Abstract. The level of the urokinase plasminogen activator receptor (uPAR) is elevated in tumor tissue from several forms of cancer. uPAR is shed from the cell surface and the soluble form, soluble urokinase plasminogen activator receptor (suPAR), has been detected in several body fluids. High plasma levels of suPAR in patients with colorectal cancer and high serum levels of suPAR in patients with recurrent metastatic breast cancer have been associated with poor prognosis. In patients with ovarian cancer (OC) it has been shown that the level of suPAR is very high in ascites and cystic fluid and that high serum levels of suPAR were associated with shorter survival of the patients. We evaluated suPAR preoperatively in plasma from primary OC stage III patients and tested for association with prognosis. The prognostic significance of suPAR was also compared to two biochemical markers; cancer antigen 125 (CA125) and tetranectin (TN). No significant differences were found between patients who died of OC compared to patients still alive regarding median plasma suPAR levels (p=0.62) and median serum CA125 levels (p=0.26). In contrast, a significant difference was found between dead and alive OC patients for the median serum TN level (p<0.0001). Dividing the patients into two groups, corresponding to preoperative plasma suPAR levels below or equal to 2.0 ng/ml and higher than 2.0 ng/ml, no significant difference in survival was found between the two groups (p=0.49). When different cut-off levels of plasma suPAR were considered (2.74 ng/ml, 3.25 ng/ml and 4.18 ng/ml), no significant differences in survival could be detected (p=0.58, p=0.68 and p=0.05). Multivariate Cox regression analysis showed that the only independent prognostic factors were radicality after primary surgery (RH=5.34; 95% CI, 2.34-12.20; p<0.0001) and preoperative serum TN (RH=0.69, 95% CI, 0.57-0.82; p<0.0001), whereas plasma suPAR (4.18 ng/ml), age, histological type of tumour and serum CA125 had no independent prognostic value. In conclusion, preoperative plasma suPAR level was of no prognostic value in this cohort of Danish stage III OC patients.

Ovarian cancer (OC) is the most lethal of the gynaecologic malignancies, ranking the fifth most frequent female cancer type and the fourth most frequent cause of death from cancer among women in Denmark (1). Every year, nearly 600 new cases appear, yielding an age-standardized incidence rate of 13.2 per 100,000 (1997) (2, 3), which is among the highest incidence rates observed worldwide. Published five-year survival rates for OC patients range from more than 80 percent for FIGO (The International Federation of Gynaecology and Obstetrics) stage I OC to less than 20 percent for stage III and IV OC’s (4, 5).

uPAR (urokinase plasminogen activator receptor) is a cell surface glycoprotein with a molecular mass of 55-60 kd, composed of three homologous domains of approximately 90 amino acids each (6-8). The protein is, through the
carboxy-terminal attachment on domain 3 of a glycosyl phosphatidyl-inositol moiety, attached to the cell membrane of a variety of cells such as fibroblasts, neutrophils, and macrophages (9-11) and, in some, cases cancer cells (12, 13). The ligand uPA (urokinase plasminogen activator) catalyses the conversion of plasminogen to plasmin (14). The uPA-mediated plasminogen activation system is active in the tissue remodelling processes that occur under various normal physiological conditions and in connection with cancer invasion (15, 16). It has been reported that cells in tumour progression express uPAR at a higher level compared to the level expressed by normal cells (7).

suPAR (soluble urokinase plasminogen activator receptor) is the soluble form of uPAR and is shed from the cell surface by an, as yet, unidentified mechanism. It has been shown that high expression levels of extra-cellular suPAR within the OC cell significantly reduce tumour cell growth and cancer progression in vivo (18, 19).

Lower levels of plasma suPAR have been found in blood from healthy individuals, compared to the levels of suPAR found in plasma from certain types of cancer, among these OC (20). High levels of suPAR in preoperatively collected serum are significantly related to shorter survival of recurrent metastatic breast cancer patients (21) and one study has shown that high levels of plasma suPAR are related to shorter survival of rectal cancer patients (22). In patients with OC it has been shown that the level of suPAR is very high in ascites and cystic fluid (23) and, furthermore, that high serum levels of suPAR were associated with shorter survival of the patients (20).

The aim of the study was to examine whether the plasma suPAR levels correlate with clinicopathological parameters and to evaluate the prognostic value of preoperative plasma suPAR levels in a homogeneous group of 47 Danish OC stage III patients.

Materials and Methods

Study design. According to the study design preoperative blood samples were left on the clot or on the blood cells at room temperature and sent from all the participating hospitals to Statens Serum Institute in Copenhagen, Denmark. Here serum and plasma were separated by centrifugation at 2000 g for 10 minutes and serum and plasma samples were stored at -80°C in aliquots until analyses were performed.

Serum and plasma suPAR levels are dependent on the method of sample collection. As reported in a previous study, blood samples must be processed into plasma (EDTA) within 8 hours at room temperature or into serum in less than 3 hours at room temperature. If this is not possible, the blood samples must be stored at 4°C until processed. In the MALOVA study (‘MALignant OVarian cancer study’) a total of 108 patients with stage III OC were enrolled from hospitals close to Statens Serum Institute. However, only 47 blood samples had been processed into plasma (EDTA) within 8 hours from venipuncture (24).

Patients. The study comprised 47 women (median age 64 years, range 37-77 years) with a reviewed FIGO stage III (5 stage IIIa, 8 stage IIIb and 34 stage IIIc) epithelial OC included in the MALOVA study, covering 4 undifferentiated adenocarcinoma, 4 papillary adenocarcinoma, 33 serous adenocarcinoma, 3 mucinous adenocarcinoma and 3 endometroid adenocarcinoma. The patients were included from December 1994 to May 1999 and at the follow-up (November 2001), a total of 34 patients had died of OC (median follow-up time: 21 months, range 1-80 months) and 13 patients were still alive (median follow-up time: 55 months, range 44-80 months). MALOVA study. The MALOVA study is a multidisciplinary Danish study on OC, covering epidemiology (lifestyle factors), biochemistry and molecular biology with the purpose of identifying risk factors and prognostic factors for OC, described in detail elsewhere (25-27). Briefly, preoperative blood samples as well as tumour tissue samples were obtained from most of these patients. Histopathological classifications of the ovarian tumours were based on the typing criteria of the WHO (World Health Organization). Pathology reports and tissue specimens were collected from the different participating hospitals and reviewed blindly by one pathologist specialized in gynaecologic tumours. FIGO stages were obtained from clinical records and were reviewed by two gynaecologists, both specialized in OC. The Science Ethics Committees in the study area, KF01-384/95, had approved the study. After reviewing procedures, a total of 683 OC and 235 ovarian borderline tumours had been enrolled in the MALOVA study.

In Denmark all inhabitants are registered in the computerized Central Population Register with a unique personal identification number, which comprises information on date of birth, sex, dates of death and emigration. Cases in this study were traced in this register and followed until date of death or follow-up (November 2001). Women who died during follow-up were linked to a Danish hospital reference system and information about the last hospital admission was obtained. The relevant hospital files were collected and scrutinized and the cause of death was registered.

Biochemical analysis. Plasma suPAR concentrations were determined by an ELISA analysis (kinetic enzyme-linked immunosorbent assay) (24). The intra-assay coefficient of variation (CV) of an EDTA-plasma pool was 9.6% whereas the inter-assay CV of two controls was 5.0% (3.8 ng/ml, N=30) and 5.6% (2.1 ng/ml, N=21), respectively.

Serum levels of the tumour-associated antigen CA125 were determined by immunoassay (EIA) (Abbott CA125-EIA, Abbott Laboratories, Chicago, IL, USA) according to the manufacturer’s
No significant differences were found, respectively, between patients who died of OC and patients still alive, for the median suPAR level in plasma from patients with an OC of another histological type (median 3.65 ng/ml, range 1.87-7.16 ng/ml, N=14) (p=0.30).

When dividing the patients into two groups with respect to substage of disease, stage III (a+b) (N=13) and stage IIIc (N=34), no significant difference in median plasma suPAR levels were found (p=0.44).

Univariate survival analysis showed that serum TN levels below 6.0 mg/L were significantly correlated to shorter survival of OC patients (p<0.0001), whereas serum CA125 levels showed no significant differences in survival between patient groups (p=0.56).

When different cut-off levels of plasma suPAR were considered (2.0 ng/ml, 2.74 ng/ml, 3.25 ng/ml and 4.18 ng/ml), no significant differences in survival could be detected (p=0.49, p=0.58, p=0.68 and p=0.05). As the cut-off of 4.18 ng/ml led to an almost significant result (p=0.05), this cut-off limit for plasma suPAR was used in a multivariate Cox regression analysis including 41 of the stage III OC patients (in 6 patients there was not enough serum for CA125 and TN analyses). The analysis showed that the only independent prognostic factors were radicality after primary surgery (macroscopically optimal vs. suboptimal initial cytoreductive surgery) (RH=5.34; 95% CI, 2.34-12.20; p<0.0001) and preoperative serum TN (RH=0.69; 95% CI, 0.57-0.82; p<0.0001) whereas plasma suPAR (p=0.53), age (p=0.16), histological type of the tumour (p=0.88) and serum CA125 (p=0.28) had no independent prognostic value (Table II). Finally, no significant correlation between plasma suPAR and serum TN levels was found (Spearman’s rho test p=0.29).

Discussion

No prognostic factors of OC have yet been shown to be unequivocally effective though several studies on the subject have been published (20, 28, 32-34). It has been reported that the concentration of suPAR in cystic fluid from OC patients was extremely high, approximately 10-fold higher than that in blood from the patients (23). In contrast to the cell bound uPAR, the biological function of suPAR, if any, in cancer progression is not known. Cell bound uPAR binds uPA at the cell surface and activates plasminogen to plasmin. Plasmin degradation of several extra cellular matrix components and basement membrane contributes to the pathological processes of cancer cell invasion and metastasis, which require tissue destruction and cell migration. TN stimulates the activity of the tissue-type plasminogen activator (tPA) (35). The enhanced activation is suggested to be caused by TNs ability to bind and accumulate tPA in an active conformation (35). TN could thereby also be related to cancer by the plasminogen system (36) and has, in previous studies, been found to be a strong prognostic marker in OC (37, 38). Our results showed preoperative serum TN

---

**Table II. Results of Cox regression analysis including 41 of the stage III OC patients**.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>RH</th>
<th>95% CI</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radicality</td>
<td>5.34</td>
<td>2.34-12.20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tetranectin</td>
<td>0.69</td>
<td>0.57-0.82</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>suPAR</td>
<td>0.90</td>
<td>0.25-2.52</td>
<td>NS</td>
</tr>
<tr>
<td>CA125</td>
<td>1.00</td>
<td>1.00-4.00</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td>1.01</td>
<td>0.98-1.11</td>
<td>NS</td>
</tr>
<tr>
<td>Histology</td>
<td>0.96</td>
<td>0.63-1.54</td>
<td>NS</td>
</tr>
</tbody>
</table>

*: In 6 patients there was not enough serum for CA125 and TN analyses
**: NS; not significant

---

Begum et al.: Prognostic Value of suPAR Levels in OC Patients

---

The median preoperative level of plasma suPAR in the stage III OC patients was 3.25 ng/ml (range 1.87-7.16 ng/ml, N=47). No significant differences were found, respectively, between patients who died of OC and patients still alive, for the median plasma suPAR level (p=0.62) and for median serum CA125 level (p=0.26). In contrast, a significant difference in the preoperative median serum TN values was found in patients who died during follow-up and in patients who stayed alive (p=0.0001) (Table I).

No significant difference was found in the median suPAR level in plasma from patients with a serous adenocarcinoma (median 3.05 ng/ml, range 1.97-5.45 ng/ml, N=33) compared to the median suPAR level in plasma from patients with another histological type (median 3.65 ng/ml, range 1.87-7.16 ng/ml, N=14) (p=0.30).

When dividing the patients into two groups with respect to substage of disease, stage III (a+b) (N=13) and stage IIIc (N=34), no significant difference in median plasma suPAR levels were found (p=0.44).

Univariate survival analysis showed that serum TN levels below 6.0 mg/L were significantly correlated to shorter survival of OC patients (p<0.0001), whereas serum CA125 levels showed no significant differences in survival between patient groups (p=0.56).

When different cut-off levels of plasma suPAR were considered (2.0 ng/ml, 2.74 ng/ml, 3.25 ng/ml and 4.18 ng/ml), no significant differences in survival could be detected (p=0.49, p=0.58, p=0.68 and p=0.05). As the cut-off of 4.18 ng/ml led to an almost significant result (p=0.05), this cut-off limit for plasma suPAR was used in a multivariate Cox regression analysis including 41 of the stage III OC patients (in 6 patients there was not enough serum for CA125 and TN analyses). The analysis showed that the only independent prognostic factors were radicality after primary surgery (macroscopically optimal vs. suboptimal initial cytoreductive surgery) (RH=5.34; 95% CI, 2.34-12.20; p<0.0001) and preoperative serum TN (RH=0.69; 95% CI, 0.57-0.82; p<0.0001) whereas plasma suPAR (p=0.53), age (p=0.16), histological type of the tumour (p=0.88) and serum CA125 (p=0.28) had no independent prognostic value (Table II). Finally, no significant correlation between plasma suPAR and serum TN levels was found (Spearman’s rho test p=0.29).

Discussion

No prognostic factors of OC have yet been shown to be unequivocally effective though several studies on the subject have been published (20, 28, 32-34). It has been reported that the concentration of suPAR in cystic fluid from OC patients was extremely high, approximately 10-fold higher than that in blood from the patients (23).

In contrast to the cell bound uPAR, the biological function of suPAR, if any, in cancer progression is not known. Cell bound uPAR binds uPA at the cell surface and activates plasminogen to plasmin. Plasmin degradation of several extra cellular matrix components and basement membrane contributes to the pathological processes of cancer cell invasion and metastasis, which require tissue destruction and cell migration. TN stimulates the activity of the tissue-type plasminogen activator (tPA) (35). The enhanced activation is suggested to be caused by TNs ability to bind and accumulate tPA in an active conformation (35). TN could thereby also be related to cancer by the plasminogen system (36) and has, in previous studies, been found to be a strong prognostic marker in OC (37, 38). Our results showed preoperative serum TN
levels to be of prognostic value in contrast to plasma suPAR levels. Another study has shown that both log serum TN and log plasma suPAR have independent prognostic value for survival in colorectal cancer patients (39). This study showed that colorectal cancer patients with a combination of low serum TN levels and elevated plasma suPAR levels had a significant 2.43-increased risk of dying compared to colorectal cancer patients with median levels of serum TN and plasma suPAR (39). The reduced TN level in colorectal cancer and OC patients is possibly due to an uptake of TN from the blood to the extracellular matrix in the stroma of most proteolytically active cancers.

Even though the biological role of suPAR still remains unclear, some potential functions have been indicated in recent studies; Chavakis et al. (40) showed that vitronectin could concentrate the uPA/suPAR-complex to cell surfaces and extracellular matrix, which leads to the accumulation of plasminogen activator activity required for cell migration and tissue remodelling. Resnati et al. (41) found that suPAR was capable of inducing chemotaxis in cells. However, this activity required proteolytic cleavage of suPAR between domain 1 and domain 2. Through its interaction with uPAR binding to vitronectin, PAI-1 can facilitate cell migration. The role in cell migration of suPAR could indicate that it was related to metastatic spread. Nevertheless, in an in vitro study, Wilhelm et al. (18) showed that suPAR can serve as a scavenger for uPA, thereby inhibiting cell proliferation and in vitro invasion of human cells.

Patients with a serous adenocarcinoma had lower plasma suPAR levels compared to patients with a tumour of another histology. The highest median plasma suPAR levels were found in patients with undifferentiated adenocarcinoma and papillary adenocarcinoma, which are also known to be histology groups with a generally severe prognosis (42). However, multivariate Cox regression analysis showed that the histological type of tumour had no independent prognostic value of survival. These results can be explained by the fact that the studied population includes small number of patients in each histological subgroup, which limits the power of this analysis.

In summary, our study indicates that preoperative measurement of plasma suPAR level in Danish stage III OC patients may not be useful to identify a subgroup of patients with poor prognosis.

Acknowledgements

The expert technical assistance of Vibeke Reese, Department of Clinical Biochemistry, Statens Serum Institute, Copenhagen, is greatly appreciated. All nurses and doctors on the gynaecological and pathological wards are thanked for their tremendous work. This study was supported by grants from Erik Hørsløv and hustru Birgit Hørsløvs Fond, Arvid Nielsøns’ Fond, Overlæge Johan Boserup og Lise Boserups Legat, Apotekerfonden af 1991, Harboe Fonden, Civilingenier Bent Bøgh og hustru Inge Bøgh Fond, Direktor Michael Hermann Nielsens mindelegat, afd B, The Danish Cancer Society and by The National Cancer Institute (RO1 CA 61107).

References


