9. Fisher ER, Costantino J, Fisher B, Redmont C and Collaborating National Surgical Adjuvant Breast and Bowel Project investigators. Pathologic findings from the National Surgical Adjuvant Breast Project (Protocol 4). Discriminants for 15-year survival. *Cancer* 1993, 71, 2141–2150.
10. Elwood JM, Moorehead WP. Delay in diagnosis and long-term survival in breast cancer. *Br Med J* 1980, **280**, 1291–1294.
11. Fisher ER, Redmond C, Fisher B. A perspective concerning the relation of duration of symptoms to treatment failure in patients with breast cancer. *Cancer* 1977, **40**, 3160–3167.
12. Layfield LJ, Glasgow BJ, Cramer H. Fine-needle aspiration in the management of breast masses. *Pathol Annu* 1989, **24**, 23–62.
13. Pain JA, Ebbs SR, Hern RP, Lowe S, Bradbeer JW. Assessment of breast cancer size: a comparison of methods. *Am J Roentgenol* 1992, **18**, 44–48.

Acknowledgements—The study was supported by Cancer Society of Finland, Turku University Foundation and Academy of Finland. The study was carried out by the Finnish Breast Cancer Study Group.

Pergamon

European Journal of Cancer Vol. 30A, No. 9, pp. 1302–1309, 1994

0959-8049/94 $7.00+0.00

0959-8049(94)E0018-Y

Plasminogen Activators and Plasminogen Activator Inhibitors in Blood and Tumour Fluids of Patients with Ovarian Cancer

B. Casslén, T. Bossmar, I. Lecander and B. Åstedt

We quantitated urokinase and tissue plasminogen activator (u-PA, t-PA), plasminogen activator inhibitor 1 and 2 (PAI-1, PAI-2), and fibrinolytic activity in peripheral blood (PB), tumour blood (TB), peritoneal/ascitic fluid (PAF) and cystic fluid (CF) from 104 patients with benign and 36 patients with malignant ovarian tumours, and in peripheral blood from 62 healthy controls. PB levels of u-PA were higher in patients with benign and malignant tumours than in controls. High concentrations of u-PA were found in CF, but not in TB, suggesting that u-PA is released by the tumour tissue, but not by the tumour vasculature. PB levels of t-PA were higher in both tumour groups than in controls. Increased levels of t-PA were found in TB, but not in CF, indicating that t-PA is released by the tumour vasculature, but not by the tumour tissue. PB levels of PAI-1 were higher in patients with both benign and malignant tumours than in controls. High levels of PAI-1 were present in both TB and CF from malignant tumours, suggesting that PAI-1 is released from the tumour vasculature as well as the tumour tissue. Elevated concentrations of PAI-2 were found in CF, but not in TB, indicating release from the tumour tissue, but not from the vasculature. High levels of t-PA, PAI-1 and PAI-2 were found in PAF of malignant tumours, and resorption from this compartment may explain elevated PB levels in patients with ascites. None of the PAs/PAIs proved useful as a PB marker for detection of early stage ovarian cancer. However, an index based on PAF levels of t-PA and PAI-1 discriminated between malignant and benign ovarian cysts in the absence of ascites. In addition, our study stresses the importance of including patients with benign tumours as well as healthy controls when markers for malignant tumours are evaluated.

Key words: ovarian cancer, ovarian cyst, ascites, plaminogen activator, plasminogen activator inhibitor, tumour marker

Eur J Cancer, Vol. 30A, No. 9, pp. 1302–1309, 1994

INTRODUCTION

TUMOUR GROWTH and spread involves proteolytic degradation of surrounding intercellular matrix by tumour cells. Several enzymes, e.g. collageneses, elastase, cathepsins and plasmin, are involved in this process [1]. Plasmin has a central function, since it acts both directly on certain matrix proteins i.e. fibronectin, laminin, entactin and fibrin, and indirectly as an activator of latent collagenases [2, 3]. Plasmin is formed after activation of the zymogen plasminogen, which is abundant throughout the extracellular space. Activation is catalysed by two specific serine proteases, tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA). t-PA is synthesised in a number of cell types, like vascular endothelial cells, macrophages and some tumour cells [4]. It binds with high affinity to fibrin, and is mainly involved in fibrinolysis and thrombolysis. u-PA, in contrast, is synthesised by many cell types, such as tumour cells and macrophages, during proliferation and migration [4]. It binds to specific receptors on the cell membrane, and activation

of plasminogen takes place on the cell surface. Tumour tissues have a higher content of extractable u-PA (5–8) and a higher number of u-PA receptors [9–11] than normal tissues. Receptor binding of u-PA is crucial for the invasive growth potential of tumour cells, and inhibition of receptor-bound u-PA reduces such growth [12–14].

The activity of u-PA and t-PA is balanced by specific plasminogen activator inhibitors (PAIs). PAI-1 is the main inhibitor of t-PA, and thereby the main regulator of vascular thrombolysis and fibrinolysis. It is also the main regulator of u-PA-induced degradation of extracellular matrix during processes like tissue growth and remodelling [15]. PAI-1 is produced by a number of cell types, including various tumour cells and endothelial cells, and it is stored as an integrated part of the extracellular matrix. PAI-2, the other inhibitor of both t-PA and u-PA, has a slightly different inhibitory profile to PAI-1 [15, 16]. PAI-2 is found in the trophoblastic tissue of the placenta, in pregnancy plasma [17, 18], and in monocyte macrophages, partly accumulated intracellularly and partly released [19, 20]. It is also found in ovarian tumours and ascitic fluid [21, 22]. The PAI-2 content of tumour tissue may indicate presence of tumour macrophages, since high levels are reportedly associated with good prognosis in breast cancer [23].

The identity of tumour plasminogen activator with u-PA was first described in cultures of ovarian cancer tissue, and these tumours were shown to have more membrane receptors for u-PA than benign ovarian tumours [10, 24]. PA activity is localised to the blood vessels in ovarian tumours, and it correlates with vascularity of the tumours [25]. Ovarian tumours release thromboplastic activity, and fibrin deposits are frequently associated with the tumour [26, 27]. Degradation of such fibrin is initiated by t-PA and results in fibrin degradation products, which are present in high concentrations in ascitic fluid and in detectable levels in peripheral blood of these patients [28].

Ovarian cancer has a good prognosis in early stages, but a poor prognosis in late stages. It is responsible for the majority of deaths in gynaecological malignancies in western countries. This is primarily due to the fact that most tumours are diagnosed in advanced stages, but also to the fact that long-term survival has not increased much despite extensive use of chemotherapy [29]. Improved detection of the early stages of ovarian cancer would be a significant step towards reducing mortality and morbidity rate in this disease. So far, however, no method has proved both specific and sensitive enough, and there is an ongoing search for better screening methods [30, 31].

This study explored the plasminogen activating system in malignant and benign ovarian tumours. The concentration of each component was assayed in peripheral and tumour blood, and in cystic and peritoneal/ascitic fluid. The objective of the study was primarily to understand the function and significance of the plasminogen activating system in ovarian tumours, but also to analyse whether any component can possibly be used as a marker for early stages of ovarian cancer, or to discriminate between benign and early malignant tumours.

MATERIALS AND METHODS

The clinical material consisted of patients admitted for surgery for ovarian tumours. Altogether 140 patients with primary ovarian tumours were included in the study. Classification of the patients according to the histopathological diagnosis (FIGO) and distribution in the pre- and postmenopausal groups is given in Table 1. Borderline tumours were included as benign tumours, unless otherwise stated. Peripheral blood samples from 62 healthy women served as a control group. This control group included blood donors and postmenopausal women, who had passed a gynaecological screening programme, including transvaginal ultrasonographic examination.

Table 1. Histopathological classification (FIGO) of ovarian tumours in 75 premenopausal (preMP) and 65 postmenopausal (postMP) patients

	Histology	PreMP	PostMP	Total
Benign ovarian tumours				
Neoplastic	Serous	8	8	16
	Mucinous	17	5	22
	Adenofibroma	6	8	14
	Brenner	0	1	1
	Simple cyst	5	8	13
	Granulosacell	0	1	1
	Fibroma	0	3	3
	Dermoid	5	4	9
	Total	41	38	79
Functional	Follicular	12	0	12
	Corpus luteum	13	0	13
	Total	25	0	25
	Sum total	66	38	104
Malignant ovarian tumours				
Neoplastic	Serous	5	14	19
	Endometroid	1	7	8
	Mucinous	1	4	5
	Clear cell	1	0	1
	Mixed Müllerian	0	1	1
	Teratoma	1	1	2
	Total	9	27	36

Hormonal status was classified on the basis of menstrual data, age, peripheral blood levels of oestradiol and progesterone, and endometrial histology, whenever hysterectomy was performed [32]. Postmenopausal patients had 1 year or more of amenorrhoea. Premenopausal patients were subgrouped as being in the perimenopausal period (irregular bleedings, age 46–54 years), in the menstrual phase (ongoing menstruation), in the follicular phase (serum progesterone < 8 nmol/l and proliferative endometrial histology), in the luteal phase (progesterone ≥ 8 nmol/l and secretory endometrial histology), or being oral contraceptive users. The study was approved by the review board for studies on human subjects at the University Hospital of Lund.

Blood and fluid sampling

Peripheral blood samples were obtained the day before surgery. Peritoneal/ascitic fluid was aspirated immediately as the abdominal cavity was opened. Careful haemostasis prevented contamination of the samples with blood. The total volume of fluid was measured. Ascites was defined as fluid volumes ≥ 100 ml. Tumour blood samples were aspirated from veins on the surface of the tumours. Cystic fluid was aspirated after removal of the tumour from the abdominal cavity. All blood and fluid samples were immediately centrifuged at 2000 *g* for 20 min, and frozen at −20°C until assayed.

Correspondence to B. Casslén.
The authors are at the Department of Obstetrics and Gynecology, University Hospital, S-221 85 Lund, Sweden.
Revised 19 Nov. 1993; accepted 25 Nov. 1993.

Assay methods

u-PA was measured with a commercial kit, Chromolize™ u-PA (Biopool, Umeå, Sweden), in the blood samples. This activity assay measures single chain u-PA and two chain u-PA, but not u-PA in complex with PAI. Some of the blood samples were also assayed with TintElize™ u-PA (Biopool), which measures free as well as complexed u-PA. All peritoneal/ascitic fluid and cystic fluid samples, having higher concentrations of u-PA, were assayed in a radioimmunoassay, which measures both free and complexed u-PA [33].

t-PA was assayed in a commercial ELISA-kit, Imulyse™ t-PA (Biopool) which, according to the manufacturer, measures free t-PA as well as t-PA in complex with PAI-1.

PAI-1 was assayed in a commercial ELISA-kit, Imulyse™ PAI-1 (Biopool), which detects the active and latent forms of PAI-1, as well as PAI-1 in complex with PAs.

PAI-2 was assayed in an ELISA, which detects free PAI-2, and about 60% of PAI-2 in complex with PAs [34]. The detection level was 5 μg/l. Blood concentrations are given as the percentage of patients with detectable levels of PAI-2 in each group. For the calculation of PAI-2 levels in cystic fluid and peritoneal/ascitic fluid, each sample without detectable PAI-2, i.e. PAI-2 < 5 μg/l, was given the value 2 μg/l.

The fibrinolytic activity of blood and fluid samples was measured on plasminogen-containing fibrin plates using a standardised procedure [35]. Results were expressed as the product of two perpendicular diameters of the lytic area.

Oestradiol and progesterone were assayed in routine radioimmunoassays.

Statistical methods

The distribution within groups was often skewed, and all results are given as median and 25th–75th percentiles. Whenever Student's *t*-test was used to compare groups, we employed the logarithm of individual values to induce normality. Comparison between groups was also performed with analysis of variance. Correlation between variables was estimated with correlation coefficients. In comparing groups with high frequency of observations below detection limit, Fisher's exact test was used. When comparing levels in different compartments, we employed the Student's *t*-test for paired observations. A significance level of 5% was used, and all tests were two-sided.

RESULTS

Peripheral blood

Peripheral blood levels of u-PA did not correlate with age of the patients or histology of the tumours. Patients with benign as well as malignant tumours had higher levels than controls (Figure 1a). Postmenopausal patients with malignant tumours and ascites had lower levels than patients with benign tumours ($P = 0.005$). Premenopausal patients with benign tumours had higher ($P = 0.003$) u-PA levels than controls. In addition, postmenopausal patients in all tumour groups had higher ($P = 0.003$, $P = 0.006$, $P < 0.0001$, respectively) u-PA levels than controls.

The concentration of t-PA in peripheral blood correlated to age in the tumour groups as well as in the control group (Figure 1b). The correlation was present within both the premenopausal ($r = 0.24$; $P = 0.02$) and the postmenopausal group ($r = 0.28$; $P = 0.002$), indicating a gradual increase with age, rather than a menopause related increase. In the postmenopausal group, peripheral blood levels of t-PA were higher in patients with malignant tumours and ascites than in those without ascites

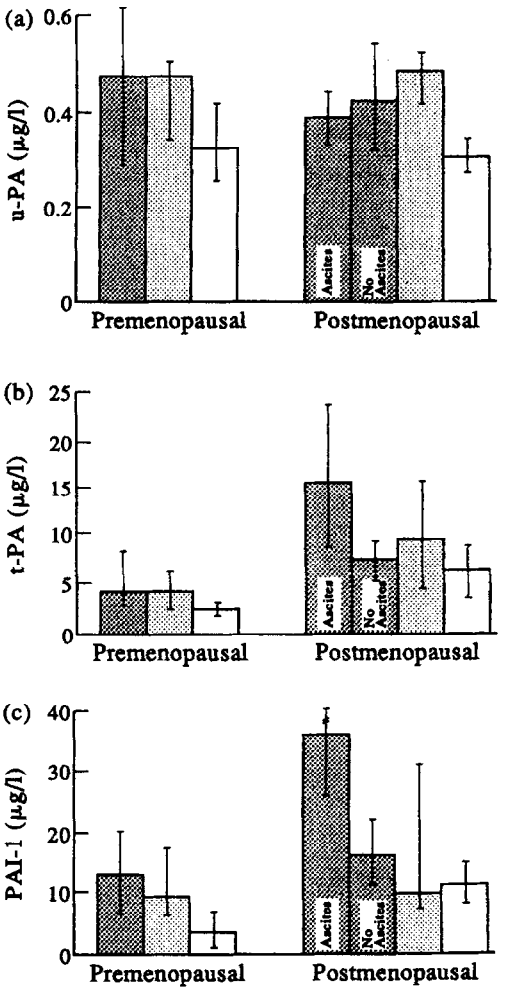

Figure 1. Peripheral blood levels of (a) u-PA, (b) t-PA and (c) PAI-1 in premenopausal and postmenopausal patients with malignant (dark grey bars) and benign (light grey bars) ovarian tumours, and in controls (white bars) are presented as median and 25th–75th percentiles. The group of postmenopausal patients with malignant tumours was further divided in those with ($n = 16$) and those without ($n = 11$) ascites. The level of u-PA did not correlate with age. There was a correlation to age for t-PA ($r = 0.49$; $P < 0.001$) and for PAI-1 ($r = 0.35$; $P < 0.001$), when all groups were included and data had been adjusted for malignancy. Age was subsequently adjusted for when groups were compared.

($P = 0.01$), in those with benign tumours ($P = 0.01$) and in controls ($P < 0.001$). The number of advanced malignancies in the premenopausal group was too low for such evaluation. Premenopausal patients with benign tumours had higher peripheral levels of t-PA ($p < 0.001$) than controls. Patients with large benign tumours tended to have high levels of t-PA.

The concentration of PAI-1 increased with age, even when the confounding effect of increasing numbers of malignant tumours with age was adjusted for (Figure 1c). This increase was mainly due to a difference between the pre- and postmenopausal groups, since no significant correlation to age was found within each group. There was no significant difference between patients with malignant and benign tumours in the premenopausal group, but both tumour groups had higher PAI-1 levels than controls ($P < 0.001$). Postmenopausal patients with malignant tumours and ascites had higher levels of PAI-1 than those without ascites ($P = 0.02$), those with benign tumours

(P = 0.001) and controls (P < 0.001). As for t-PA, patients with large benign tumours tended to have high levels of PAI-1.

PAI-2 was not detectable in the peripheral blood of most patients. However, the percentage of patients with detectable levels of PAI-2 was higher in the group of patients with malignant tumours and ascitic fluid than in the benign and control groups (Figure 2). Age and histological groups did not correlate with detection of PAI-2.

No or very little fibrinolytic activity was found in peripheral blood, and there was no difference between the groups.

Tumour blood

Tumour blood was obtained from 83 patients; 8 malignant and 38 benign tumours in the premenopausal group; 16 malignant and 21 benign tumours in the postmenopausal group. Tumour blood levels were compared to the corresponding peripheral blood levels.

There was no significant difference between the levels of u-PA in tumour blood and peripheral blood for any of the groups. The median of tumour blood/peripheral blood ratios was 0.93 in the malignant group and 0.98 in the benign group. In order to exclude the possibility that u-PA was undetected due to complex formation with any of the PAIs, the level of u-PA in tumour blood and peripheral blood was assayed further with the TintElize™ assay. The concentrations obtained with the TintElize™ assay did not differ significantly from those obtained with Chromolize™.

The concentration of t-PA was not significantly different between blood obtained from malignant and benign tumours. The level in tumour blood was, however, higher than that in corresponding peripheral blood in all patient groups (premenopausal patients: malignant, P = 0.02; benign, P < 0.001; postmenopausal patients, benign, P = 0.004) except the postmenopausal malignant group, where the peripheral blood levels were as high as the tumour blood levels (Figure 3a).

PAI-1 levels were significantly higher (P = 0.005) in blood from malignant than from benign tumours (Figure 3b), and tumour blood concentrations were also higher than peripheral blood concentrations in patients with malignant tumours (P = 0.002 for premenopausal; P = 0.002 for pre- and postmenopausal together). High concentrations in tumour blood were not related to the presence of ascitic fluid. No significant difference was found between tumour and peripheral blood levels for patients with benign tumours.

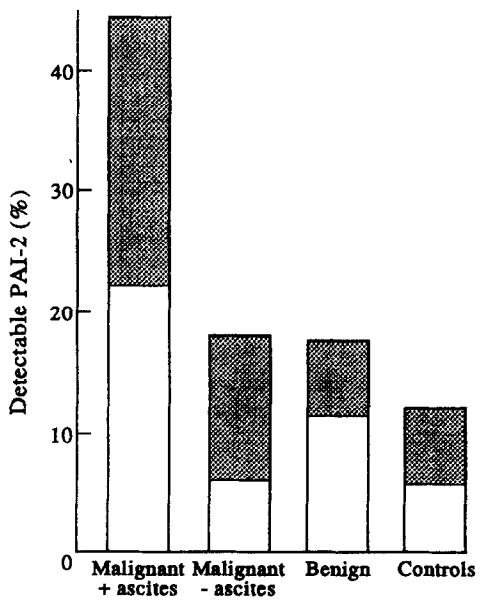

Figure 2. The percentage of detectable levels of PAI-2 (open bars: 5–9 μg/l; filled bars: ≥ 10 μg/l) was higher in patients with malignant tumours and ascites than in patients with benign tumours (P = 0.03), and in controls (P = 0.004).

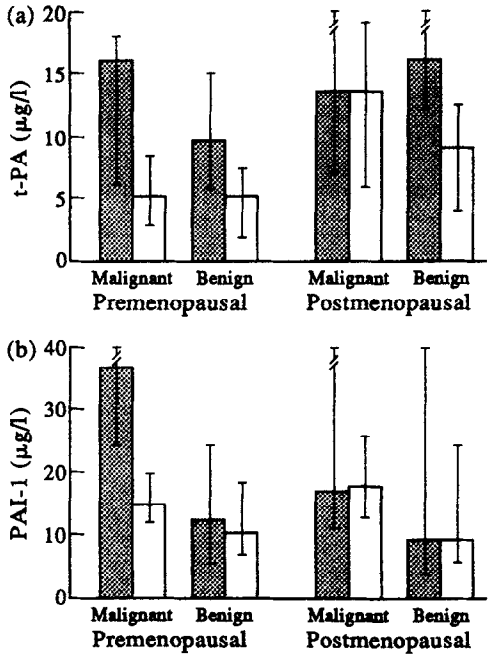

Figure 3. Concentrations of (a) t-PA and (b) PAI-1 in tumour blood (filled bars) and peripheral blood (open bars).

The percentage of detectable PAI-2 was not significantly higher in tumour blood samples than in peripheral blood samples for any of the groups.

The fibrinolytic activity of tumour blood was not significantly higher than that of peripheral blood.

Peritoneal/ascitic fluid

This was obtained from 34 patients with malignant tumours, and 78 patients with benign tumours. Apart from fibrinolytic activity (see below), there was no significant correlation to age or stage of the menstrual cycle for any of the parameters, and none correlated to histology of the tumour.

The concentration of u-PA in peritoneal/ascitic fluid was not significantly different between patients with malignant and benign tumours (Figure 4a). The level was, however, higher than that of peripheral blood in both groups (P < 0.001).

The t-PA concentration was much higher in the peritoneal/ascitic fluid of malignant than benign tumours (Figure 4b). However, high levels of t-PA did not correlate with high levels of PAI-1 or PAI-2, nor did they correlate with poor tumour differentiation, but tended to be higher in more advanced stages of the disease (Figure 5). The peritoneal/ascitic fluid levels were higher than the peripheral blood levels both in patients with benign (P < 0.001) and malignant (P < 0.001) tumours.

The level of PAI-1 was higher in peritoneal/ascitic fluid from malignant than benign ovarian tumours (Figure 4c). Within the group of malignant tumours, the level correlated to histological differentiation of the tumour (Figure 6a) with poor differentiation correlating to high levels of PAI-1 (P = 0.005), but not to the clinical stage of the disease. However, the concentration was higher (P = 0.04) in the presence (55 μg/l range 37–146)

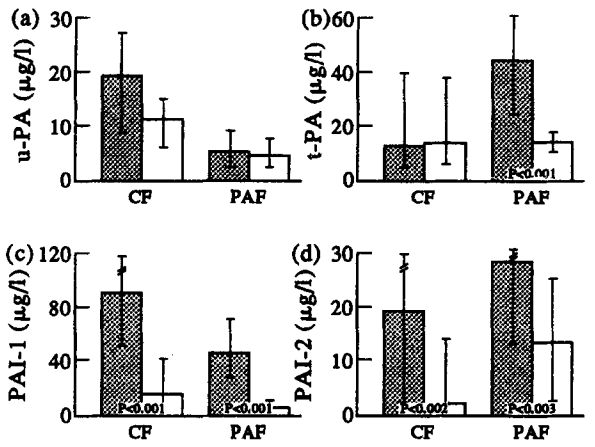

Figure 4. Concentrations of (a) u-PA, (b) t-PA, (c) PAI-1, and (d) PAI-2 in cystic fluid (CF) and peritoneal/ascitic fluid (PAF) of malignant (filled bars) and benign (open bars) ovarian tumours. Higher concentrations for malignant than benign tumours are indicated with p values in the figure. A similar pattern with higher concentrations in cystic fluid than in peritoneal/ascitic fluid was seen for u-PA (malignant $P < 0.001$, benign $P < 0.001$) and PAI-1 (malignant non-significant, benign $P < 0.001$). The opposite pattern with higher levels in peritoneal/ascitic fluid that in cystic fluid was seen for PAI-2 (malignant non-significant, benign $P = 0.003$), and for t-PA (malignant $P = 0.001$).

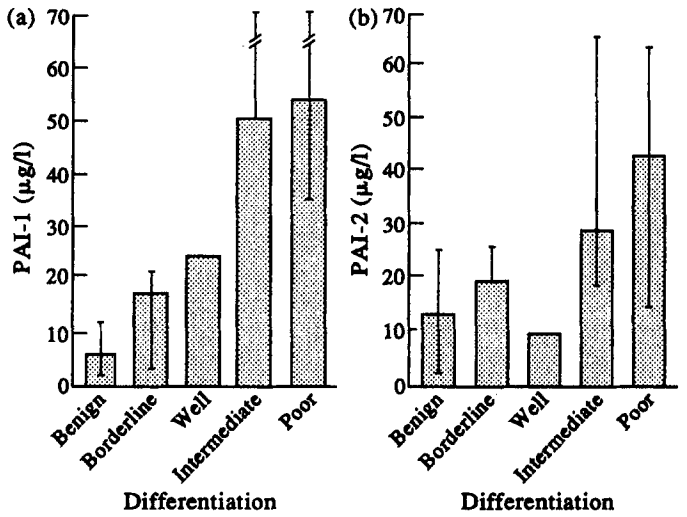

Figure 6. The levels of (a) PAI-1 and (b) PAI-2 in peritoneal/ascitic fluid from benign and malignant ovarian tumours are grouped according to histological differentiation of the tumour. Benign tumours were separated into benign ($n = 97$) and borderline ($n = 5$) groups, and malignant tumours were separated into well differentiated ($n = 2$), intermediately differentiated ($n = 15$) and poorly differentiated ($n = 19$). Borderline and true malignant tumours were tested for trend. There was a correlation between the levels of PAI-1 and PAI-2 in peritoneal/ascitic fluid of malignant tumours ($r = 0.54$, $P = 0.002$).

than in the absence (35 μg/l, range 23–64) of ascitic fluid. In patients with malignant tumours, the level was higher ($P < 0.001$) than that of peripheral blood, whereas in those with benign tumours the peritoneal/ascitic fluid level was lower ($P = 0.001$) than the corresponding peripheral blood level.

The concentration of PAI-2 was higher in the peritoneal/ascitic fluid of malignant as compared to benign tumours (Figure 4d). There was a trend for correlation with tumour differentiation similar to that of PAI-1 (Figure 6), but not with the clinical stage or with the presence of ascitic fluid. The level of PAI-2 correlated with the level of PAI-1, but neither of them correlated with the level of t-PA. The levels in peritoneal/ascitic fluid were higher than peripheral blood levels (benign $P < 0.001$, malignant $P < 0.001$).

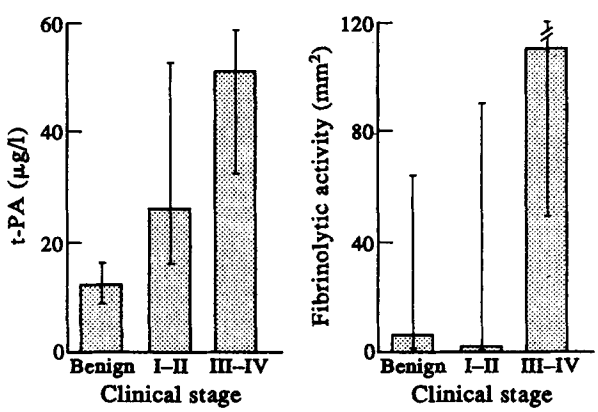

Figure 5. The concentration of t-PA and the fibrinolytic activity of peritoneal/ascitic fluid in benign and malignant ovarian tumours. Malignant tumours were grouped with respect to the clinical stage of the disease. Stages I ($n = 7$) and II ($n = 3$) were pooled, as were stages III ($n = 22$) and IV ($n = 4$). Fibrinolytic activity was significantly higher ($P = 0.002$) in the more advanced stages of the disease, and t-PA values showed the same trend.

Fibrinolytic activity of peritoneal/ascitic fluid was higher in patients with malignant than benign tumours ($P = 0.005$), and within the malignant group, the level correlated significantly to the clinical stage of the disease (Figure 5). In patients with benign tumours, fibrinolytic activity was lower ($P = 0.001$) in postmenopausal (0 mm², range 0–3), than in premenopausal (37 mm², range 0–93) women.

Ovarian malignancy index (log t-PA + log PAI-1) discriminated between benign cysts and possibly malignant cysts without ascites (Figure 7).

Cystic fluid

This was obtained from 22 malignant and 87 benign tumours. None of the parameters correlated to age.

u-PA was higher in fluid from malignant than benign cysts ($P = 0.02$) (Figure 4a). The concentration of u-PA was higher in cystic fluid than in peritoneal/ascitic fluid and peripheral blood (both $P < 0.001$).

The content of t-PA was not significantly different between fluids from malignant and benign cysts (Figure 4b). Benign cystic fluid had a higher level than corresponding peripheral blood ($P < 0.001$), but was not significantly different from the peritoneal/ascitic fluid level. In contrast, the level of t-PA in malignant cysts was not significantly different from that of the peripheral blood, but was much lower than that of the peritoneal/ascitic fluid ($P = 0.001$).

Malignant cysts had a higher content of PAI-1 than benign cysts (Figure 4c). Cystic fluid levels were higher than peripheral blood levels (malignant $P < 0.001$, benign $P = 0.02$) and peritoneal/ascitic fluid levels ($P < 0.001$ both groups).

PAI-2 was found in higher concentrations in malignant than in benign cysts (Figure 4d). The levels of PAI-2 in the cystic fluid were higher than those in the peripheral blood (malignant $P < 0.001$, benign $P < 0.001$), but lower than those in the peritoneal/ascitic fluid ($P = 0.003$ both groups).

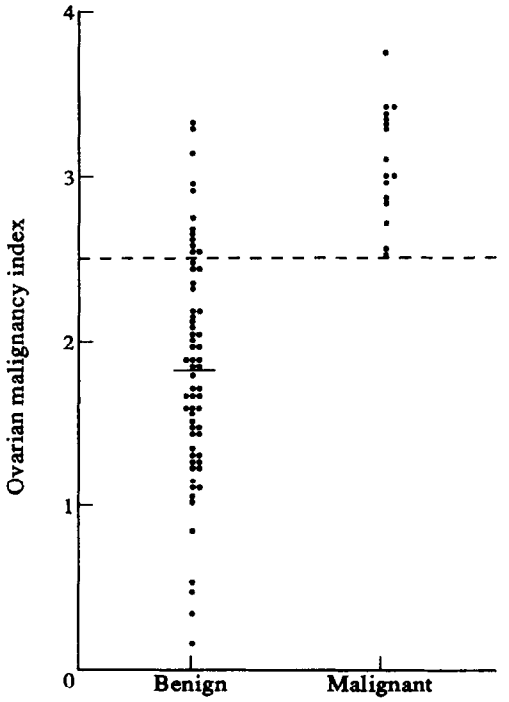

Figure 7. Ovarian malignancy index was based on the levels of t-PA and PAI-1 in peritoneal fluid (log t-PA + log PAI-1). An arbitrary cut-off level at 2.5 discriminated between benign tumours and malignant tumours without ascites: sensitivity 100%, specificity 81%, positive predictive value 57%, and negative predictive value 100%. Each individual value is given, and horizontal bars indicate medians.

The variation of fibrinolytic activity within the groups was substantial, and no significant differences were found.

DISCUSSION

Ovarian cancer tissue is known to release considerable amounts of u-PA *in vitro*, and to contain a higher density of u-PA receptors than benign tumours [10, 24]. This study found u-PA in higher concentrations in cystic fluids than in the other body fluids, suggesting that u-PA is preferentially released from the epithelial surface of the tumour. Correspondingly, higher levels of u-PA have been found in the uterine cavity of patients with endometrial carcinomas than in those with benign endometria [36]. However, there was no indication that the tumours released u-PA into the blood stream. This was not the result of undetected complexes of u-PA and PAIs, because similar results were obtained with an assay, that measured u-PA complexed with PAI-1 or PAI-2. We were thus not able to confirm our own early finding of elevated tumour blood levels of u-PA in a small series of patients [37]. This is most likely due to superiority of the presently used ELISA techniques, and a much larger patient number. The cellular localisation of u-PA within the tumour remains to be established, since apparently in some tumours the stromal component is the main source of u-PA [38].

However, patients with benign as well as malignant tumours had slightly, but significantly, higher peripheral levels of u-PA than controls, suggesting that u-PA can reach the blood stream by routes other than the tumour vasculature, e.g. being release from activated macrophages [39]. The lower peripheral blood levels seen in patients with advanced malignancies, as compared to patients with benign tumours, presumably result from elimination of u-PA in complex with PAI-1 or PAI-2, since these inhibitors were increased in the peripheral blood of these patients. Such complexes may be eliminated via u-PA receptors [40, 41], which are present in macrophages [14], ovarian tumour tissue [10], and possibly endothelial cells [42].

Peripheral blood levels of u-PA have been reported to be increased in patients with carcinomas of the breast, uterine cervix, and endometrium [43, 44]. However, these studies compared the malignant tumour groups only with healthy control groups, and not with benign tumour groups. Shimada and colleagues in contrast, found no significant difference in peripheral blood levels of u-PA between patients with malignant and benign ovarian and uterine cervical tumours [45]. These results are thus altogether compatible with our own results, and stress the importance of comparing data from patients with malignant tumours not only with controls, but also with data obtained in patients with benign tumours. Saito and colleagues found no significant difference between patients with benign uterine tumours and controls, whereas patients with cervical cancer had lower levels of u-PA than controls [46]. Hienert and colleagues found higher levels of u-PA in prostatic cancer patients with distant metastasis than in those without metastasis [47]. The corresponding levels of PAI-1 were, however, not studied by these authors.

Peripheral blood levels of t-PA increased with age. The increase occurred both in pre- and postmenopausal patients, and was thus not related to the menopause *per se*, a finding in accordance with an earlier report [48]. Ovarian tumour blood concentrations were higher than peripheral blood concentrations in patients with benign or malignant tumours, indicating that the release of t-PA from tumour vessels is not limited to malignant tumours. The finding of higher peripheral blood levels of t-PA in patients with either benign or malignant tumours compared with controls might indicate such release. The concentration of t-PA in cystic fluid was not significantly different from that in peripheral blood, suggesting that t-PA is released by the tumour vasculature but not by the tumour tissue. This distribution agrees with an earlier report on the localisation of PA activity in vessel walls of malignant ovarian tumours [25]. The concentration of t-PA in peritoneal/ascitic fluid was generally higher in the group with malignant tumours, even when the fluid volume was low. Although the primary tumours did not produce t-PA, the metastatic implants may do so, and thus contribute to the high t-PA in peritoneal/ascitic fluid. Alternatively, other intraperitoneal cells, e.g. macrophages, may release t-PA in response to fibrin, which is generated by thromboplastic activity from the tumour [26–28, 49]. Higher peripheral blood levels of t-PA in patients with ascites than in those without ascites suggests resorption of t-PA from the intraperitoneal compartment. Increased peripheral blood levels of t-PA were thus not indicative for early stage malignant tumours, but for the presence of asitic fluid and late stage tumours. In accordance with our findings, increased peripheral t-PA levels have previously been reported in patients with advanced gynaecological malignancies [45, 46]. The amount of t-PA in malignant mammary tumour tissue is, in contrast to that of u-PA, not correlated with a poor prognosis, but with a good prognosis [50, 51].

The concentration of PAI-1 was increased in blood from malignant tumours, when compared to blood from benign tumours, and peripheral blood, suggesting that PAI-1 is released from the vasculature in malignant tumours. Neovascularisation within the malignant tumours, which involves endothelial cell proliferation, may be associated with release of PAI-1, and PAI-1 mRNA has, in fact, been located in endothelial cells in the stroma of malignant tumours [52]. Peripheral blood levels of

PAI-1 were, however, increased not only in patients with malignant tumours, but also in patients with benign tumours as compared to controls. Possible mechanisms may include release of PAI-1 from vascular endothelial cells stimulated by the increased concentrations of t-PA in these patients [53]. Production and/or clearance of PAI-1 may be oestrogen dependent, since peripheral blood levels are lower in women than in men under 50 years of age, whereas above that age, levels are equal [54]. The lack of correlation to age within the pre- and postmenopausal groups suggests that the menopause and the loss of oestrogen *per se* is critical. The level of PAI-1 was only slightly higher in cystic fluid than in peripheral blood of patients with benign tumours, but was markedly increased in cystic fluid of patients with malignant tumours. This observation suggests that PAI-1 is produced by malignant tumour tissue, which also reflects the function of PAI-1 as an inhibitor of u-PA. This is in accordance with the localisation of PAI-1 to tumour cells in mammary cancer tissues [55], but contrary to the localisation in colon cancer tissue [52]. These data strongly support the involvement of PAI-1 in malignant tumour growth. High levels of PAI-1 in the peritoneal/ascitic fluid of malignant tumours may originate either in the tumour cells or in other intraperitoneal cells, and may serve to balance the high levels of t-PA in this fluid. High peripheral blood levels of PAI-1 in patients with malignant tumours and ascites are likely to result from resorption from the ascitic fluid. Thus, the peripheral blood level of PAI-1 is not recommended as a marker of early ovarian cancer.

We found that patients with advanced malignant tumours had detectable levels of PAI-2 in the peripheral blood more often than patients with early stage malignant or benign tumours, and healthy controls. Since PAI-2 was not released into the tumour blood, and was present in higher concentrations in the ascitic fluid than in the cystic fluid, it is likely that PAI-2 originates in cells other than the tumour cells, e.g. activated macrophages [19, 20]. Higher peripheral blood levels may thus result from a generalised macrophage activation and/or resorption from the ascitic fluid. The significance of high levels of PAI-2 in some patients with malignant tumours is not known, but may have prognostic implications, since there are indications that PAI-2 has tumour restricting properties. Thus, PAI-2 inhibits tumour cell surface bound u-PA, and thereby restricts extracellular matrix degradation by these cells [56]. Furthermore, absence of lymphatic node involvement is reportedly associated with high levels of PAI-2 in mammary tumour tissue [51]. Such implications, however, need further evaluation.

Screening programmes for the detection of early stage ovarian cancer produce a number of patients with ovarian abnormalities. A few of these are malignant, but the majority are benign lesions [30]. A parameter which clearly discriminates between benign and malignant lesions would be helpful to avoid surgery in the majority of these patients. Although peripheral blood is the most accessible body fluid, it was obvious from our results that these parameters could be used neither as a marker in screening for early cancer, nor as a discriminator between malignant and benign lesions. Ovarian cystic fluid as well as peritoneal fluid is, however, accessible for aspiration via the vaginal route under ultrasonographic guidance. We tested cystic and peritoneal fluid parameters for their discriminating ability. Patients with ascites were not included in these tests, since the presence of ascites itself indicates advanced malignancy. The best discrimination was obtained with an index, which was based on the peritoneal fluid levels of t-PA and PAI-1. We suggest that this index, which would require peritoneal fluid sampling through culdocenthesis at ultrasonographic examination, may be helpful to discriminate between benign and possibly malignant cysts in women detected with ovarian pathology.

1. Goldfarb RH. Proteolytic enzymes in tumor invasion and degradation of host extracellular matrices. In Honn, KV, Powers WE, Sloane BF eds. *Mechanisms in Cancer Metastasis*. Martinus Nijhoff Publishing, 1986, 341–375.
2. Danoe K, Andreasen PA, Grøndahl-Hansen J, Kristensen P, Nielsen LS, Skriver L. Plasminogen activators, tissue degradation, and cancer. *Adv Cancer Res* 1985, **44**, 139–266.
3. Duffy MJ. Plasminogen activators and cancer. *Blood Coagulat Fibrinol* 1990, **1**, 681–687.
4. Hart DA, Rehemtulla A. Plasminogen activators and their inhibitors: regulators of extracellular proteolysis and cell function. *Comp Biochem Physiol* 1988, **90B**, 691–708.
5. Sapppino A-P, Busso N, Belin D, Vassalli JD. Increase of urokinase-type plasminogen activator gene expression in human lung and breast carcinomas. *Cancer Res* 1987, **47**, 4043–4046.
6. Suzumiya J, Hasui Y, Kohga S, Sumiyoshi A, Hashida S, Ishikawa E. Comparative study of plasminogen activator antigens in colonic carcinomas and adenomas. *Int J Cancer* 1988, **42**, 627–632.
7. Saito K, Nagashima N, Iwata M, *et al.* The concentration of tissue plasminogen activator and urokinase in plasma and tissues of patients with ovarian and uterine tumors. *Thromb Res* 1990, **58**, 355–366.
8. Jänicke F, Schmitt M, Hafter R, *et al.* Urokinase-type plasminogen activator (u-PA) antigen is a predictor of early relapse in breast cancer. *Fibrinolysis* 1990, **4**, 69–78.
9. Needham GK, Sherbet GV, Farndon JR, Harris AL. Binding of urokinase to specific receptor sites on human breast cancer membranes. *Br J Cancer* 1987, **55**, 13–16.
10. Casslén B, Gustavsson B, Åstedt B. Cell membrane receptors for urokinase plasminogen activator are increased in malignant ovarian tumours. *Eur J Cancer* 1991, **27**, 1445–1448.
11. Jankun J, Maher VM, McCormick JJ. Malignant transformation of human fibroblasts correlates with increased activity of receptor-bound plasminogen activator. *Cancer Res* 1991, **51**, 1221–1226.
12. Ossowski L. *In vivo* invasion of modified chorioallantoic membrane by tumor cells: the role of cell surface-bound urokinase. *J Cell Biol* 1988, **107**, 2437–2445.
13. Schlechte W, Murano G, Boyd D. Examination of the role of the urokinase receptor in human colon cancer mediated laminin degradation. *Cancer Res* 1989, **49**, 6064–6069.
14. Kirchheimer JC, Remold HG. Endogenous receptor-bound urokinase mediates tissue invasion of human monocytes. *J Immunol* 1989, **143**, 2634–2639.
15. Andreasen PA, Georg B, Lund LR, Riccio A, Stacey SN. Plasminogen activator inhibitors: hormonally regulated serpins. *Mol Cell Endocrin* 1990, **68**, 1–19.
16. Thorsen S, Philips M, Selmer J, Lecander I, Åstedt B. A comparison of the kinetics of inactivation of tissue-type plasminogen activator or urokinase by plasminogen activator inhibitors 1 and 2. *Eur J Biochem* 1988, **175**, 33–39.
17. Lecander I, Åstedt B. Isolation of a new specific plasminogen activator inhibitor from pregnancy plasma. *Br J Haematol* 1986, **62**, 221–228.
18. Åstedt B, Hägerstrand I, Lecander I. Cellular localisation in placenta of placental type plasminogen activator inhibitor. *Thromb Haemost* 1986, **56**, 63–65.
19. Chapman HA, Stone OL. Characterization of a macrophage-derived plasminogen activator inhibitor. Similarities with placental urokinase inhibitor. *Biochem J* 1985, **230**, 109–116.
20. Schwartz BS, Monroe MC, Levin EG. Increased release of plasminogen activator inhibitor type 2 accompanies the human mononuclear cell tissue factor response to lipopolysaccharide. *Blood* 1988, **71**, 734–741.
21. Reith A, Bennett B, Booth NA. Plasminogen activator inhibitor (PAI-2) in pregnancy plasma and ovarian cysts, studied by zymography after non-denaturing gel electrophoresis. *Fibrinolysis* 1989, **3**, 159–163.
22. Lecander I, Martinsson G, Casslén B, Andreasen PA, Åstedt B. Occurrence of the specific plasminogen activator inhibitor of placental type, PAI-2, in ascitic fluid and tumor vessel blood from patients with ovarian carcinoma. *Fibrinolysis* 1990, **4**, 221–224.

23. Sumiyoshi K, Serizawa K, Urano T, Takada Y, Takada A, Baba S. Plasminogen activator system in human breast cancer. *Int J Cancer* 1992, **50**, 345–348.
24. Åstedt B, Holmberg L. Immunological identity of urokinase and ovarian carcinoma plasminogen activator released in tissue culture. *Nature* 1976, **261**, 595–597.
25. Svanberg L, Linell F, Pandolfi M, Åstedt B. Plasminogen activators in ovarian tumours. *Acta Pathol Microbiol Scand* 1975, **83**, 193–198.
26. Svanberg L. Thromboplastic activity of human ovarian tumours. *Thromb Res* 1975, **6**, 307–313.
27. Åstedt B, Glifberg I, Mattsson W, Tropé C. Arrest of growth of ovarian tumor by tranexamic acid. *J Am Med Assoc* 1977, **237**, 154–155.
28. Åstedt B, Svanberg L, Nilsson IM. Fibrin degradation products and ovarian tumours. *Br Med J* 1971, **4**, 458–459.
29. Pettersson F. Annual report on the results of treatment in gynecological cancer. *Int J Gynecol Obstet* 1991, **36**, (suppl), 1–315.
30. Campbell S, Bhan V, Royston P, Whitehead MI, Collins WP. Transabdominal ultrasound screening for early ovarian cancer. *Br Med J* 1989, **299**, 1363–1366.
31. Bast RC, Knauf S, Epenetos A, *et al.* Coordinate elevation of serum markers in ovarian cancer but not in benign disease. *Cancer* 1991, **68**, 1758–1763.
32. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. *Fertil Steril* 1950, **1**, 3–25.
33. Åstedt B, Holmberg L, Lecander I, Thorell J. Radioimmunoassay of urokinase for quantification of plasminogen activators released in ovarian tumour cultures. *Eur J Cancer Clin Oncol* 1981, **17**, 239–244.
34. Lecander I, Åstedt B. Specific plasminogen activator inhibitor of placental type, PAI-2, occurring in amniotic fluid and cord blood. *J Lab Med* 1987, **110**, 602–605.
35. Nilsson IM, Olow B. Fibrinolysis induced by streptokinase in man. *Acta Chir Scand* 1962, **123**, 247–266.
36. Niklasson O, Svanberg L, Thorell J, Lecander I, Åstedt B. Urokinase-like plasminogen activator as a marker of endometrial neoplasia. *Cancer* 1981, **48**, 1484–1486.
37. Åstedt B. Immunological detection of tumor plasminogen activator *in vitro* and *in vivo*. In Ruddon RW, ed. *Biological Markers of Neoplasia: Basic and Applied Aspects*. North Holland Elsevier, 1978, 481–489.
38. Pyke C, Kristensen P, Ralfkiaer E, *et al.* Urokinase-type plasminogen activator is expressed in stromal cells and its receptor in cancer cells at invassive foci in human colon adenocarcinomas. *Am J Pathol* 1991, **138**, 1059–1067.
39. Hart PH, Vitti GF, Burgess DR, *et al.* Activation of human monocytes by granulocyte-macrophage colony stimulating factor: Increased urokinase-type plasminogen activator activity. *Blood*, 1991, **77**, 841–848.
40. Jensen PH, Christensen EI, Ebbesen P, Gliemann J, Andreasen PA. Lysosomal degradation of receptor-bound urokinase-type plasminogen activator is enhanced by its inhibitors in human trophoblastic choriocarcinoma cells. *Cell Reg* 1990, **1**, 1043–1056.
41. Olson D, Pöllänen J, Hoyer-Hansen G, *et al.* Internalization of the urokinase-plasminogen activator inhibitor type-1 complex is mediated by the urokinase receptor. *J Biol Chem* 1992, **267**, 9129–9133.
42. Barnathan E, Kuo A, Karikó K. Characterization of human endothelial cell urokinase-type plasminogen activator receptor protein and messenger RNA. *Blood* 1990, **76**, 1795–1806.
43. Grøndahl-Hansen J, Agerlin N, Munkholm-Larsen P, *et al.* Sensitive and specific enzyme-linked immunosorbent assay for urokinase-type plasminogen activator and its application to plasma from patients with breast cancer. *J Lab Clin Med* 1988, **11**, 42–51.
44. Koelbl H, Kirchheimer JK, Tatra G, Christ G, Binder BR. Increased plasma levels of urokinase-type plasminogen activator with endometrial and cervical cancer. *Obstet Gynecol* 1988, **72**, 252–256.
45. Shimada H, Ikeuchi M, Suwa M, *et al.* Study on the plasma plasminogen activators in patients with malignant gynecologic tumors. *Acta Obst Gynaec Jpn* 1989, **41**, 1353–1359.
46. Saito K, Nagashima M, Iwata M, *et al.* The concentration of tissue plasminogen activator and urokinase in plasma and tissues of patients with ovarian and uterine tumors. *Thromb Res* 1990, **58**, 355–366.
47. Hienert G, Kirchheimer JC, Pflüger H, Binder BR. Urokinase-type plasminogen activator as a marker for the formation of distant metastases in prostatic carcinomas. *J Urol* 1988, **140**, 1466–1469.
48. Sundell I, Nilsson T, Rånby M, Hallmans G, Hellsten G. Fibrinolytic variables are related to age, sex, blood pressure, and body build measurements: a cross-sectional study in Norsjö, Sweden. *J Clin Epidemiol* 1989, **42**, 719–723.
49. Hart PH, Vitti GV, Burgess DR, Singelton DK, Hamilton JA. Human monocytes can produce tissue-type plasminogen activator. *J Exp Med* 1989, **169**, 1509–1514.
50. Duffy MJ, O'Grady P, Devaney D, O'Siorain L, Fennelly JJ, Lijnen HR. Tissue-type plasminogen activator, a new prognostic marker in breast cancer. *Cancer Res* 1988, **48**, 1348–1349.
51. Sumiyoshi K, Serizawa K, Urano T. Plasminogen activator system in human breast cancer. *Int J Cancer* 1992, **50**, 345–348.
52. Pyke C, Kristensen P, Ralfkiaer E, Eriksen J, Danoe K. The plasminogen activation system in human colon cancer: messenger RNA for the inhibitor PAI-1 is located in endothelial cells in the tumor stroma. *Cancer Res* 1991, **51**, 4067–4071.
53. Fujii S, Lucore CL, Hopkins WE, Billadello JJ, Sobel BE. Induction of synthesis of plasminogen activator inhibitor type-1 by tissue-type plasminogen activator in human hepatic and endothelial cells. *Thromb Haemost* 1990, **64**, 412–419.
54. Rånby M, Sundell IB, Johnson O, Ledin MC, Dahlen G. Activity of plasminogen activator inhibitor-1 (PAI-1) in a population of northern Sweden. *Fibrinolysis* 1990, **4**, 54–55.
55. Sumiyoshi K, Baba S, Sakayuchi S, Urano T, Takada Y, Takada A. Increase in levels of plasminogen activator and type 1 plasminogen activator inhibitor in human breast cancer: possible roles in tumor progression and metastasis. *Thromb Res* 1991, **63**, 59–71.
56. Baker M, Bleakley P, Woodrow G, Doe W. Inhibition of cancer cell urokinase plasminogen activator by its specific inhibitor PAI-2 and subsequent effects on extracellular matrix degradation. *Cancer Res* 1990, **50**, 4676–4684.

Acknowledgements—This study was supported by grants from the Swedish Cancer Fund (2693-B91-04XAA and 3339-B93-02XBB), the Swedish Medical Research Council (04523-19B), the Medical Faculty of the University of Lund, the University of Lund Hospitals Fund for Cancer Research, the Gunnar, Arvid and Elisabeth Nilsson's Fund for Cancer Research, the Anna-Lisa and Sven-Eric Lundgren's Fund for Medical Research and the Crafoord's Fund.