive cancers of all stages, patients with uPA concentrations below 5.5 ng/mg protein ( $n = 62$ ) had an improved median PFS and OS compared with patients with

elevated levels ( $n = 18$ ; median PFS, 16 versus 10 months,  $P = 0.003$ ; median OS, 40 versus 15 months,  $P = 0.0002$ ; Fig. 3). Similarly, patients with PAI-1 levels below 18.8 ng/mg protein ( $n = 40$ ) had an improved PFS and OS compared with patients with elevated levels ( $n = 40$ ; median PFS, 19 versus 13 months,  $P = 0.039$ ; median OS not reached for PAI-1-negative patients versus 21 months for PAI-1-positive patients,  $P = 0.007$ ; Fig. 4). uPA concentration retained prognostic significance for OS in patients with residual tumor ( $n = 54$ ; median OS, 24 versus 14 months;  $P = 0.012$ ) and achieved borderline significance among patients with no residual tumor ( $n = 28$ ; mean OS, 53 versus 20 months;  $P = 0.06$ ). Neither estrogen and progesterone receptor status nor the volume of ascites ( $> 500$  versus  $\leq 500$  ml) or CA 125 values ( $> 35$  versus  $\leq 35$  units/ml) were significant prognostic factors in this cohort (data not shown). In multivariate analysis of patients with FIGO stages I–IV disease, which included the parameters of the FIGO stage, residual tumor volume, uPA, PAI-1, age and grading, the parameters of residual tumor volume, uPA, and age remained independent prognostic factors for PFS and OS (Table 3).

To compare this study with previous studies in which only patients with advanced disease stages were analyzed, the prognostic significance of uPA and PAI-1 in the subset of FIGO stages III and IV patients ( $n = 62$ ) was analyzed. Among these invasive cancers, residual tumor volume ( $P = 0.0007$ ), age ( $P = 0.005$ ), and uPA ( $P = 0.037$ ) were significant prognostic factors for PFS, whereas FIGO stage ( $P = 0.10$ ) was only of borderline significance. FIGO stage ( $P = 0.024$ ), residual tumor volume

**Fig. 4** Kaplan-Meier plots for PFS and OS of ovarian cancer patients, FIGO stages I–IV. Patients with PAI-1 levels below the cutoff value (18.8 ng/mg protein) had improved median PFS and OS compared with patients with elevated levels (PFS, 19 versus 13 months;  $P = 0.039$ ; OS, median OS not reached for patients with PAI-1 levels  $\leq 18.8$  ng/mg protein versus 21 months;  $P = 0.007$ ).



( $P = 0.012$ ), and uPA ( $P = 0.003$ ) were significant prognostic factors for OS, whereas age ( $P = 0.056$ ) and PAI-1 ( $P = 0.066$ ) were of borderline significance. Interestingly, FIGO stage III or IV patients with optimal surgical cytoreduction ( $n = 28$ ) had significantly lower uPA and PAI-1 concentrations than those with higher volumes ( $> 1$  cm;  $n = 36$ ) [uPA (mean  $\pm$  SD),  $2.4 \pm 2.4$  versus  $4.9 \pm 3.7$  ng/mg protein,  $P = 0.001$ ; PAI-1 (mean  $\pm$  SD),  $22.9 \pm 32.1$  versus  $33.1 \pm 30.0$  ng/mg protein,  $P = 0.016$ ]. This suggests that the inability to optimally debulk patients could be related to the increased proteolytic activity in tumors observed in patients with higher residual volumes. In multivariate analysis of advanced ovarian cancer, including FIGO stage, residual tumor volume, age, and uPA, the parameters of residual tumor volume (PFS,  $P = 0.0004$ ; OS,  $P = 0.025$ ), uPA (PFS,  $P = 0.07$ ; OS,  $P = 0.007$ ) and age (PFS,  $P = 0.0006$ ; OS,  $P = 0.025$ ) retained independent prognostic importance.

## DISCUSSION

In the present study, uPA concentrations were significantly higher in invasive tumors compared with LMP tumors, which have been recognized as a separate entity, as the clinical course of these tumors is far more favorable when compared with their invasive counterparts (23). Similarly, uPA levels were higher in metastatic lesions as compared with their respective primary tumors. Increasing levels of uPA were also significantly associated with advanced disease stages and with the amount ( $> 1$  cm) of residual tumor. Taken together, these findings demonstrate that uPA is associated with the malignant progression of epithelial ovarian cancer. The results are consistent with the hypothesis that elevated levels of uPA may contribute to invasiveness and metastasis of ovarian cancer. In contrast, PAI-1 content was not correlated with disease stage, which is in accordance with previous reports (16, 17), and no significant difference was found in PAI-1 content between primary and metastatic ovarian cancers as compared with uPA.

The present study is the first study to evaluate the prognostic significance of uPA and PAI-1 in uni- and multivariate analyses in a representative cohort of primary ovarian cancers of all stages. The level of uPA was an independent prognostic marker for both PFS and OS in multivariate analysis, using the cutoff values established for this cohort.

Consistent with previous reports, we also confirmed the independent prognostic relevance of residual tumor volume and age in ovarian cancer (24–28). This is the first study to demonstrate the independent prognostic relevance of uPA in a nonselected group of ovarian cancer patients. In a recent report on 77 patients with primary ovarian cancer (FIGO stages I–III), Hoffmann *et al.* (19) were also able to demonstrate prognostic relevance of uPA for OS; however, they only performed univariate analysis. In contrast, van der Burg *et al.*, (16) who assessed uPA and PAI-1 among 90 patients ranging from stage I to stage IV disease, reported no correlation of uPA and PAI-1 with PFS or OS. The negative findings of that study possibly are attributable to the different laboratory assays, extraction procedures, and cutoff values used, as van der Burg *et al.* based median values as cutoff values for uPA and PAI-1 and measured uPA and PAI-1 concentrations in cytosols routinely prepared for ER and PR determinations.

Two previous studies support a poor prognosis associated with high PAI-1 content (17, 18). Chambers *et al.* (17) determined PAI-1 levels by immunohistochemistry in samples from 119 patients with FIGO stages I–IV disease, and PAI-1 was an independent prognostic factor among the 99 patients with FIGO stages III or IV disease; however uPA was not included in this analysis. Kuhn *et al.* (18) recently demonstrated the importance of PAI-1 as an independent prognostic marker for survival by assessing PAI-1 by ELISA among 84 ovarian cancer patients with FIGO stage IIIc disease. In the present study, the prognostic relevance of high PAI-1 levels for PFS and OS was confirmed in univariate analysis only when all disease stages were included in the analysis; however, this was not the case among the subset of patients with advanced disease for PFS, and for OS the effect was of borderline significance ( $P = 0.066$ ), possibly because of the small sample size ( $n = 64$ ) in subset analysis.

Not only did the previously mentioned studies use different extraction procedures or assay methods and different antibodies, but, also, different cutoff values were applied to separate patients into low- versus high-risk groups. Hoffmann *et al.* (19), who analyzed a comparable cohort (FIGO stages I–III) of primary ovarian cancer patients, detected uPA and PAI-1 levels in tissue pellets and selected a slightly lower

optimized cutoff value of 4.8 ng/ml for uPA. However, they did indicate that the uPA concentrations were ~30% lower if detection was performed in tissue pellets, as done in their study, compared with tissue homogenates, which were used in the present study.

The optimal cutoff value for uPA in this study was 5.5 ng/mg protein, meaning that 18 of 82 patients (22%) whose tumors revealed elevated uPA concentrations had a significantly shorter OS compared with those with lower uPA levels. To further establish the prognostic impact of uPA in ovarian cancer, the previously described cutoff value of 4.8 ng/ml protein for uPA was analyzed (data not shown). This cutoff value also provided clinically significant results in this study population, in which 22 of 82 patients (27%) had a significantly shorter survival compared with those with lower uPA concentrations ( $P = 0.006$ ). uPA also retained independent prognostic significance with the selected cutoff value of 4.8 ng/mg protein in multivariate analysis, including FIGO stage, residual tumor volume, levels of PAI-1, age, and histological grade ( $P = 0.0088$ ). These data, however, also demonstrate that suitable cutoff values for both uPA and PAI-1 must be further defined and validated in a prospective setting.

This study presents evidence that elevated levels of the uPA protease and, to a lesser extent, its inhibitor, PAI-1, are associated with the capacity of ovarian cancer cells to invade then metastasize in the peritoneum. Additional research, however, is necessary to understand the role of PAI-1 in this process. It has been suggested that PAI-1 can promote internalization of receptor-bound uPA, which allows recycling of the uPA receptor to the cell surface (29). In this way, the proteolytically active areas of the cell surface can be modified by PAI-1, thus regulating directed proteolytic activity of tumor cells (4). It recently has been described that competitive displacement of uPA from the cellular binding sites results in decreased proteolysis *in vitro*. Metastatic capacity was similarly inhibited when animals were given intermittent i.p. injections of uPA/IgG fusion protein capable of displacing uPA activity from the tumor cell surface (30). Assessment of uPA and PAI-1 levels may therefore allow identification of ovarian cancer patients at high risk and provide a rationale for a biologically directed therapy.

## ACKNOWLEDGMENTS

We thank Sandra Lude and Margret Felber for their expertise, Wendy Aft for her kind support in preparing the manuscript, and Dr. Beth Karlan (Department of Obstetrics and Gynecology, Jonsson Comprehensive Cancer Center, University of California at Los Angeles, Los Angeles, CA) for critically reviewing the manuscript.

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