Cleaved Forms of the Urokinase Plasminogen Activator Receptor in Plasma Have Diagnostic Potential and Predict Postoperative Survival in Patients with Ovarian Cancer

Emir Henic, Christer Borgfeldt, Ib Jarle Christensen, Bertil Casslén, and Gunilla Høyer-Hansen

Abstract

Purpose: To evaluate the plasma level of different forms of soluble urokinase plasminogen activator receptor (suPAR) as discriminators between malignant, borderline, and benign ovarian tumors and as prognostic markers in patients with ovarian cancer.

Experimental Design: The different suPAR forms were measured in preoperative plasma samples obtained from 335 patients with adnexal lesions using three different time-resolved fluorescence assays (TR-FIA): TR-FIA 1 measuring intact suPAR, suPAR(I-III), TR-FIA 2 measuring the total amount of suPAR(I-III) and the cleaved form, suPAR(II-III), and TR-FIA 3 measuring the liberated uPAR(I). Tumors were classified as benign (n = 211), borderline (possibly malignant; n = 30), and well (n = 19), moderately (n = 15), and poorly (n = 60) differentiated malignant.

Results: All uPAR forms as well as CA125 were statistically significant in univariate analysis discriminating between benign, borderline, and invasive tumors. Restricting the analysis of invasive tumors to early stage (I and II) showed similar results. A combination of CA125 and suPAR(I-III) + suPAR(II-III) discriminated between malignant (all stages) and benign tumors [AUC, 0.94; 95% confidence interval (95% CI), 0.90–0.98] as well as borderline and benign tumors (AUC, 0.78; 95% CI, 0.67–0.89). All suPAR forms were markers for poor prognosis in univariate analyses, and high preoperative plasma level of uPAR(I) is an independent predictor of poor prognosis (hazard ratio, 1.84; 95% CI, 1.15–2.95; P = 0.011) in multivariate analyses including age and CA125.

Conclusions: High concentration of plasma uPAR(I) is an independent preoperative marker of poor prognosis in patients with ovarian cancer. The combination of plasma suPAR(I-III) + suPAR(II-III) and CA125 discriminates between malignant and benign tumors with an AUC of 0.94.

Ovarian cancer is the third leading cause of death in cancer among women ages 45 to 64 years in Sweden, and the incidence in Sweden is comparable with other western countries (1). Due to mild symptoms, the majority of patients with ovarian cancer are not diagnosed until the disease is in advanced stages, which is consequently reflected in poor outcome (2). In contrast, early-stage ovarian cancer (before the tumor has spread in the peritoneal cavity) has excellent curability. Thus, any marker, which could be used for screening of asymptomatic women in age groups at risk, would promote early detection and thus increase curability. Several tumor markers have been tried, either alone or in combinations. However, even the most useful one, CA125, is not reliable due to low sensitivity in patients with early-stage ovarian cancer (3–8). Gynecologic ultrasound has high sensitivity and acceptable specificity but is too labor intensive to be employed for screening.

The urokinase plasminogen activator (uPA) system is involved in tissue remodeling processes, such as wound healing and cancer cell invasion. In addition, the components of the uPA system are up-regulated in many types of malignant tumors (9). The uPA receptor (uPAR) has a central function in these processes, because binding of the zymogen pro-uPA initiates activation of plasminogen leading to other proteolytic events in the extracellular matrix. Intact uPAR, uPAR(I-III), consists of three domains denoted uPAR(I), uPAR(II), and uPAR(III) connected by two linker regions and uPAR(III) is attached to cell membrane by a glycosylphosphatidylinositol anchor. Two crystal structures of soluble forms of the human uPAR(I-III) have recently been reported (10, 11). Intact uPAR is required for efficient binding of ligands like uPA and vitronectin (12–14). uPAR(I-III) can be cleaved in the linker region between domains I and II by uPA, liberating uPAR(I) and leaving the cleaved form, uPAR(II-III), on the cell surface (15, 16). In vivo uPAR has been shown to be responsible for cleavage of uPAR (17, 18), but in vitro uPAR can also be cleaved...
Translational Relevance
Ovarian cancer is the leading cause of death among gynecologic malignancies. Due to mild symptoms, the majority of patients are already in advanced stage at diagnosis, facing poor long-term survival despite radical surgery and improved chemotherapy. In contrast, early-stage ovarian cancer has excellent curability. Early diagnosis is the only measure that can radically improve prognosis in patients with ovarian cancer. Unfortunately, there is no reliable marker to be used in screening for early-stage ovarian cancer. CA125 has too low sensitivity for early-stage tumors, and gynecologic ultrasound is too labor intense to be employed in screening. We show that plasma levels of suPAR(I-III) + suPAR(II-III) have diagnostic potential in patients with ovarian cancer. The product of suPAR(I-III) + suPAR(II-III) and CA125 detects early-stage tumors more accurately than each marker separately. This variable may help to identify early-stage ovarian tumors among the numerous ovarian cysts detected by transvaginal ultrasonography. In addition, uPAR(I) is an independent marker for postoperative survival in patients with ovarian cancer. It is available already preoperatively and can be used to guide the effort of surgery as well as to individualize chemotherapy.

Materials and Methods

Patients and Treatment
Peripheral blood samples were obtained preoperatively in 335 patients admitted for primary surgery because of adnexal masses at the Department of Obstetrics and Gynecology in Lund from 1993 to 2005. Blood was collected in citrate tubes and centrifuged, and the plasma was stored at -20°C until analyzed. The standard surgical procedure included resection of the cyst or unilateral oophorectomy in benign cases and abdominal hysterectomy, bilateral salpingo-oophorectomy, and infracolic omentectomy in the malignant cases. Cytologic analyses of ascitic fluid, or when absent, of peritoneal washing were done. All diagnoses were verified by histopathology of the tumors. Histopathologic grade and stage of the disease (FIGO) were available in all malignant cases as shown in Tables 1 and 2. Postoperative adjuvant treatment was given according to clinical standards in patients with invasive cancer. Patients with stage Ic or higher stage received platinum-based chemotherapy, either alone or combined with paclitaxel or cyclophosphamide. Survival status of all patients (alive or dead including date of death) was obtained on September 27, 2006 from the Swedish Population Register (Tumor Registry Center in Lund). For patients with benign cysts, the median age was 50 years (range, 16.6-88); for borderline patients, the median age was 52.2 years (range, 30.6-85.7); and for ovarian cancer patients, the median age was 62.6 years (range, 31-88). The median follow-up time for patients alive on September 27, 2006 was 64 months (range, 20-154).

The study was approved by the Regional Ethical Board, Faculty of Medicine, University of Lund.

Immuoassays
uPAR. Three uPAR immunoassays, time-resolved fluorecence assays (TR-FIA) 1 to 3, have been designed for the specific measurement of uPAR(I-III), uPAR(I-III) + uPAR(II-III), and uPAR(I), respectively (21). The detection limits were 0.3 pmol/L for uPAR(I-III) for TR-FIA 1 and 2 and 1.9 pmol/L for uPAR(I) for TR-FIA 3. The assays were previously validated for use in citrate plasma diluted 1:10 (21). Because the amounts of uPAR(I) in citrate plasma diluted 1:10 is close to the limit of quantification, we decided to only dilute our samples 1:5 in assay buffer (DELFIA assay buffer 1244-111). The assays were therefore validated for their use in citrate plasma diluted 1:5. The limit of quantification was determined by spiking suPAR-depleted citrate plasma with purified suPAR and examining the coefficient of variation (CV). suPAR depletion of plasma diluted 1:5 was achieved as described previously (21). The depleted plasma was spiked with a concentration range from 0.016 to 10 ng/ml purified standards [0.5-325 pmol/L suPAR(I-III) and 1.5-961 pmol/L uPAR(I)]. The limit of quantification was defined as the concentration at which CV exceeded 20%.

Intra-assay precision was determined by measuring the donor citrate plasma pool in TR-FIA 1 (n = 26) and TR-FIA 2 (n = 28) and calculating the CVs. For TR-FIA 3 (n = 27), we used donor citrate plasma pool spiked with 480 pmol/L uPAR(I). The same samples were employed for determination of inter assay precision (n = 24).

The amount of suPAR(I-III) was obtained by subtracting the moles of suPAR(I-III) measured in TR-FIA 1 from those of suPAR(I-III) and suPAR(II-III) measured in TR-FIA 2.

CA125. Preoperative plasma samples were routinely assayed for CA125 using a commercial electrochemiluminescence immunoassay Elesys CA125 kit (Roche). The assay was done according to the manufacturer’s instructions.

Statistical Methods
Descriptive statistics for the plasma content of the different suPAR forms and CA125 stratified by the histopathologic group and stage are presented by box-whisker plots showing their medians, quartiles, and extreme values. Tests for location comparing the histopathologic groups have been done using the Kruskal-Wallis test, and if significant, pairwise
comparisons have been done using the Mann-Whitney U test. The component uPAR(I) was lower than the detection limit in 75% of the analyzed samples; therefore, we chose to dichotomize uPAR(I) at the detection limit considering uPAR(I) levels above the limit to be elevated. Tests for independence between the histopathologic groups and uPAR(I) were done using the $\chi^2$ test. Trend tests for ordered groups were done using linear regression with the dependent variable log transformed. Spearman’s rank correlation has been used as a measure of association between the studied biomarkers.

Analysis of discrimination between the benign, borderline, and invasive has been done using a proportional odds model with biomarkers either log transformed or dichotomized. Backwards selection was used to identify the significant biomarkers (<5%). The initial model did not include suPAR(II-III), as this marker is highly correlated to suPAR(I-III) + suPAR(II-III). Results are presented by the respective odds ratios and 95% confidence intervals (95% CI). The receiver operating characteristic (ROC) curve and the area under the ROC curve with 95% CI are presented for comparison of borderline and invasive tumors to benign tumors. Furthermore, the specificity for fixed sensitivities of 85%, 90%, and 95% was calculated for the combination of suPAR(I-III) + suPAR(II-III) and CA125 as well as the false-positive and false-negative rates computed as posterior probabilities using Bayes’ theorem. The Cox proportional hazards model was used for univariate and multivariate analysis. The uPAR variants and CA125 were entered as a continuous covariate on the log scale. uPAR(I) levels below the detection limit have been set at the limit for the analysis of continuous levels. Point estimates are reported as hazard ratios (HR) with 95% CI. Assumptions of proportional hazards were checked using Schoenfeld’s test or verified graphically where applicable. Significant departures from proportionality were not observed for dichotomized soluble uPAR forms or for other covariates used in the Cox regression. For graphical presentation of overall survival, probabilities were estimated using the Kaplan-Meier method, dichotomizing biomarker levels by their respective medians. Multivariate analysis of the suPAR components using backwards selection was used to identify a model for use in a final model including CA125, grade, stage, age, and residual tumor, all significant in univariate analysis. Histology type was not significant in univariate analysis and is therefore not included in the multivariate analysis. The final model was also reduced using backwards selection. All comparisons were two sided, and a 5% level of significance was used. The statistical analyses were done using SPSS (11.5.1) and SAS (v9.1; SAS Institute).

### Results

The limit of quantification was determined in suPAR-depleted citrate plasma pool spiked with the analytes and was for suPAR(I-III) <1.3 pmol/L in TR-FIA 1 and 1.3 pmol/L in TR-FIA 2. For uPAR(I) in TR-FIA 3, the limit of quantification was 2.9 pmol/L. The CVs calculated for the intra-assay precision was 4.9% for TR-FIA 1, 6.4% for TR-FIA 2, and 7.9% for TR-FIA 3. The CVs for the interassay precision of TR-FIA 1 and TR-FIA 2 were 10.2% and 7.3%, respectively, whereas the CV for TR-FIA 3 was 10.6%. Accuracy was previously determined and the recoveries in 20% citrate plasma of TR-FIA 1 to 3 were 93%, 101%, and 95%, respectively (21).

The plasma levels of suPAR(I-III) + suPAR(II-III) were higher in patients with borderline ($P < 0.0001$) and invasive ($P < 0.0001$) tumors than in those with benign tumors (Fig. 1; Table 3). Furthermore, the levels were higher in patients with invasive tumors than in those with borderline tumors ($P = 0.03$). It was, however, not significantly different between the grades of invasive tumors. The plasma concentrations of suPAR(I-III) + suPAR(II-III) were not different in patients with different clinical stages of invasive tumors (data not shown).

The content of suPAR(I-III) in plasma was higher in patients with malignant ($P < 0.0001$) and borderline ($P = 0.0002$) tumors than in those with benign tumors. However, the levels of suPAR(I-III) in samples from patients with borderline and malignant tumors were not different ($P = 0.36$), and there was no difference between the histologic grades of invasive tumors. Also, the levels of suPAR(I-III) were not different between the clinical stages of the disease.

The calculated plasma levels of suPAR(II-III) were higher in patients with malignant ($P < 0.0001$) and borderline ($P < 0.0001$) tumors compared with patients with benign tumors. The levels were also higher in patients with malignant compared with those with borderline tumors ($P = 0.009$) and were not vary with the clinical stages of the disease.

The number of patients with uPAR(I) above detection limit was 47% in the malignant group, 50% in the borderline group, and 12% in the benign group. Concentrations were significantly higher in the malignant ($P < 0.0001$) and borderline ($P < 0.0001$) groups than in the benign group, but there was no difference between plasma samples from patients with malignant and borderline tumors ($P = 0.76$). There was also no difference between the histologic grades or clinical stages within the malignant group.

### Table 1. Histopathologic diagnoses in relation to differentiation and grade of the ovarian tumor

<table>
<thead>
<tr>
<th>Differentiation</th>
<th>Serous</th>
<th>Mucinous</th>
<th>Endometroid</th>
<th>Clear cell</th>
<th>Functional</th>
<th>Endometriosis</th>
<th>Teratoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>91</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>39</td>
<td>25</td>
<td>211</td>
</tr>
<tr>
<td>Borderline</td>
<td>17</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Well</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Moderately</td>
<td>9</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
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<tr>
<td>Poor</td>
<td>41</td>
<td>4</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>166</td>
<td>64</td>
<td>22</td>
<td>16</td>
<td>39</td>
<td>25</td>
<td>335</td>
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### Table 2. Histopathologic grade differentiation in relation to stage

<table>
<thead>
<tr>
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<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>27</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Well</td>
<td>12</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Moderately</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Poor</td>
<td>5</td>
<td>6</td>
<td>44</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>11</td>
<td>57</td>
<td>7</td>
<td>124</td>
</tr>
</tbody>
</table>

Plasma suPAR and Prognosis in Ovarian Cancer
The content of CA125 in plasma was evaluated in 278 patients only, because this variable is missing in 35 patients with benign tumors, 6 patients with borderline tumors, and 16 patients with malignant tumors. The concentration was higher in patients with malignant \((P < 0.0001)\) and borderline \((P < 0.0001)\) tumors compared with those with benign tumors. Also, the concentration was higher in patients with well \((P = 0.02)\), moderately \((P = 0.008)\), and poorly \((P < 0.0001)\) differentiated tumors compared with those with borderline tumors. Plasma CA125 increased with loss of histologic differentiation \((P_{\text{trend}} < 0.001)\). Patients with endometriosis had significantly higher CA125 values compared with other benign cysts \((P < 0.001)\). In contrast, however, there were no differences in plasma levels of suPAR(I-III) + suPAR(II-III), suPAR(I-III), or uPAR(I) between patients with endometriosis and other benign ovarian cysts.

The Spearman rank correlation coefficients between different uPAR forms range from 0.40 to 0.70, except that between suPAR(I-III) + suPAR(II-III) and suPAR(II-III), which is 0.94. Correlation coefficients between uPAR forms and CA125 range from 0.32 to 0.50. All correlations are significantly different from 0.

Univariate analysis using the proportional odds model showed that all uPAR forms were statistically significant \((P < 0.0001)\) with suPAR(I-III) + suPAR(II-III) as the best discriminator. In addition, CA125 was also significant \((P < 0.0001)\). The results of the proportional odds model discriminating the three ordered categories showed that suPAR(I-III) + suPAR(II-III) and CA125 were retained in the final model \((P = 0.0002 \text{ and } P < 0.0001, \text{ respectively})\). The score test for the proportional odds assumption yielded \(P = 0.46\), showing that the assumption could not be rejected. The odds ratios were 7.94 (95% CI, 2.64-23.85) for suPAR(I-III) + suPAR(II-III) and 3.46 (95% CI, 2.59-4.61) for CA125. Note that these odds are on the log scale (natural) showing the odds comparing patients differing by one unit on the log scale. Restricting the analysis to include only early invasive cancers (stages I and II) also showed suPAR(I-III) + suPAR(II-III) to be significant (odds ratio, 6.41; 95% CI, 2.12-19.42) as well as CA125 (odds ratio, 2.34; 95% CI, 1.70-3.24). Further analysis using the ROC curve comparing benign with invasive tumors yields an AUC of 0.94 (95% CI, 0.90-0.98) and between benign and borderline the AUC is 0.78 (95% CI, 0.67-0.89; Fig. 2). From this model, the specificities for borderline versus benign were 11.9%, 19.9%, and 23.9% when the sensitivity was set at 95%, 90%, and 85%, and increased to 52.3%, 82.4%, and 89.2% when invasive versus
benign was analyzed (Table 4). Restricting the invasive group to early stage, stages I and II (n = 32 and n = 25 with CA125 available) resulted in 44.9%, 48.9%, and 68.2% specificities at 95%, 90%, and 85% sensitivities. The AUC of this model is 0.87 (95% CI, 0.78-0.96).

Overall survival was analyzed in patients with invasive malignant tumors (n = 94), thus excluding borderline tumors. The different variables of plasma suPAR were dichotomized at the median to discriminate between high and low risk for poor overall survival using univariate Cox regression analysis and Kaplan-Meier curves (Fig. 3A-D; P values are for the log-rank test; the HR and 95% CI estimated using the Cox model are shown). Using backwards selection in multivariate analysis including all variants of suPAR dichotomized by their respective medians, uPAR(I) was selected as the only covariate (HR, 2.49; 95% CI, 1.40-4.41; P = 0.002). A similar analysis using the log-transformed values resulted in uPAR(I) being retained (HR, 2.38; 95% CI, 1.58-3.57; P < 0.0001). Therefore, uPAR(I) levels were used in the multivariate analyses with other risk factors in ovarian cancer included. The results obtained by replacing the covariates uPAR(I) and CA125 by their actual values on the log scale are shown in Table 5, model 1. The number of patients in this analysis was 78 with 38 deaths, mainly due missing CA125 values. Removing CA125 from the multivariate analysis shows that age is now included as well as residual tumor and uPAR(I) (Table 5, model 2, 48 deaths). The number of events in a full multivariate model including all covariates could result in unstable estimates of these; however, the final models have a sufficient number of events. A multivariate model including preoperatively available covariates CA125 and uPAR(I) showed that CA125 is nonsignificant (HR, 1.18; 95% CI, 0.97-1.43; P = 0.10) as well as age (HR, 2.04; 95% CI, 0.97-4.31; P = 0.06), whereas uPAR(I) is significant (HR, 1.84; 95% CI, 1.15-2.95; P = 0.011).

Discussion

The aim of the study was to investigate whether concentrations of the individual suPAR forms in peripheral blood plasma from women with adnexal lesions can be used for diagnosing ovarian cancer or for predicting postoperative prognosis in patients with ovarian cancer. We identified the product of suPAR(I-III) + suPAR(II-III) and CA125 as a diagnostic marker for malignant and possibly malignant (borderline) ovarian tumors with higher accuracy than each of these variables separately. We also identified high plasma levels of uPAR(I) as an independent preoperative prognostic marker for poor overall survival in multivariate analyses.

Plasma levels of all separately analyzed suPAR variants were increased in patients with borderline and invasive tumors compared with those with benign tumors. The levels of suPAR(I-III) + suPAR(II-III) and suPAR(II-III) were also higher in invasive tumors than in borderline tumors. On the other hand, among invasive tumors, none of the suPAR forms varied with histologic differentiation or clinical stage. In a previous study of a much smaller number of ovarian tumor patients, we measured suPAR (all forms) with an ELISA in serum prepared from both peripheral blood and tumor blood and found increased levels of suPAR in invasive but not in borderline tumors (22). Apparently, the TR-FIAs, which are used in this article, allow us to distinguish benign from borderline tumors, which is very important in a diagnostic context. The present observations suggest that cleavage of uPAR as well as shedding of uPAR from the cell surface is increased already in borderline tumors but is most pronounced in the invasive tumors. Because plasma suPAR was independent of clinical stage, it appears not to be dependent on tumor burden. Although stage does not alone determine tumor burden, it reflects tumor extension with involvement of peritoneal surfaces, formation of ascitic fluid, etc.

The level of suPAR in peripheral blood from patients with ovarian cancer has been assayed previously using ELISA, which measure the total amount of all suPAR forms (22, 29, 36–38). In contrast to our findings, two of these studies found a correlation between the concentration of suPAR and the clinical stage. Sier et al., analyzing serum samples from 87 patients with ovarian cancer, found the highest levels in stage II (111 ± 20 pmol/L) with decreasing levels in stage III (98 ± 10 pmol/L) and stage IV (72 ± 7 pmol/L), and this was compared with healthy controls (49 ± 3 pmol/L; ref. 38). Riisbro et al., who studied citrate plasma from 53 ovarian cancer patients, reported that the level of suPAR increased gradually from clinical stage I (36 pmol/L) to stage IV (52 pmol/L) and that elevated level of suPAR was associated with poor postoperative prognosis (29).

We have shown previously that uPAR mRNA in the tumor tissue increase gradually when histology progress from well-differentiated to poorly differentiated tumors (34). However, tumor tissue content of uPAR protein is actually highest in borderline and well-differentiated malignant tumors and is substantially reduced in poorly differentiated tumors (39). Because of this inverse correlation with tumor differentiation, high tumor tissue content of uPAR is actually a marker for good postoperative prognosis in these patients. We also found the same inverse correlation between histologic differentiation and suPAR concentration in cystic fluid (22). The lack of correlation

\[
\begin{array}{|c|c|c|c|}
\hline
 & Benign vs borderline & Borderline vs malignant & Benign vs malignant \\
\hline
suPAR(I-III) + suPAR(II-III) & <0.0001 & 0.03 & <0.0001 \\
suPAR(I-III) & 0.0002 & 0.36 & <0.0001 \\
suPAR(II-III) & <0.0001 & 0.009 & <0.0001 \\
uPAR(I) & <0.0001 & 0.76 & <0.0001 \\
CA125 & <0.0001 & <0.0001 & <0.0001 \\
\hline
\end{array}
\]
between the levels of uPAR mRNA and uPAR protein in tumor tissue and cystic fluid could be due to increased shedding of uPAR from poorly differentiated tumor cells to blood and body fluids (other than cystic fluid), because ovarian cancer cells shed uPAR (40). Alternatively, this may result from increased degradation of uPAR(I-III) during internalization with the uPA/plasminogen activator inhibitor-1 complex (41), but this would not hold for uPAR(II-III) because it cannot be internalized by this mechanism (19). A third possibility is that only a fraction of the uPAR mRNA is translated.

Ovarian cancer has a high mortality rate due to few symptoms in the early stage often resulting in diagnosis in late stages and subsequent poor prognosis. In addition, long-term outcome for these patients with advanced disease has not improved significantly despite introduction of more radical surgery and improved chemotherapy. The only measure that could radically change the prognostic situation in ovarian cancer would be diagnosis of the tumors in early stages. This can be achieved provided a biomarker with high enough sensitivity and specificity can be found. Because the composition of ovarian cystic fluid largely reflects the content of the tumor tissue, we previously measured the collective amounts of suPAR forms in cystic fluids from 68 patients admitted for surgery of ovarian tumors (22). The concentrations were generally high (651-8,468 pmol/L) and separated clearly benign and malignant cysts with both sensitivity and specificity above 90%. Furthermore, concentrations in borderline cysts were as high as in malignant cysts. However, cystic fluid cannot be aspirated without leakage in the abdomen and up-staging the patient, not even when fine-needle technique is used.

Thus, we conclude that any marker, which is intended to for screening purpose, should be found in the peripheral blood. The previously well-studied marker CA125 has not met the requirements mainly because of too low sensitivity in patients with early-stage ovarian cancer. Any potential marker needs to prove sufficient sensitivity in early-stage and borderline tumors, which is the ultimate target group for detection because it has excellent survival data. Analysis of ROC curves showed that suPAR(I-III) + suPAR(II-III) and CA125 clearly discriminated between invasive and benign tumors as well as between borderline and benign. Similar results is found when restricting the analysis to early-stage invasive tumors; however, the result should be interpreted with caution as the number of patients in the invasive group is small. This means that surgery has to be done in less than two women to find an invasive ovarian cancer in women with adnexal lesion and high levels of the linear combination of suPAR(I-III) + suPAR(II-III) and CA125. Thus, the combination of these two markers detects early-stage tumors more accurately than each marker separately. Because comparisons between early stages + borderline tumors and benign tumors are not readily available in the literature, we compared AUC between all stages malignant tumors and benign tumors. In fact, AUC 0.94 is higher than most other proposed plasma markers as well as combinations of transvaginal ultrasonography and plasma marker algorithms (42). The linear combination of suPAR(I-III) + suPAR(II-III) and CA125 may in patients with serious comorbidity be a way of monitoring the patient with adnexal lesion and eventually avoid a stressful laparotomy or laparoscopy. This variable may also, in conjunction with transvaginal and abdominal ultrasonography, be a way to select those patients with ovarian cysts, who should be sent to centers with oncologist expertise to have optimal primary surgery.

**Table 4.** ROC analysis: sensitivities and specificities in discriminating borderline from benign and invasive ovarian cancer from benign cases

<table>
<thead>
<tr>
<th></th>
<th>Specificity at 85% sensitivity</th>
<th>Specificity at 90% sensitivity</th>
<th>Specificity at 95% sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borderline vs benign</td>
<td>23.9%</td>
<td>19.9%</td>
<td>11.9%</td>
</tr>
<tr>
<td></td>
<td>87.0%,* 8.7% †</td>
<td>86.5%,* 5.4% †</td>
<td>87.1%,* 4.5% †</td>
</tr>
<tr>
<td>Invasive vs benign</td>
<td>89.2%</td>
<td>82.4%</td>
<td>52.3%</td>
</tr>
<tr>
<td></td>
<td>22.4%,* 7.1% †</td>
<td>30.7%,* 5.2% †</td>
<td>53.2%,* 4.2% †</td>
</tr>
</tbody>
</table>

*False-positive rate.
†False-negative rate.
We found that high levels of uPAR(I) in the plasma samples correlated with poor survival of the patients. In fact, uPAR(I) was an independent marker of poor prognosis in multivariate analyses. The level of uPAR(I) reflects the activity of uPA, because uPA has been shown to cleave uPAR with high efficiency on the cell surface both in vitro and in vivo (17–19). Interestingly, high uPA levels in tumor tissue extracts reportedly associate with poor histologic differentiation (39) and with short progression-free and overall survival in patients with primary ovarian cancer all stages (n = 82; ref. 43). In contrast, the plasma level of the other cleavage product suPAR(II-III) is dependent both on uPA-mediated cleavage of uPAR(I-III) at the cell surface and on subsequent shedding of uPAR(II-III) from the cell surface.

The long-term follow-up time and the consistent treatment regimes in this study are advantages, which increase reliability. Overall survival was chosen as the only endpoint, because progression-free survival is dependent on variables such as follow-up intervals and other variables chosen to indicate progression (increased CA125, CT scan findings, positive cytology or histopathology, or use of follow-up symptom questionnaires). Furthermore, death among patients diagnosed with ovarian cancer is to a large extent related to progression of the malignant disease. The Swedish Population Register, which includes all citizens, made a complete follow-up of all the patients. However, the results of the multivariate analysis should be interpreted with caution, because the power of the study is limited with 49 events in the analyses. Future studies...

Fig. 3. Kaplan-Meier estimates of survival probabilities using peripheral blood concentrations dichotomized by the median of suPAR(I-III) + suPAR(II-III) (A), suPAR(I-III) (B), suPAR(II-III) (C), and uPAR(I) (D) dichotomized by the detection limit. P value is the log-rank statistic with HR (95% CI) calculated using the Cox proportional hazards model. The number of patients at risk in each stratum at time 0 and 60 mo after surgery are shown below the axis with the number of deaths (events) to the left.
Table 5. Multivariate Cox regression analysis models of overall survival estimating HR for included variables

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Model 1</th>
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<th>Model 2</th>
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<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
<td>HR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>suPAR(1)</td>
<td>1.58 (1.01-2.48)</td>
<td>0.046</td>
<td>2.02 (1.33-3.07)</td>
<td>0.0009</td>
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<tr>
<td>CA125</td>
<td>0.84* Not included</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age &gt;70 vs &lt;70</td>
<td>0.06* 2.66 (1.37-5.16)</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td>0.21*</td>
<td></td>
<td>0.07*</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>0.65*</td>
<td></td>
<td>0.69*</td>
<td></td>
</tr>
<tr>
<td>Residual tumor</td>
<td>4.64 (2.37-9.11) &lt;0.0001 3.94 (2.16-7.20) &lt;0.0001</td>
<td></td>
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*P value to include in model.

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# Cleaved Forms of the Urokinase Plasminogen Activator Receptor in Plasma Have Diagnostic Potential and Predict Postoperative Survival in Patients with Ovarian Cancer

Emir Henic, Christer Borgfeldt, Ib Jarle Christensen, et al.

*Clin Cancer Res* 2008;14:5785-5793.

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