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Prognostic value of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitors PAI-1 and PAI-2 in breast carcinomas

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Summary It is now clearly established that proteolytic enzymes, including plasminogen activator (uPA), play an important role in breaking down the extracellular matrix, which is considered to be a step in metastasis formation. Plasminogen activators are controlled at various levels. Two inhibitors, PAI-1 and PAI-2, have been identified, the latter being more specific for uPA. In attempts to determine their prognostic value, it is essential to investigate the relative importance of these parameters and their interactions. We used an immunoenzymatic method to assay uPA, PAI-1 and PAI-2 antigens in cytosols prepared from 314 primary breast tumours. The patients were followed up for a minimum of 6 years and all relevant clinical and laboratory findings were recorded. Univariate analysis confirmed the poor outcome of patients whose tumours contained large amounts of uPA and PAI-1. In addition, low levels of PAI-2 correlated with shorter disease-free survival in the overall population ($P = 0.02$), post-menopausal women ($P = 0.02$) and women without lymph node involvement ($P = 0.02$). Multivariate analysis in the 'main effects' Cox model identified node involvement, macroscopic tumour size and PAI-2 as significant variables. The 'interactive' model, taking into account interactions between uPA and its two inhibitors, identified a first subgroup with a very poor prognosis associating either high levels of PAI-1 with low levels of PAI-2 in the overall population and the women with no node involvement or high levels of uPA with low levels of PAI-2 in the group of menopausal women. We conclude that PAI-1 provides the same prognostic information as uPA, and does not appear to play a role as an inhibitor. In contrast, PAI-2 increases the prognostic value of uPA, particularly in post-menopausal women, and PAI-1 in patients with no node involvement.

Tumour cell invasion and metastasis formation is a multifactorial process. The remodelling it involves requires the coordinated action of cell-secreted proteolytic enzymes and their inhibitors. Elevated levels of urokinase-type plasminogen activator (uPA) have been implicated in these invasive processes (Dano *et al.*, 1985; Duffy, 1987; Layer *et al.*, 1987; Markus *et al.*, 1988; Pyke *et al.*, 1991b), and plasminogen activator inhibitor type 1 (PAI-1) has been found in many types of malignant tissue (Kruithof *et al.*, 1988; Cubellis *et al.*, 1990; Foucré *et al.*, 1991; Tanaka *et al.*, 1991; Reilly *et al.*, 1992). Plasminogen activator inhibitor type 2 (PAI-2) is one of the primary physiological inhibitors of uPA. Individual prognostic values have been reported in breast cancer (Duffy *et al.*, 1990; Jänicke *et al.*, 1990, 1993; Grondahl-Hansen *et al.*, 1992; Spyratos *et al.*, 1992; Sumiyoshi *et al.*, 1992). The purpose of this study was to analyse the antigen levels of uPA, PAI-1 and PAI-2 by means of an enzyme-linked immunosorbent assay (ELISA) technique and to evaluate their relative value in predicting disease-free and metastasis-free survival rates of primary breast cancer patients.

Materials and methods

Patients

The 314 patients in this study (mean age 55 years; range 30–86 years) were selected according to the following criteria: primary, unilateral breast carcinoma and treatment at the Centre René Huguenin between 1980 and 1985. The median follow-up was 7 years (maximum 11 years) at the time of the study. Fifty-nine per cent of patients had undergone a total mastectomy and 41% of patients a partial mastectomy; both groups had undergone axillary lymph node clearance. Two hundred and three patients had received post-operative irradiation. Patients ($n = 115$) regarded as high risk [i.e. those with more than three involved axillary lymph nodes or those with at least one involved axillary lymph node and Scarff, Bloom and Richardson (SBR) (Bloom & Richardson, 1957; Scarff & Torloni, 1968) histological grade III tumours and those aged below 35 years] had received post-operative chemotherapy or hormone therapy. All patients received peroperative thiotepa. Two hundred and ninety-two patients underwent clinical, radiological and laboratory tests every 3 months for the first 2 years and yearly thereafter. At the time of analysis, 91 patients had relapsed (37 had recurrences, 78 had distant metastases); of these, 54 died of breast cancer.

Tissue extract samples

Tumour specimens were obtained at surgery, selected by the pathologist and stored in liquid nitrogen until extraction. Age, SBR histological grade, number of involved lymph nodes (mean number of lymph nodes examined = 14; range 7–40), macroscopic tumour size, menopausal status and oestrogen and progesterone receptor status (EORTC, 1980) were known in every case (Table I). For extraction, tissues pieces of 250–300 mg wet weight were pulverised at 4°C in 10 mM Tris–HCl buffer pH 7.4 containing 1.5 mM EDTA, 0.5 mM dithiothreitol and 10% glycerol. The suspension was centrifuged (100,000 *g* at 4°C for 60 min). The cytosols were collected and stored in liquid nitrogen until use.

Assay of uPA, PAI-1 and PAI-2

Levels of uPA, PAI-1 and PAI-2 antigen were measured in cytosols by an immunoenzymatic method (Biopool Tint Elize, Umea, Sweden). Monoclonal anti-uPA antibody was raised against pro-uPA, two forms of uPA (low and high molecular weight) and uPA bound to plasminogen activator inhibitor type 1 or 2 (PAI-1 or PAI-2). Monoclonal anti-PAI-1 antibody recognises active and inactive forms of PAI-1 and PAI-1 bound to uPA or tPA. Monoclonal anti-PAI-2 antibody recognises low molecular weight PAI-2 (44.6 kDa) and glycosylated high molecular weight PAI-2 (60 kDa). After incubation of cytosols for 2 h at 25°C with agitation,

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the second polyclonal antibody labelled with peroxidase was added. Absorbance was measured at 405 nm in a microtitre plate reader (Milenia Kinetic Analyser). Antigen levels (ng ml^{-1}) were obtained from standard curves. Protein levels (mean = 1.8 ng ml^{-1}) were assayed using the Bradford method (Bradford, 1976; Bio Rad, CA, USA). Results were expressed in ng per mg of protein and assays were performed in triplicate. The Biopool Tint Elize Kit commercial standards for sera were optimised by diluting cytosols (1:6 for the assay of PAI-1 and 1:2 for PAI-2) in phosphate-buffered saline (PBS) solution pH 7.4, EDTA/Tween 20, containing bovine serum albumin (BSA) 1:1,000. The detection limit is about 0.1 ng ml^{-1} for uPA, 2.5 ng ml^{-1} for PAI-1 and 4.0 ng ml^{-1} for PAI-2.

Statistical methods

Differences in the distribution of characteristics among patient subgroups were analysed using the χ^2 test. Spearman correlation coefficients (ρ) were computed for pairwise combinations of uPA, PAI-1, and PAI-2. The cut-off points for uPA, PAI-1 and PAI-2 were determined independently of prognosis by the *K*-means clustering method (Hartigan, 1975), which separates patients into clusters so that the within-cluster sum of squares is minimised. For each parameter, actuarial disease-free survival (DFS) and metastasis-free survival (MFS) rates were calculated according to the method of Kaplan and Meier (1958) and compared by the log-rank test. DFS was defined as the time from diagnosis to the detection of the first local relapse or distant metastasis, and MFS was defined as the time from diagnosis to the occurrence of the first distant metastasis (locoregional relapse was excluded) or to the end of the study. A forward stepwise multivariate Cox 'main effects' was used to determine the importance of uPA, PAI-1 and PAI-2 relative to known classical prognostic factors (Cox, 1972). Table I presents the patient characteristics candidates for inclusion in the Cox model. It should be noted that variables with more than two outcome states were recoded using dummy variables. In addition to these variables, the potential for interaction between uPA, PAI-1 and PAI-2 was evaluated in a second Cox model, the 'interactive' model. The two models were then compared using Akaike's criterion (Akaike, 1974; Lawleff & Singhal, 1987*a,b*) which gives the best-fit model as the one with minimum AIC (Akaike information criterion), defined as: AIC = -2 log (likelihood) + 2 (number of parameters).

Results

The distribution of clinical, histological and biological factors in the overall study population is shown in Table I. Addi-

Table I Description of the population and results of the univariate prognostic analysis

Factor	*Number of patients*	*Number of events (%)*	P-*values*	*Number of metastases (%)*	P-*values*
Age					
< 50 years	118	34 (29)	0.8	30 (25)	0.9
50–64 years	119	37 (31)		30 (25)	
> 64 years	77	20 (26)		18 (23)	
Menopausal status					
Premenopausal	141	38 (27)	0.2	34 (24)	0.4
Post-menopausal	173	53 (31)		44 (26)	
Stage					
I	55	13 (24)	0.04	10 (18)	0.02
II	219	61 (28)		52 (24)	
II	40	17 (42)		16 (40)	
Clinical tumour size (mm)					
< 34	157	35 (22)	0.005	27 (17)	0.001
⩾ 34	157	56 (35)		51 (32)	
Macroscopic tumour size (mm)					
< 29	221	56 (25)	0.01	46 (21)	0.01
⩾ 29	93	35 (38)		32 (34)	
Path. axillary lymph nodes					
0	146	32 (22)	< 0.0005	24 (16)	< 0.0005
1–3	98	25 (25)		21 (21)	
> 3	70	34 (48)		33 (47)	
Scarff, Bloom and Richardson grade					
I	30	6 (20)	0.5	4 (13)	0.3
II	199	61 (31)		51 (26)	
III	85	24 (28)		23 (27)	
Oestrogen receptor level					
< 10 fmol mg^{-1} protein	95	29 (30)	0.6	28 (29)	0.2
⩾ 10 fmol mg^{-1} protein	219	62 (28)		50 (23)	
Progesterone receptor level					
< 10 fmol mg^{-1} protein	137	45 (33)	0.2	40 (29)	0.1
⩾ 10 fmol mg^{-1} protein	177	46 (26)		38 (21)	
uPA status					
uPA − ⩽ 0.52 ng mg^{-1} protein	214	55 (25)	0.06	46 (21)	0.03
uPA + > 0.52 ng mg^{-1} protein	100	36 (35)		32 (32)	
PAI-1 status					
PAI-I − ⩽ 3 ng mg^{-1} protein	231	60 (26)	0.04	49 (21)	0.007
PAI-1 + > 3 ng mg^{-1} protein	83	31 (37)		29 (35)	
PAI-2 status					
PAI-2 − ⩽ 14.5 ng mg^{-1} protein	271	85 (31)	0.02	73 (27)	0.04
PAI-1 + > 14.5 ng mg^{-1} protein	43	6 (14)		5 (12)	
Combination					
PAI-1 − PAI-2 −	205	56 (27)	0.004	46 (22)	0.001
PAI-1 − PAI-2 +	26	4 (15)		3 (11)	
PAI-1 + PAI-2 −	66	29 (44)		27 (41)	
PAI-1 + PAI-2 +	17	2 (12)		2 (12)	

Table II Distribution of uPA, PAI-1 and PAI-2 in the overall population of breast cancer patients

Variable	*Median*	*Mean*	*Standard deviation*	*Range*	*Cut-off*
uPA (ng mg^{-1} protein)	0.32	0.48	0.53	0–4.4	0.52
PAI-1 (ng mg^{-1} protein)	1.86	2.60	2.66	0–28.9	3
PAI-2 (ng mg^{-1} protein)	2.72	8.70	19.18	0–171	14.5

tional details on the biological factors are presented in Table II. The cut-off points were 0.52 ng per mg of protein for uPA, 3 ng per mg of protein for PAI-1 and 14.5 ng per mg of protein for PAI-2.

Relation between uPA, PAI-1, PAI-2 and clinical and histological factors

uPA was not related to any clinical or histological variable. High levels of uPA were related to high levels of PAI-1 and PAI-2 ($P<0.0001$, $\rho = 0.47$, and $P<0.001$, $\rho = 0.35$, respectively). High levels of PAI-1 and PAI-2 were related to post-menopausal status ($P = 0.008$ and $P<0.01$ respectively). Higher levels of PAI-1 were related to SBR histological grade II and III ($P<0.01$) and showed borderline significance with high values of PAI-2 ($P = 0.06$) (not shown).

Univariate prognostic analysis in the overall population (Table I)

High levels of uPA showed borderline significance with DFS ($P = 0.06$) and were associated with MFS ($P = 0.03$). High levels of PAI-1 were associated with significantly shorter DFS ($P = 0.04$) and MFS ($P = 0.007$) (Figure 1a). In contrast, high values of PAI-2 were associated with longer DFS ($P = 0.02$) (Figure 1b) and MFS ($P = 0.04$). When PAI-1 and PAI-2 were combined, high levels of PAI-1 and low levels of PAI-2 correlated with poor DFS ($P = 0.004$) and MFS ($P = 0.001$) (Figure 1c).

Univariate prognostic analysis according to menopausal status (Table III)

In premenopausal patients, uPA, PAI-1 and PAI-2 levels were not related to DFS or MFS. In post-menopausal patients, high levels of uPA showed borderline significance with DFS ($P = 0.06$) and were associated with shorter MFS ($P = 0.04$). High levels of PAI-1 were only associated with shorter MFS ($P = 0.02$) (Figure 2a). In contrast, patients with low levels of PAI-2 had shorter DFS ($P = 0.02$) and MFS ($P = 0.03$) (Figure 2b). The combination of high levels of PAI-1 and low levels of PAI-2 correlated with shorter DFS ($P = 0.002$) and MFS ($P = 0.001$) (Figure 2c).

Univariate prognostic analysis according to axillary node status (Table IV)

In node-postive patients, uPA, PAI-1 and PAI-2 were not related to DFS or MFS. In node-negative patients, uPA was not correlated with DFS or MFS. However, high levels of PAI-1 were associated with shorter DFS ($P = 0.02$) and MFS ($P = 0.0008$). High levels of PAI-2 were only associated with longer DFS. The combination of high levels of PAI-1 and low levels of PAI-2 was related to shorter DFS ($P = 0.0008$) (Figure 3a) and MFS ($P = 0.00005$) (Figure 3b).

Cox multivariate analysis of clinical and histological factors and uPA, PAI-1 and PAI-2 in the overall population (Table V)

The two models took into account the main effects and interactions respectively. With regard to DFS, the 'main effects' model identified, in the following order, node involvement, PAI-2 and PAI-1 as independent variables. The 'interactive' model increased the individual prognostic value

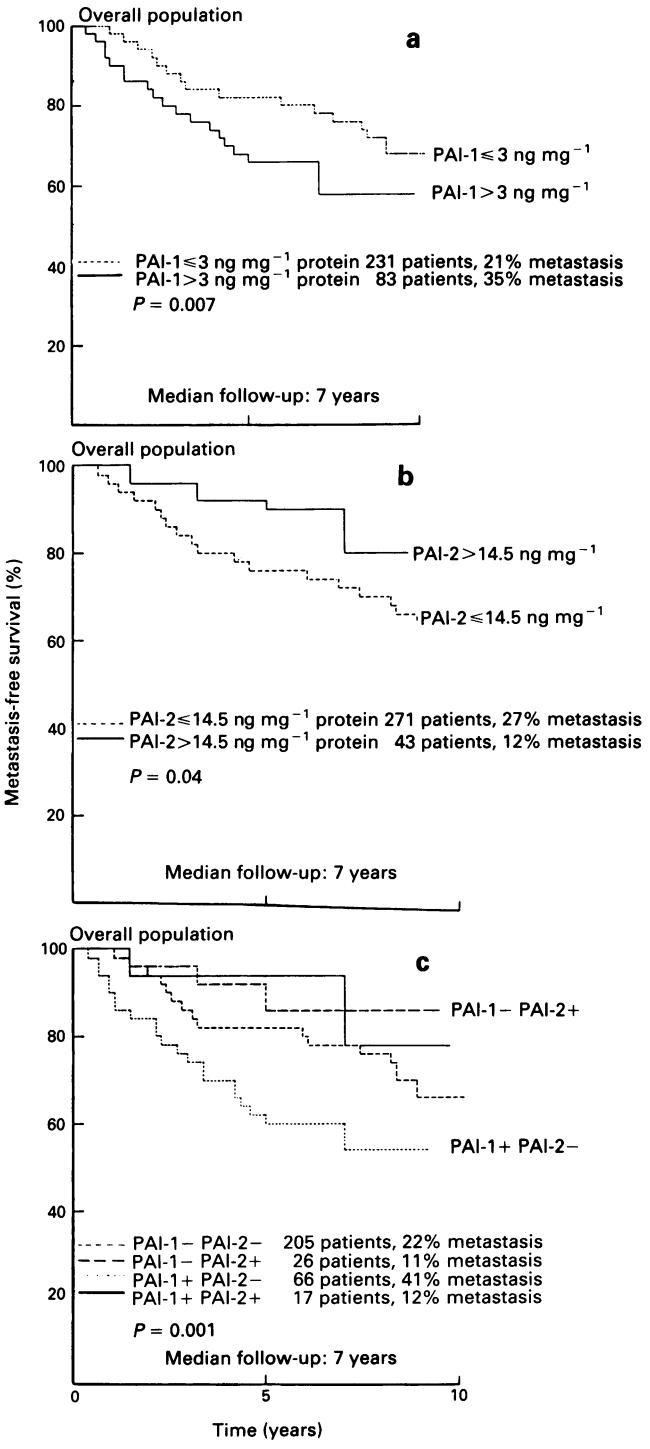

Figure 1 Metastasis-free survival as a function of **a**, PAI-1; **b**, PAI-2; and **c**, their association in the overall population. **a**, Patients with high PAI-1 levels had a significantly lower rate of MFS than those with low levels. **b**, Patients with high PAI-2 levels had a significantly higher rate of MFS than those with low levels. **c**, A particularly favourable prognostic group was identified by an association between low PAI-1 and high PAI-2 levels.

Table III Univariate prognostic analysis according to DFS and MFS in post-menopausal patients ($n = 173$)

		DFS			MFS		
Variable	*Number of patients*	*Number of events (%)*	*Relative risk*	P-*value*	*Number of metastases (%)*	*Relative risk*	P-*value*
UPA							
$\leqslant 0.52$ ng mg^{-1} protein	119	31 (26)	1.0	0.06	25 (21)	1.00	0.04
>0.52 ng mg^{-1} protein	54	22 (41)	1.70		19 (35)	1.86	
PAI-1							
$\leqslant 3$ ng mg^{-1} protein	117	31 (26)	1.00	0.1	24 (20)	1.00	0.02
>3 ng mg^{-1} protein	56	22 (39)	1.56		20 (36)	1.91	
PAI-2							
$\leqslant 14.5$ ng mg^{-1} protein	142	49 (35)	1.00	0.02	41 (29)	1.00	0.03
>14.5 ng mg^{-1} protein	31	4 (13)	0.32		3 (9)	0.30	
Combination							
PAI-1 – PAI-2 –	100	28 (28)	1.00	0.002	22 (22)	1.00	0.001
PAI-1 – PAI-2 +	17	3 (18)	0.62		2 (12)	0.56	
PAI-1 + PAI-2 –	42	21 (50)	2.12		19 (45)	2.54	
PAI-1 + PAI-2 +	14	1 (7)	0.20		1 (7)	0.27	

Table IV Univariate prognostic analysis according to DFS and MFS in axillary node-negative patients ($n = 146$)

		DFS			MFS		
Variable	*Number of patients*	*Number of events (%)*	*Relative risk*	P-*value*	*Number of metastases (%)*	*Relative risk*	P-*value*
UPA							
$\leqslant 0.52$ ng mg^{-1} protein	97	18 (18)	1.00	NS	12 (12)	1.00	NS
>0.52 ng mg^{-1} protein	49	14 (28)	1.59		12 (24)	2.12	
PAI-1							
$\leqslant 3$ ng mg^{-1} protein	102	17 (16)	1.00	0.02	10 (10)	1.00	0.0008
>3 ng mg^{-1} protein	44	15 (34)	2.22		14 (32)	3.64	
PAI-2							
$\leqslant 14.5$ ng mg^{-1} protein	121	31 (26)	1.00	0.02	23 (19)	1.00	NS
>14.5 ng mg^{-1} protein	25	1 (4)	0.13		1 (4)	0.19	
Combination							
PAI-1 – PAI-2 –	90	17 (19)	1.00	0.0008	10 (11)	1.00	0.00005
PAI-1 – PAI-2 +	12	0 (0)	0		0 (0)	0	
PAI-1 + PAI-2 –	31	14 (45)	2.85		13 (42)	4.66	
PAI-1 + PAI-2 +	13	1 (8)	0.36		1 (8)	0.64	

of PAI-1 and PAI-2 by identifying a subpopulation with a particularly poor prognosis, in which elevated PAI-1 levels were associated with low PAI-2 levels. With regard to MFS, the main effects model identified, in the following order, node involvement, PAI-1, clinical tumour size and PAI-2 as independent variables. The 'interactive' model identified a subpopulation with a particularly poor prognosis, in which elevated PAI-1 levels were associated with low PAI-2 levels, as for DFS. According to Akaike's criterion, the interactive model was equivalent to the 'main effects' model.

Cox multivariate analysis of clinical and histological factors and uPA, PAI-1 and PAI-2 according to menopausal status (Table VI)

In the premenopausal patients, macroscopic tumour size was the only variable associated with DFS ($P = 0.005$). For MFS, the only independent variable was node involvement ($P = 0.004$) (not shown).

In the post-menopausal patients, the 'main effects' model identified, in the following order, node involvement, uPA and PAI-2 as independent variables for DFS. When interactions were taken into account, the prognostic value of uPA and PAI-2 was increased, identifying a subpopulation with a particularly poor prognosis, in which high levels of uPA were associated with low levels of PAI-2. With regard to MFS, the 'main effects' model identified, in the following order, uPA, PAI-2, clinical tumour size and PAI-1 as independent variables. The 'interactive' model increased the individual prognostic value of uPA and PAI-2, identifying a subpopulation with a particularly poor prognosis, in which high levels of uPA were associated with low levels of PAI-2.

Cox multivariate analysis of clinical and histological factors and uPA, PAI-1 and PAI-2 according to axillary node status (Table VII)

In the patients with node involvement, only the number of affected nodes was selected by the Cox model, both for DFS ($P = 0.0008$) and for MFS ($P = 0.0002$). This also held true for DFS ($P = 0.0008$) when the interactions between the relevant variables were taken into account. The 'interactive' model identified an MFS population in which low levels of PAI-1 were associated with low levels of uPA ($P = 0.05$); this minimised the pejorative effect of node involvement, which remained the most important factor ($P = 0.0002$) (not shown).

In the patients free of lymph node involvement, the 'main effects' model identified PAI-2 and PAI-1 as independent variables for MFS and DFS. Similarly, when interactions were taken into account in the DFS and MFS models, the prognostic value of PAI-2 and PAI-1 was increased; a subpopulation was identified in which the prognosis was particularly poor and in which high levels of PAI-1 were associated with low levels of PAI-2.

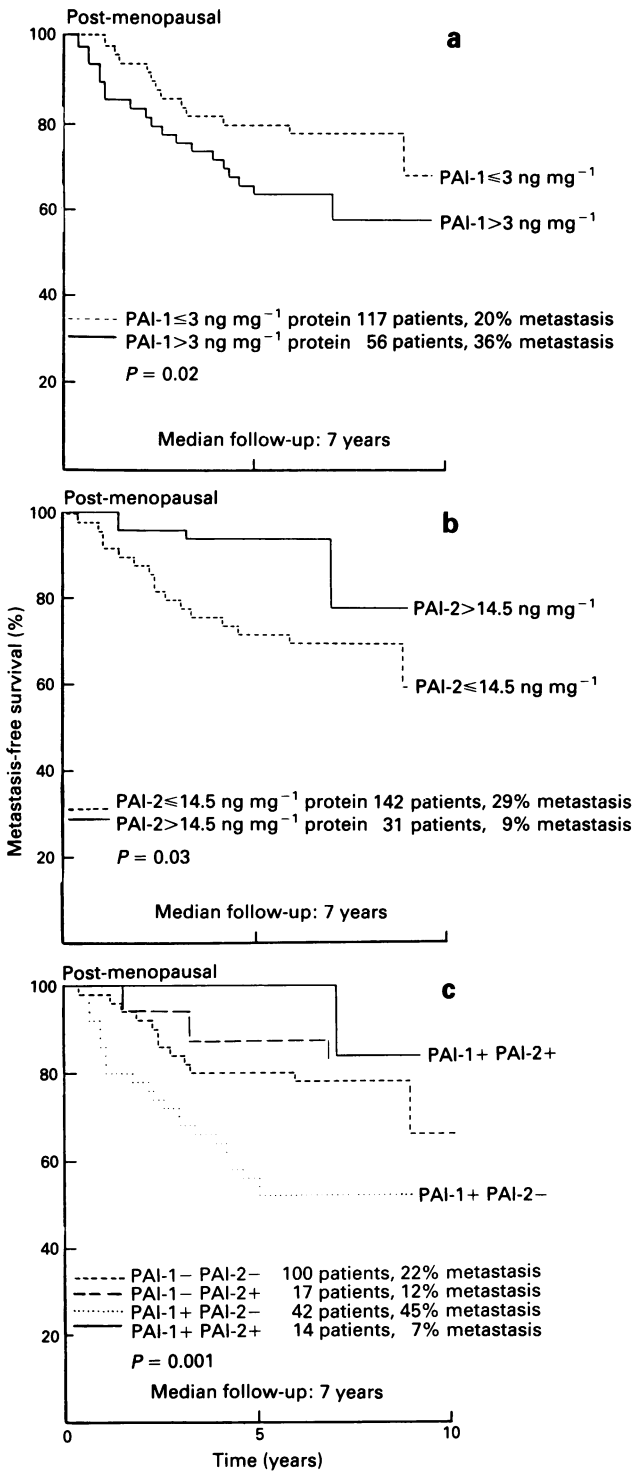

Figure 2 Metastasis-free survival as a function of **a**, PAI-1; **b**, PAI-2; and **c**, their association in post-menopausal women. **a**, Patients with high PAI-1 levels had a significantly lower rate of MFS than those with low levels. **b**, Patients with high PAI-2 levels had a significantly higher rate of MFS than those with low levels. **c**, A particularly favourable prognostic group was identified by an association between high PAI-1 and high PAI-2 levels.

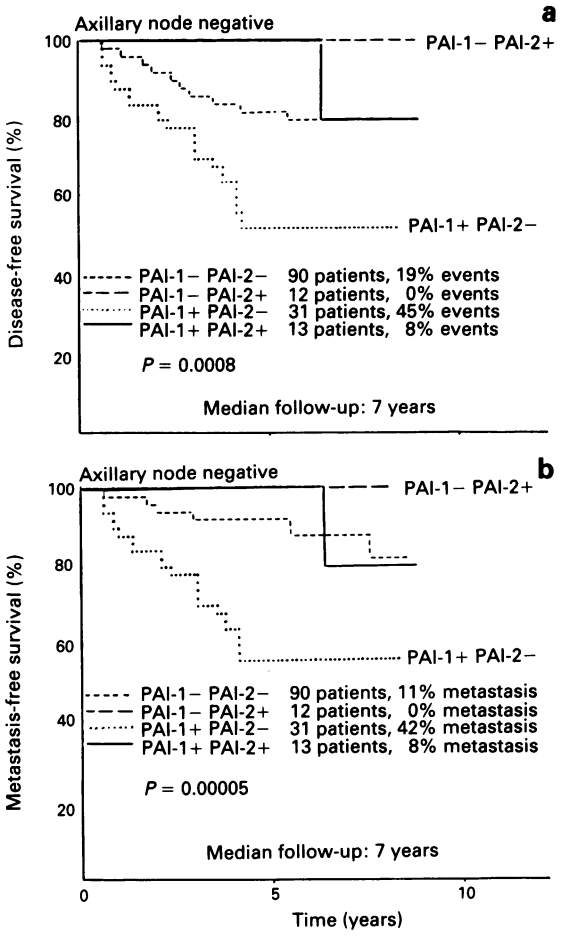

Figure 3 **a**, Disease-free survival; and **b**, Metastasis-free survival as a function of the association of PAI-1 and PAI-2 in axillary node-negative women. DFS and MFS were particularly good in women with low PAI-1 and high PAI-2 levels.

Discussion

The involvement of uPA in the invasion and metastatic mechanisms is well documented (De Bruin *et al.*, 1987; Sappino *et al.*, 1987; Jänicke *et al.*, 1990; Quax *et al.*, 1990; Hollas *et al.*, 1991), but the exact role of the two inhibitors PAI-1 and PAI-2 is less well known.

To better analyse their prognostic value, it is important to evaluate the relative importance of these three parameters and their interactions. To this end, we measured uPA, PAI-1 and PAI-2 antigen levels in a series of 314 breast cancer specimens and studied their relationships with classic parameters, as well as their prognostic value.

We confirmed that uPA and PAI-1 were independent of classical prognostic factors (Duffy *et al.*, 1990; Jänicke *et al.*, 1990; Grondhal-Hansen *et al.*, 1992; Reilly *et al.*, 1992), as well as the strong relationship between uPA and PAI-1 (Reilly *et al.*, 1992). We also confirmed the poor prognosis of patients with tumours containing high levels of uPA and PAI-1 (Duffy *et al.*, 1990; Jänicke *et al.*, 1990; Grondhal-Hansen *et al.*, 1992; Spyratos *et al.*, 1992; Sumiyoshi *et al.*, 1992). In contrast, high levels of PAI-2 were associated with a favourable prognosis not only in the overall population, but also in the subgroup of post-menopausal women and those with no node involvement. PAI-2, which has highest affinity constant for uPA, appears to be a true inhibitor, contrary to PAI-1. Sumiyoshi *et al.* (1992) reported that PAI-2 levels were higher in node-negative patients, but did not provide a prognostic analysis.

The correlation between uPA and PAI-1 antigen levels suggests one of two possibilities (Reilly *et al.*, 1992): either PAI-1 is a defence mechanism against tumoral invasion that has been inactivated at the time of analysis, or it plays a role in plasminogen activation. Our results, showing a link between high levels of PAI-1 and a poor outcome, tend to support the second hypothesis and to confirm the redundancy of the information provided by PAI-1 and uPA. PAI-1 could also be a marker of neovascularisation, as it is abun-

Table V Cox multivariate analysis in 'main effects' model and 'interactive' model of clinical, histological and biological factors in breast cancer in overall population ($n = 314$) for DFS and MFS

Criterion	*Variable*	*Regression coefficient*	*Relative risk (95% CI)*	P-*value*
DFS	*'Main effects' model*			
	Node status: $\leqslant 3$ *vs* >3	0.970	2.6 (1.7–4.0)	<0.0001
	PAI-2	− 0.956	0.4 (0.2–0.9)	0.02
	PAI-1	0.554	1.7 (1.1–2.7)	0.02
	'Interactive' model			
	Node status: $\leqslant 3$ *vs* >3	0.868	2.4 (1.5–3.7)	<0.0001
	PAI-1 + PAI-2 −	0.737	2.1 (1.3–3.3)	0.003
	Clinical tumour size: <34 *vs* $\geqslant 34$ mm	0.451	1.6 (1.0–2.4)	0.04
MFS	*'Main effects' model*			
	Node status: $\leqslant 3$ *vs* >3	1.066	2.9 (1.8–4.6)	<0.0001
	PAI-1	0.796	2.2 (1.4–3.5)	0.007
	Clinical tumour size: <34 *vs* $\geqslant 34$ mm	0.577	1.8 (1.1–2.9)	0.02
	PAI-2	− 0.962	0.4 (0.2–1.0)	0.01
	'Interactive' model			
	Node status: $\leqslant 3$ *vs* >3	1.106	2.9 (1.8–4.6)	<0.0001
	PAI-1 + PAI-2 −	0.920	2.5 (1.6–4.0)	0.0008
	Clinical tumour size: <34 *vs* $\geqslant 34$ mm	0.593	1.8 (1.1–2.9)	0.02

Table VI Cox multivariate analysis in 'main effects' model and 'interactive' model of clinical, histological and biological factors in breast cancer in menopausal patients ($n = 173$) for DFS and MFS

Criterion	*Variable*	*Regression coefficient*	*Relative risk (95% CI)*	P-*value*
DFS	*'Main effects' model*			
	Nodal status: $\leqslant 3$ *vs* >3	1.028	2.8 (1.6–4.9)	0.0003
	uPA	0.872	2.4 (1.4–4.2)	0.03
	PAI-2	− 1.280	0.3 (0.1–0.8)	0.005
	'Interactive' model			
	Nodal status: $\leqslant 3$ *vs* >3	1.115	3.0 (1.7–5.3)	0.0003
	UPA + PAI-2 −	0.992	2.7 (1.5–4.7)	0.001
MFS	*'Main effects' model*			
	Nodal status: $\leqslant 3$ *vs* >3	1.147	3.1 (1.7–5.8)	<0.0001
	uPA	0.668	2.0 (1.0–3.8)	0.02
	PAI-2	− 1.215	0.3 (0.1–1.0)	0.01
	Clinical tumour size: <34 *vs* $\geqslant 34$ mm	0.892	2.4 (1.2–5.1)	0.04
	PAI-1	0.725	2.1 (1.1–4.0)	0.04
	'Interactive' model			
	Nodal status: negative *vs* positive	1.189	3.3 (1.8–6.0)	<0.0001
	UPA + PAI-2 −	1.047	2.9 (1.5–5.3)	0.001
	Clinical tumour size: <34 *vs* $\geqslant 34$ mm	0.720	2.1 (1.0–4.2)	0.04

Table VII Cox multivariate analysis in 'main effects' model and 'interactive' model of clinical, histological and biological factors in breast cancer in axillary node-negative patients ($n = 146$) for DFS and MFS

Criterion	*Variable*	*Regression coefficient*	*Relative risk (95% CI)*	P-*value*
DFS	*'Main effects' model*			
	PAI-2	− 2.362	0.1 (0.0–0.7)	0.005
	PAI-1	1.080	2.9 (1.5–5.9)	0.003
	'Interactive' model			
	PAI-1 + PAI-2 −	1.263	3.5 (1.8–7.1)	0.0008
MFS	*'Main effects' model*			
	PAI-1	1.573	4.8 (2.1–10.9)	0.002
	PAI-2	− 2.171	0.1 (0.0–0.9)	0.003
	'Interactive' model			
	PAI-1 + PAI-2 −	1.706	5.5 (2.5–12.3)	<0.0001

dantly secreted by endothelial cells (Loskutoff & Edginton, 1977).

The Cox model identified subgroups of patients at risk, in whom low levels of PAI-2 were associated, on the one hand, with high levels of PAI-1, both in the overall population and in the node-negative patients, and, on the other hand, with high levels of uPA in the post-menopausal women. The significant association observed in the overall population was in fact due to the subgroup of post-menopausal women and node-negative women. Indeed, PAI-1 and PAI-2 levels were

linked to hormonal status: high levels of both proteins were found preferentially in the post-menopausal women. Scarabin *et al.* (1990) suggested that decreased oestrogen production could influence PAI-1 activity and reported that serum levels of PAI-1 were elevated in post-menopausal women relative to premenopausal women, reflecting a degree of control of PAI-1 secretion by oestrogen.

If one analyses the populations studied by Duffy *et al.* (1990), Jänicke *et al.* (1991), Foekens *et al.* (1992) and Jänicke *et al.* (1993), it can be seen that the median follow-up periods are very different (35, 25, 5 and 30 months respectively). Discrepancies between the results of these studies and our own can partly be explained by differences in the populations. In the study by Jänicke *et al.* (1993) (a prospective study) the proportion of premenopausal patients (36%) was lower than in our work (45%). In addition, there were differences in the treatment regimens (chemotherapy, hormone therapy). In the study by Duffy *et al.* (1990), 69% of the patients received adjuvant treatment, compared with 28% in the study by Foekens *et al.* (1992) and 37% in our work. The number of patients also varied considerably. Jänicke *et al.* (1991) studied respectively 54 and 50 patients with and without node involvement. The numbers in our work were 168 and 146 respectively.

In addition, the choice of buffer (presence or absence of Triton X-100) directly influences the uPA antigen level measured (Schmitt *et al.*, 1991). However, the comparative study by Schmitt *et al.* (1991) showed no significant difference for PAI-1 (median PAI-1 = 1.0 ng per mg of protein without Triton X-100 and median PAI-1 = 0.95 ng per mg of protein with Triton X-100). The influence of this ionic detergent on PAI-2 levels is unknown, but the results of a prospective study by Foucrè *et al.* (1991) and those of our study are similar for PAI-1 and PAI-2, although Triton X-100 was used only in the former.

The determination of the cut-off could be the reason for the slight discrepancies between the various reported data. However, in the case of PAI-1, if one compares the cut-offs determined by Jänicke *et al.* (1993) (2.18 ng per mg of protein) and our population (3.0 ng per mg of protein), there is a certain similarity despite the use of different approaches. It is, however, very difficult to compare the cut-offs in other series given the lack of standardisation.

In situ hybridisation studies of colonic cancer (Pyke *et al.*, 1991*a*) have shown a very heterogeneous distribution of PAI-1 within the tumour and an association with uPA in areas showing no signs of breakdown, suggesting a protective effect of PAI-1. In contrast, our results obtained with breast tumour cytosols suggest no such protective effect, although further analysis using the same approach as Pyke *et al.* (1991*b*)will be necessary. Indeed, tumours contain a complex mixture of epithelial cells, stromal cells and vascular elements, which could lead to interactions with an influence on tumour development.

The potential prognostic factors PAI-1 and PAI-2, together with the PAI-1–PAI-2 and uPA–PAI-2 associations, add to an evolving list of biological markers of breast cancer, including oestrogen and progesterone receptors, epidermal growth factor (EGF) receptor, uPA and cathepsin D (Spyratos *et al.*, 1992). A better understanding of the regulation of uPA and its receptor and inhibitors (PAI-1 and PAI-2) in breast carcinomas may lead to other ways of interrupting tumour invasion and metastasis formation.

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