Determination of the Complex between Urokinase and Its Type-1 Inhibitor in Plasma from Healthy Donors and Breast Cancer Patients

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Background: The complex between urokinase (uPA) and its type-1 inhibitor (PAI-1) is formed exclusively from the active forms of these components; thus, the complex concentration in a biological sample may reflect the ongoing degree of plasminogen activation. Our aim was to establish an ELISA for specific quantification of the uPA:PAI-1 complex in plasma of healthy donors and breast cancer patients.

Methods: A kinetic sandwich format immunoassay was developed, validated, and applied to plasma from 19 advanced-stage breast cancer patients, 39 age-matched healthy women, and 31 men.

Results: The assay detection limit was <2 ng/L, and the detection of complex in plasma was validated using immunoabsorption, competition, and recovery tests. Eighteen cancer patients had a measurable complex concentration (median, 68 ng/L; range, <16 to 8700 ng/L), whereas for healthy females and males the median signal values were below the detection limit (median, <16 ng/L; range, <16 to 200 ng/L; P < 0.0001). For patient plasma, a comparison with total uPA and PAI-1 showed that the complex represented a variable, minor fraction of the uPA and PAI-1 concentrations of each sample.

Conclusion: The reported ELISA enables detection of the uPA:PAI-1 complex in blood and, therefore, the evaluation of the complex as a prognostic marker in cancer.

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The plasminogen activation system plays a central role in several tissue remodeling processes, including cancer invasion. Of the two plasminogen activators known, tissue-type plasminogen activator is involved mainly in intravascular fibrinolysis, whereas urokinase plasminogen activator (uPA)5 is most important in pericellular matrix degradation processes, such as in wound healing, inflammatory disease, and cancer invasion (1, 2). uPA is a 52-kDa serine proteinase secreted as an inactive proenzyme (pro-uPA), which constitutes the major portion of uPA in tissues and blood (3). A specific cell-surface receptor (uPAR) binds pro-uPA and uPA, leading to localization and potentiation of plasminogen activation (4). The plasmin thus generated mediates broad spectrum proteolysis, facilitating cell migration, proliferation, and invasion (5). uPA activity is rapidly neutralized by specific high-affinity plasminogen activator inhibitors (PAIs) (6). PAI-1, the principal physiological inhibitor, is a 52-kDa protein secreted in an active but conformationally unstable form, gradually losing activity unless stabilized by binding to extracellular matrix- and plasma-protein vitronectin (7). Active PAI-1 forms an equimolar, sodium dodecyl sulfate-stable 100-kDa complex with active uPA in solution as well as with uPA, which is receptor bound (6, 8), whereas the inactive forms of these two components cannot form a complex. By an internalization process dependent on uPAR and the α2-macroglobulin or VLDL receptors, cell-surface uPA:PAI-1 complex is cleared from the pericellular space and blood (2, 9) or released by proteolytic cleavage of uPAR or uPA (10–12).

The concentrations of uPA and PAI-1 in cancer extracts, as determined by ELISAs, have been shown to be

5 Nonstandard abbreviations: uPA, urokinase plasminogen activator; pro-uPA, proenzyme form of uPA; uPAR, uPA receptor; PAI, plasminogen activator inhibitor; MAb, monoclonal antibody; PAb, polyclonal antibody; TNP, trinitrophenyl hapten; and NPGB, p-nitrophenyl guanidinobenzoate.
independent prognostic indicators (2, 13). The ELISAs applied measure the total amount of the given molecule, including proforms and active, inactive, and complex-bound forms. However, to fully realize its proteolytic potential, the uPA system requires the presence of active components, and selective measurement of the active form of a component may more closely reflect the ongoing proteolytic activity and, therefore, more closely relate to disease progression. Because the complex between uPA and PAI-1 can only be formed from the active forms of both components, quantification of this complex may indirectly reflect the concentrations of active components produced and therefore be particularly valuable for clinical studies of patient prognosis.

We recently established a uPA:PAI-1 ELISA for tissue extracts, and application of the assay to breast cancer extracts has demonstrated substantial amounts of complex in such tissues (14, 15). Soluble complex released from the tissue may find its way into the peripheral blood (16); thus, assay of blood may be a more accessible method of measuring the activity of the tumor uPA system. We now report the first ELISA specifically developed for plasma measurements of the low uPA:PAI-1 concentrations present in healthy individuals and cancer patients; in addition, we report preliminary findings of increased plasma complex concentrations in patients with advanced breast cancer.

Materials and Methods

Subjects and Plasma Preparation

Blood samples were collected from 19 stage IV breast cancer patients (ages, 45–70 years) receiving antineoplastic therapy at the Department of Oncology, Herlev Hospital, Herlev, Denmark. In addition, blood samples from 73 age-matched apparently healthy women and men (ages, 45–65 years) were obtained from the Blood Bank, Hvidovre Hospital, Hvidovre, Denmark, and the Department of Oncology, Herlev Hospital. Informed consent was obtained from all healthy donors and patients, blood samples were obtained in accordance with the Helsinki Declaration of 1975, and permission was granted by the local ethics committees (permission no. KA93032).

Peripheral venous blood was drawn from resting individuals into ice-chilled citrate collection tubes (Becton-Dickinson). If necessary, a tourniquet at a maximum +2 kPa was applied before venipuncture to locate a peripheral vein. Blood samples were quickly mixed by repeated inversion and immediately chilled on ice. Within 1 h, plasma was separated from blood cells by centrifugation at 1800g for 30 min at 4 °C, and the resulting supernatant was aliquoted and stored at −80 °C until use. On the day of analysis, the samples were thawed quickly in a water bath at 37 °C and subsequently chilled on ice until diluted with assay dilution buffer. Two plasma pools were prepared with equal volumes of plasma from individual samples: one pool of 6 samples representing various concentrations of uPA:PAI-1 in plasma from healthy donors and patients (98 ng/L), and another pool of 10 samples representing undetectable concentrations of complex.

Antibodies

Five different mouse monoclonal antibodies (MAbs) against human uPA were tested alone or in combination (17, 18), of which a combination of the clones designated 5 and 6 was shown to be superior for the capture of uPA:PAI-1 in the ELISA. These MAbs were also used for immunoabsorption experiments. Clone 5 reacts with an epitope in the carboxy-terminal region of uPA, whereas clone 6 binds an epitope within the amino-terminal sequence of uPA. Five different preparations of rabbit polyclonal antibodies (PAb) against human PAI-1 were available, of which the one with the strongest response combined with a low background was selected as the detection reagent in the ELISA. This PAb was further purified to remove reactivity with the capture MAbs by immunoabsorption on Sepharose 4B-coupled (Pharmacia) anti-uPA clones 5 and 6. An anti-PAI-1 MAb designated clone 2, directed against PAI-1 residues 110–145 (19, 20), was used for immunoabsorption specificity tests with plasma. The control MAb against irrelevant 2,4,6-trinitrophenyl (TNP) hapten has been described previously (21). The MAbs were all of the IgG1 subclass and were purified from hybridoma culture fluids by affinity chromatography on protein A-Sepharose (Pharmacia). The specific PAb as well as the non-immune control PAb were purified from rabbit sera using protein A-Sepharose.

UPA:PAI-1 Calibrator

uPA:PAI-1 was prepared and purified as described previously (14) and was used as calibrator in the ELISA and Western blotting. In short, high-molecular weight two-chain uPA (Serono) and activated PAI-1, purified from conditioned media of HT-1080 fibrosarcoma cells, were co-incubated to form complex. Purification of the uPA:PAI-1 complex was performed by sequential affinity chromatography using immobilized anti-PAI-1 and anti-uPA MAbs. The concentration of the purified complex was determined by protein analysis according to the Bradford method and confirmed by the Lowry method. The calibrator was stored at −80 °C.

UPA:PAI-1 ELISA

Maxisorp™ 96-well immunoplates (Nunc) were coated overnight without shaking at 4 °C with a mixture (100 μL/well) of 2 mg/L each of anti-uPA clones 5 and 6 in 0.05 mol/L Na2CO3, pH 9.6. Both MAbs contributed substantially to the measured signal; omission of clone 5 or 6 reduced the signal of the calibrator complex by 35% and 64%, respectively. After the wells were coated, we blocked the remaining protein binding sites on the plates by manually washing the wells twice, using for each wash 200 μL of washing buffer (1.5 mmol/L KH2PO4, 8.1 mmol/L Na2HPO4, 2.7 mmol/L KCl, 0.14 mol/L NaCl,
the addition of NPGB to the dilution buffer, as we have complex formation from free components was blocked by During incubation in the assay plate at 37 °C, uPA:PAI-1 has a detection limit of 25 ng/L and detects pro-uPA, incubated with 100 g/L Tween 20. Plates were washed three times and incubated with dilution buffer (100 µL/well; containing 1 g/L Tween 20. Plates were washed three times, and the wells were incubated with 100 µL of 2 mg/L anti-PAI-1 PAb or, as a specificity control, non-immune PAb. The anti-PAI-1 PAb used at this concentration generated a high response with the calibrator and a low background. The anti-PAI-1 PAb used in the determination generated a high response with the calibrator and a low background. After the detector step, plates were washed six times and incubated with 100 µL/well of an alkaline phosphatase-conjugated MAb against rabbit IgG (Sigma) diluted 1:1000 in dilution buffer. Finally, the plates were washed six times with buffer plus an additional three times in MilliQ water (Millipore), and the alkaline phosphatase reaction was performed at 20 °C with 100 µL/well of 1.7 g/L p-nitrophenyl phosphate disodium (Sigma) freshly made in 0.1 mol/L Tris, 0.1 mol/L NaCl, 5 mmol/L MgCl₂, pH 9.5. The absorbance at 405 nm was read against an air blank at 10-min intervals for 1 h in a Ceres-900 microplate reader (Bio-Tek Instruments). Development of color in each well was a linear function of time for all concentrations of uPA:PAI-1 measured. The mean rate of change in absorbance was computed and interpreted by the KinetiCalc 2™ software (Bio-Tek) using a four-parameter fit for the calibration curve. All determinations were performed in duplicate or triplicate, and the mean value was used. All of the individual plasma sample determinations were performed in triplicate, of which one was performed with the non-immune PAb for detection, as a specificity control.

INHIBITION OF IN VITRO FORMATION OF UPA:PAI-1
To prevent ex vivo formation in blood of uPA:PAI-1 from free PAI-1 and uPA not bound to other inhibitors, such as α2-macroglobulin (22), the samples were kept at 0–4 °C until and during plasma separation; at this temperature, free PAI-1 does not form a complex with free uPA (23).

ELISAS FOR UPA AND PAI-1
The total uPA and total PAI-1 in plasma samples were measured using ELISA kits from Oncogene Science Diagnostics, as described previously (24, 25). The uPA assay has a detection limit of 25 ng/L and detects pro-uPA, uPA, uPA:PAI-1, and uPA:uPAR complexes with approximately equal efficiency (Pedersen et al, manuscript in preparation). The PAI-1 ELISA has a detection limit of 100 ng/L and measures latent PAI-1, active PAI-1, and PAI-1 in complex with plasminogen activators, although uPA:PAI-1 is detected with ~50% lower efficiency than latent PAI-1 (Pedersen et al., manuscript in preparation).

IMMUNOABSORPTION AND WESTERN BLOTTING
For immunoabsorption, pooled citrate plasma was diluted fivefold in dilution buffer containing NPGB and mixed with Sepharose 4B-coupled MAb against uPA (100 µg/L) gel in a buffer-gel ratio of 2:1. This mixture was incubated with end-over-end rotation at 20 °C overnight and subsequently centrifuged at 40g for 1 min. The resulting gel-free supernatant was diluted an additional twofold in dilution buffer containing NPGB and assayed in the uPA:PAI-1 ELISA.

For Western blotting, 6 mL of plasma pool was diluted in dilution buffer containing a proteinase inhibitor mixture of NPGB, aprotinin, and EDTA and recycled several times at 20 °C overnight through a 100 µg/L column of Sepharose-4B-coupled anti-uPA clones 5 and 6 (1 g of each per liter of gel), identical to those used for capture in the ELISA. After washing, the column outlet was plugged and 100 µL of Laemmli sample buffer (Bio-Rad) was applied. Electrophoresis of 50 µL of the resulting uPA-immunoenriched eluate was performed on a 10% sodium dodecyl sulfate-polyacrylamide Ready minigel (Bio-Rad), and the proteins were blotted electrophoretically from the gel onto a polyvinylidene difluoride membrane (Millipore). The membrane was blocked overnight at 4 °C with 10 g/L skimmed milk powder in Tris-buffered saline, washed, and incubated for 2 h with 10 mL of 20 mg/L anti-PAI-1 PAb, identical to the rabbit IgG used for detection in the ELISA. After washing, the membrane was incubated for 1 h with 10 mL of an alkaline phosphatase-conjugated anti-rabbit IgG MAb diluted 1:1000. Finally, the membrane was washed and rinsed in MilliQ water, and the color was developed with a phosphatase substrate solution, NBT/BCIP (Boehringer Mannheim).

MISCELLANEOUS MATERIALS
Recombinant nonglycosylated pro-uPA, purified from Escherichia coli, was a kind gift from Grünenthal, Aachen, Germany. High-molecular weight two-chain uPA (Se-rono), purified from human urine, was inactivated by treatment with diisopropyl fluorophosphate (26), and recombinant soluble uPAR (i.e., uPAR lacking the glycolipid anchor) capable of binding uPA lacking the glycolipid anchor) capable of binding uPA was produced as described previously (27).

Results

UPA:PAI-1 ELISA
When the ELISA protocol described above was used, there was a linear relationship between signal and uPA:PAI-1 calibrator concentration from 2 ng/L up to at least
1000 ng/L (linear correlation coefficient, $r > 0.99$; Fig. 1). The rate when no calibrator complex was used (read against air) was $0.055 \pm 0.017$ (mean $\pm$ SD) milliabsorbance units/min ($n = 27$), whereas the rate with 1000 ng/L complex was $25 \pm 2.2$ milliabsorbance units/min ($n = 9$). The limit of detection for the assay was 1.6 ng/L, defined as the lowest antigen concentration giving a signal higher than the mean of the buffer control plus 3 SD. The assay did not detect free pro-uPA, active uPA, diisopropyl fluorophosphate-uPA, latent PAI-1, active PAI-1, or soluble uPAR assayed in concentrations up to 10 mg/L. Furthermore, addition to the calibrator complex of any form of free uPA in amounts up to $\approx 50 \mu$g/L did not significantly reduce the detection of complex by the assay. In fact, addition of at least 150 $\mu$g/L and 10 mg/L uPA was required to inhibit the signal by 50% and 90%, respectively. Addition of up to 1 mg/L PAI-1 or uPAR to the calibrator complex did not interfere with the signal detected.

**ELISA PERFORMANCE WITH PLASMA**

A pool of plasma from six individuals with diverse concentrations of plasma uPA:PAI-1 complex was prepared to investigate the ELISA performance; this pool contained 98 ng/L uPA:PAI-1, hereafter designated (98 ng/L). When different dilutions of the pool were assayed in the ELISA, the signal was linearly related to dilution provided the dilution factor was $\approx 10$ (Fig. 2). Therefore, a 10-fold plasma dilution was chosen for all subsequent determinations. The analytical recovery in the ELISA was tested by adding various concentrations (4–500 ng/L) of calibrator complex to a plasma pool from 10 individuals (containing concentrations of plasma complex below the detection limit of the assay) and comparing the signal to the signal for calibrator diluted in pure dilution buffer. The recovery of signal from calibrator complex in a 10-fold dilution of this pool was virtually complete (104%) over the entire range measured. For individual plasma samples, the linearity of the dilution curves and the recoveries were similar to the above results.

The within-run imprecision of the ELISA was determined by assaying 12 independent 10-fold dilutions of plasma pool (98 ng/L) on the same ELISA plate. The calculated within-run CV was 7.4%. The between-run (total) imprecision of the assay at this plasma concentration was determined by assaying the pool on 8 separate days, which gave a CV of 23%.

**ELISA SPECIFICITY WITH PLASMA**

Omission of either capture MAb, i.e., clone 5 or clone 6, from the ELISA reduced the signal from plasma pool (98 ng/L) by 39% and 72%, respectively. For the uPA:PAI-1 calibrator, the signal reduction was comparable (35% and 64%, respectively), indicating a similar epitope pattern between plasma complex and calibrator complex.

Plasma pool (98 ng/L) was then used to prepare a sample of plasma complex. Affinity chromatography was applied, using the same MAbS used for capture in the ELISA, and the absorbed material was analyzed by Western blotting with the same batch of PAb that was used for ELISA detection. The blot showed a single band with a mobility corresponding to uPA:PAI-1 (Fig. 3, inset).

Specific depletion of uPA:PAI-1 from plasma pool (98 ng/L) was performed by immunoabsorption on an immobilized anti-PAI-1 MAb different from the capture MAbS used in the ELISA. The signal in the subsequent assay was extinguished (Fig. 3). When very high concentrations (10 mg/L) of free diisopropyl fluorophosphate-uPA were added to plasma pool (98 ng/L), the ELISA signal could be extensively reduced (87%). Finally, if the capture MAbS were replaced with an irrelevant MAb, anti-TNP, or if the detector PAb was replaced with non-immune rabbit IgG, no significant signal above background was observed with plasma pool (98 ng/L) or the calibrator complex. This latter specificity test was also applied to all subsequent assays of individual
plasma samples because occasionally a significant signal above background was observed with the non-immune detector PAb.

**UPA:PAI-1 in Plasma of Cancer Patients and Healthy Donors**

To investigate the concentration of uPA:PAI-1 in blood, we studied a set of 19 individual citrate plasma samples from advanced (stage IV) breast cancer patients and a set of 73 samples from age-matched healthy donors, comprising 39 women and 34 men. In three cases (all men), a signal above background was observed with the non-immune detector control antibody; these samples were, therefore, excluded from further analyses. For the remaining samples, uPA:PAI-1 was present in 18 of 19 (95%) samples from the cancer patients, in 8 of 39 (20%) samples from healthy women, and in 1 of 31 (3%) men. A Kruskal-Wallis test indicated a significant difference between the three groups ($P < 0.0001$). In the plasma of healthy women, the median complex concentration was below the detection limit (i.e., <16 ng/L for undiluted plasma; range, <16 to 200 ng/L), whereas for breast cancer patients, the median value was 68 ng/L (range, <16 to 8700 ng/L). A Mann-Whitney test for cancer patients vs healthy women indicated a highly significant difference with $P < 0.0001$. Fig. 4 shows the discriminatory power of the concentration of the uPA:PAI-1 complex between the healthy female donors and the cancer patients (area under the curve, 0.83). Of 31 men with assessable assay results (see above), only 1 had a measurable concentration of plasma complex (30 ng/L).

**Comparisons Between uPA:PAI-1, uPA, and PAI-1**

To elucidate the relationship between the concentrations of uPA:PAI-1, total uPA, and PAI-1, respectively, we also assayed the plasma samples from the breast cancer patients for plasma total uPA and PAI-1. For total uPA, the concentration range was 0.48–6.8 µg/L, with a median value of 0.98 µg/L. For total PAI-1, the concentration range was 6.7–79 µg/L, with a median of 23 µg/L. Quantitatively, uPA:PAI-1 complex was a variable, minor fraction of the total uPA and PAI-1 concentrations in each plasma sample; the complex represented <10% of the total uPA in 17 of 19 samples from breast cancer patients and <1% of the total PAI-1 in 18 of 19 samples. In Table 1, the correlations between the three indicators are shown. There was a moderate correlation between PAI-1 and its complex (Spearman correlation coefficient $r = 0.58$, $P = 0.01$), whereas there was no significant correlation between uPA and complex in the whole set.

**Discussion**

We have developed the first ELISA method that selectively measures preformed uPA:PAI-1 complex in blood. Plasma samples from advanced breast cancer patients and age-matched healthy individuals were included in this study, and there was a highly significant difference between complex concentrations in healthy female and breast cancer patient plasma. Although plasma uPA:PAI-1 was detectable in a minority of samples from healthy male (3%) and female (20%) donors, plasma from breast cancer patients contained measurable complex in almost all of the samples (95%). Thus, the reported ELISA

<table>
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<tr>
<th>Comparison</th>
<th>$r^a$</th>
<th>$P^b$</th>
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<tr>
<td>uPA vs PAI-1</td>
<td>0.47</td>
<td>0.05</td>
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<tr>
<td>uPA vs uPA:PAI-1 complex</td>
<td>0.34</td>
<td>0.14</td>
</tr>
<tr>
<td>PAI-1 vs uPA:PAI-1 complex</td>
<td>0.58</td>
<td>0.01</td>
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*a* $r$, Spearman rank correlation coefficient.

*b* Significance of the correlation.
is well suited for the evaluation of plasma uPA:PAI-1 in cancer prognosis.

ELISAs for plasma uPA:PAI-1, uPA:PAI-3, and uPA: uPAR complexes have been reported previously (28-30). We recently described an ELISA designed for measurement of preformed uPA:PAI-1 complex in tissue extracts (14) and evaluated the prognostic impact of the complex in breast cancer (31). This ELISA consisted of two anti-PAI-1 capture MAbs (clones 2 and 5) and three biotinylated anti-uPA detector MAbs (clones 5, 6, and 16). When the use of this assay was extended to investigations of plasma in the present study, we found it necessary to redesign the assay format to eliminate a source of non-specific signals that could be traced to the use of biotinylated reagents [Høyer-Hansen et al, manuscript submitted, and (32)]. Furthermore, to prevent assay interference from free components, anti-uPA antibodies were used for capture instead of anti-PAI-1 antibodies because uPA is present in the plasma of breast cancer patients at much lower concentrations than PAI-1. With this protocol, the assay fulfilled the requirements of sensitivity, specificity, and stability when assaying the majority of the plasma samples. We do not know, however, why a small proportion (~3%) of the samples gave rise to a nonspecific signal, but heterophilic antibodies can be a source of false signals (33).

The concentrations of uPA and PAI-1 in cancer extracts are independent prognostic indicators (2, 13). Release of these protein components of the plasminogen activation system from the tumor tissue through the hyperpermeable tumor vessels (34) leads to increased concentrations in peripheral blood, and the most aggressive tumors appear to release more of these components (17, 35, 36). As a consequence, the studies have now been extended to the determination of uPA and PAI-1 in blood because assay of soluble components in blood rather than in tissue extracts may offer wider applications. Indeed, recent prognostic studies on the plasminogen activation system in the blood of cancer patients have shown that plasma PAI-1 is significantly associated with survival (37, 38).

All of the published ELISA studies referred to above have been based on assays that have been developed to measure the total amount of the given component, including proforms and active, inactive, and complex-bound forms. A substantial amount of the measured concentration of the given component in each sample may actually represent inactive forms (3) and may, therefore, not contribute to the prognostic impact. However, selective measurements of the active form of a component—and in particular, as with uPA:PAI-1, combining the active forms of the two components—could provide a stronger prognostic indicator. It is, therefore, of considerable interest to study the prognostic value of the uPA:PAI-1 complex in cancer—in tissue extracts as well as in blood. In fact, because soluble complex released from the tissue into the peripheral blood can occur both as a consequence of uPA in solution being inhibited by PAI-1 and by uPAR-bound complex being released by proteolytic cleavage (10-12), assay of the uPA:PAI-1 complex in blood may be an even more representative method of measuring the activity of the uPA system than assay of tissue extracts.

The finding in the present study, that plasma uPA: PAI-1 concentrations were significantly increased in the breast cancer patients compared with healthy women, may suggest that complex is indeed released from tumor tissue into the peripheral blood. We cannot determine from these data, however, whether the variable concentrations of plasma complex reflect differences in tumor aggressiveness or whether they simply reflect tumor burden. Other potential sources of the increased/variable concentrations of complex in cancer patients might be accompanying stromal reactions, such as inflammation (39, 40). It is also noteworthy that, in contrast to the males, an appreciable minority of the healthy female donors displayed detectable plasma complex. Some of the healthy female donors (age range, 45-65 years) and patients were premenopausal; therefore, it may be speculated whether these women were in a phase of their menstrual cycle during which complex could be released from the remodeling uterine tissue into the blood (41, 42). We fully expect the complex to be increased in such conditions and other tissue remodeling processes because degradation of pericellular matrix in general involves the uPA system. For these reasons, we do not propose that the uPA:PAI-1 complex is a cancer-specific marker.

In the comparison of plasma uPA:PAI-1 with plasma total uPA and PAI-1, we found that, quantitatively, the uPA:PAI-1 complex appeared to be a variable, minor fraction of the total uPA as well as the total PAI-1 in each plasma sample. The plasma uPA:PAI-1 complex may, therefore, be a possible prognostic marker independent of total antigen concentrations. In this context, it is noteworthy that the complex between metalloproteinase-9 and tissue inhibitor of metalloproteinase is a prognostic marker in the plasma of patients with gastrointestinal cancer (43) and that serum prostate-specific antigen complexed to antichymotrypsin is a clinical marker for prostate cancer (44, 45). For the above reasons, we currently are conducting a study of the prognostic significance of uPA:PAI-1 in preoperative plasma from 550 patients with various stages of primary colon adenocarcinoma.

In conclusion, we would emphasize that the exclusive purpose of the ELISA for uPA:PAI-1 presented here is its use for prognostic studies. This is the first assay that selectively quantifies preformed complex in plasma, thus allowing the investigation of the plasma uPA:PAI-1 complex as a potential indicator for prognosis in cancer.
ples from healthy donors and Vivi Kielberg, Monozoyme Aps., Horsholm, Denmark, for providing monoclonal antibodies.

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