Clinical Significance of Urokinase-type Plasminogen Activator (uPA) and its Type-1 Inhibitor (PAI-1) for Metastatic Sentinel Lymph Node Involvement in Breast Cancer

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Abstract. *Urokinase-type plasminogen activator (uPA) and its type-1 inhibitor (PAI-1) are key factors for tumor invasion and development of metastases in breast cancer. Prospective studies confirmed the prognostic significance of these factors for development of distant metastases. The predictive impact of uPA and PAI-1 for metastatic sentinel lymph node involvement is unclear. Patients and Methods: Between 2006 and 2008 uPA and PAI-1 were measured in 184 out of 1,035 patients for primary breast cancer. uPA and PAI-1 were analyzed with an ELISA assay. Measured concentrations were considered as negative for uPA <3 ng/ml and for PAI-1 <14 ng/ml. Results: In a retrospective analysis, 173/184 women had a negative sentinel lymph node and 11/184 women had a metastatic sentinel lymph node. From the 11 women with a positive sentinel lymph node 7 had elevated values for uPA and 4 had elevated values for PAI-1. Four and 7 women were uPA- and PAI-1-negative, respectively. Sensitivity, specificity, positive and negative predictive values for uPA were 63.3%, 50.9%, 7.6%, 95.6% and for PAI-1 36%, 52.6%, 4.7%, 92.9%. Even the combination of both uPA and PAI-1 values did not detect 3/11 women with metastatic lymph node involvement. Conclusion: uPA and PAI-1 alone or in combination did not identify all patients with metastatic lymph node involvement. Thus, uPA and PAI-1 cannot be considered as predictive selection parameters to avoid sentinel lymph node biopsy in case of negative values for uPA or PAI-1.*

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Breast cancer is the most common female carcinoma. In Germany, approximately 70,000 women are diagnosed with breast cancer each year and about 20,000 women die from this disease each year (1). In the past decades treatment has changed considerably as surgical treatment has become less aggressive now favoring breast conservative surgical techniques (2). Axillary lymph node dissection has been abandonned as routine surgical therapy and sentinel lymph node excision has become an integral part in the treatment of patients with breast carcinoma with clinically node-negative axilla and to select patients needing subsequent axillary lymph node dissection. Even this strategy was modified because the presence of isolated tumor cells or micrometastases does not require axillary clearance and systematic axillary dissection may be reserved for patients with macrometastastic involvement of more than 2 sentinel nodes in a breast-conserving treatment strategy (ACOSOG Z0011-trial) (3). Major advantages of sentinel lymph node biopsy using radiolabelled colloids are that this is a safe procedure with detection rates usually above 95% resulting in a decrease in morbidity in comparison to axillary lymph node dissection (4).

In breast cancer, tumor proteases have been identified as risk factors for metastatic spread guiding the selection of postoperative chemotherapy (5). Urokinase-type plasminogen activator (uPA) and its inhibitor plasminogen activator inhibitor-1 (PAI-1) are key factors in tumor invasion and metastasis. Increased levels of uPA and/or PAI-1 in primary tumor tissues correlate with tumor aggressiveness and poor patient outcome (6, 7). In 1988, Duffy *et al*. were the first to show that high enzymatic activity of uPA in primary breast cancer tissues was correlated with advanced tumor stage and poor clinical outcome (8). In 1989, Jänicke *et al*. demonstrated that high uPA antigen levels in primary tumor tissue measured by enzyme-linked immunosorbent assay (ELISA) also predicted poor prognosis (9). At the same time,

it also became apparent that not only antigen levels of uPA but also those of PAI-1 are of prognostic importance in breast cancer patients (10). Subsequently, many researchers validated that primary breast cancer patients, and in particular node-negative patients with high tumor tissue antigen content of uPA and/or PAI-1, have a worse probability of disease-free survival (DFS) and overall survival (OS) than patients with low levels of either or both biomarkers (5, 11-14). uPA and PAI-1 have prognostic information independent of established prognostic factors such as tumour size, tumour grade, steroid hormone receptor status, menopausal status (15) and even HER2 status (14, 16-18). A large meta-analysis conducted by the European Organisation for Research and Treatment of Cancer (EORTC) Receptor and Biomarker Group, comprising 8377 breast cancer patients from 18 independent collectives, validated the prognostic (19) and predictive impact of uPA/PAI-1 (14, 20).

Thus, we investigated whether tumor proteases uPA and PAI-1 may be predictive for metastatic lymph node involvement in patients with breast cancer potentially obviating the need for sentinel lymph node biopsy in patients with non-elevated values for uPA and PAI-1 in their primary tumors.

Patients and Methods

Between 2006 to 2008 a total of 1,035 women with an initial diagnosis of breast cancer were treated in the breast Center of the University-Hospital of Cologne, Germany. From these 1,035 women, 947 were elegible for sentinel lymph node biopsy and of these SLN patients 184 had a subsequent analysis of the expression of uPA and PAI-1 (protease group) of the primary tumor while 763 patients did not have an analysis of uPA and PAI-1 (non-protease group). Non-protease and protease group were characterized with respect to age, T-stage and hormone receptor status of the primary tumor, and lymph node involvement. Further details are provided in Table I.

Table I. *Patients' characteristics*.

	Protease group N (%)	Non-protease group N (%)
Age		
25-34	0	20 (2.6)
35-45	25 (13.6)	141 (18.5)
46-56	62 (33.7)	217 (28.4)
57-67	73 (39.7)	256 (33.6)
68-78	24 (13.0)	111 (14.5)
79-90	0	18 (2.4)
T-stage		
T0	0	16 (2.1)
T1	139 (76)	464 (60.8)
T2	45 (24)	203 (26.6)
T3	0	29 (3.8)
T4	0	2 (0.3)
TX	0	14 (1.8)
Tis	0	35 (4.6)
Hormone receptor status		
ER-positive	176 (96)	637 (83.5)
ER-negative	8 (4.0)	126 (16.5)
PR-positive	156 (85.0)	612 (80.2)
PR-negative	28 (15.0)	151 (19.8)
Proteases		
uPA ≥3ng/ml	92 (50)	
uPA <3ng/ml	92 (50)	
PAI-1 ≥14 ng/ml	86 (46.7)	
PAI-1 <14ng/ml	98 (53.3)	
SLN involvement		
0	173 (94)	601 (78.8)
1	10 (5.5)	106 (13.9)
>1	1 (0.5)	56 (7.3)
Adjuvant therapy		
Yes	178 (97)	128 (16.8)
No	6 (3)	635 (83.2)

Measurement of uPA and PAI-1. Measurements of tumor-associated proteolytic factors uPA (urokinase-type plasminogen activator) and its type-1 inhibitor PAI-1 were done by a quality-assured enzyme-linked immunosorbent assay (ELISA) in fresh-frozen primary tumor tissue extracts (FEMTELLE® kit; American Diagnostica, Stanford, CT, USA). A value of uPA <3 ng/ml and for PAI-1 <14 ng/ml were considered as negative and values above as positive, respectively. Sentinel lymph node biopsy was performed according to published guidelines (4) and intraoperative localization of the radioactive lymph nodes were performed using a hand-held detector (Crystal Photonics®, Crystal Photonics GmbH, Albert-Einstein-Straße 16, D-12489 Berlin, GERMANY, http://crystal-photonics.com). Pathological analysis of the primary tumor and sentinel lymph node analysis were done in the Institute of Pathology according to WHO guidelines.

Statistical analysis. Descriptive statistics were used for the analysis of the protease and the non-protease group. Sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy were calculated. Comparison of groups was done by Mann-Whitney test. A *p*-value less than 0.05 was considered as significant.

Results

Mean age in the protease group (N=184) was 57.9±9 years (range=36.5-75.5 years) and in the non-protease group (N=763) 55.2±9.2 years (range=31.7-73.9 years). Only patients with T1 and T2 tumors with no suspicion of axillary lymph node involvement were elegible for sentinel lymph node biopsy. In the protease group and in the non-protease group a mean of 3 lymph nodes were excised (protease group: T1: 3.2±1.6 and T2: 2.8±1.7 *versus* non-protease group T1: 2.9±1.7 and T2: 3.1±1.8 lymph nodes).

Table II. *Age, T-stage, Estrogene and Progesterone receptor status, uPA and PAI-1 values, and number of metastatic lymph nodes of the 11 patients with metastatic sentinel lymph node involvement*

Patient	Pt.1	Pt.2	Pt.3	Pt.4	Pt.5	Pt.6	Pt.7	Pt.8	Pt.9	Pt.10	Pt.11
Age (years)	60	62	49	59	68	51	50	42	70	64	49
T-Stage	T1	T2	T2	T1	T1	T1	T1	T1	T2	T2	T1
ER(INR)	12	12	12	12	12	6	12	12	12	12	12
PR(INR)	11	12	12	12	12	12	9	12	9	12	12
uPA (ng/mg)	0,9	1,3	1,4	1,6	3,2	4,3	4,6	6,9	7,1	8,8	20,5
PAI-1 (ng/mg)	14,3	2,5	11,6	12,12	16,2	8,7	9,2	8,9	32,3	42,5	5,7
No of metastatic lymph nodes	1	4	1	1	1	1	1	1	1	4	1

In the non-protease group 601 (79%) patients had a negative and 162 (21%) a positive axillary lymph node. From 184 patients in the protease group 173 (94%) had a negative and 11 (6%) a positive axillary lymph node. From these 11 patients 7 had T1 and 4 had T2 tumors. Values for uPA and PAI-1 for these 11 patients are given in Table II. From the 11 patients with metastatic involvement of the sentinel lymph node 7 patients were positive for uPA and 4 patients were positive for PAI-1, three patients had both parameters elevated. Four patients (36%) were negative for uPA and 7 patients (64%) were negative for PAI-1. Even the combination of both parameters missed 3 patients (27%) with metastatic sentinel lymph node involvement (Table III). Because of the overall low number of metastatic sentinel lymph nodes in the protease group sub-group analysis with respect to T1 and T2 tumors was not performed.

Thus, elevation of uPA had a sensitivity of 63.3% and specificity of 50.9% and elevation of PAI-1 had values of 36% and 52.2% respectively. Though the negative predictive values were above 90% for uPA or PAI-1 or both parameters the positive predictive values were only 7.6% for uPA, 4.7% for PAI-1 and even with either parameter being positive a positive predictive value of only 7.3% was reached because most patients with a positive protease value did not have metastatic involvement of the sentinel lymph node. From the 173 patients in the protease group with a negative sentinel lymph node 85 patients were positive for uPA and 85 were positive for PAI-1 while 88 were negative for uPA and 91 for PAI-1, meaning that the Vast majority of patients with elevation of uPA or PAI-1 did not have metastatic sentinel node involvement. 59% of the node-negative patients had at least one positive protease.

Discussion

The present study demonstrated the very low positive predictive value of either uPA or PAI-1 or both parameters for metastatic sentinel lymph node involvement in our patient collective. The very low positive predictive value for uPA and PAI-1 for metastatic sentinel node involvement is explained by the fact that despite elevation of at least one protease most patients were node-negative. In addition, even elevation of both parameters missed about 1/3 of patients with metastatic sentinel lymph node involvement. Thus, neither uPA nor PAI-1 nor both parameters can be used for selection of patients in whom lymph node scintigraphy and subsequent lymph node excision could be obviated.

Sentinel lymph node scintigraphy and intra-operative localization were performed according to national guidelines (4) with high detection rates ≥98%. Pathological examination of the sentinel node was done according to WHO standards.

Antigen levels of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) are proteolytic factors associated with tumor invasion and metastasis in primary breast cancer (21). Whereas uPA facilitates tumor metastasis by proteolytic degradation of the extracellular matrix (22, 23), PAI-1 plays an important role in tumor invasion and metastasis not solely as an inhibitor of uPA. After binding of PAI-1 to receptor-bound uPA, this tertinary complex is internalized into the cell (24), thereby initiating signal transduction and cell proliferation (25).

The commercially-available uPA/PAI-1 test (Femtelle®, American Diagnostica GmbH, Kaplaneigasse 35, 64319 Pfungstadt, GERMANY, http://femtelle.de) is an ELISA with excellent standardisation and quality assurance requiring for very little tissue. Routine core biopsy specimens are sufficient, thus increasing feasibility in today's clinical environment (26, 27, 28). Concerning the results presented the same laboratory test for measurement of uPA and PAI-1 was used as in the Node-Negative Breast Cancer-3 (NNBC-3) Europe multicenter trial (14, 26).

The clinical significance of uPA and PAI-1 for breast cancer patients were first described by Duffy and Jänicke about 25 years ago (3, 4). Since then, the clinical value of the proteases uPA and PAI-1 have been validated in large clinical trials with the highest level of evidence such as the (i) Chemo N0 breast cancer trial and the (ii) Node-Negative Breast Cancer-3 (NNBC-3) Europe trial. The Chemo N0 breast cancer trial showed that node-negative breast cancer patients with low uPA and PAI-1, which account for approximately

Table III. *Contingency table (2 by 2 table) comparing absolute patient numbers for positive and negative uPA values with metastatic sentinel lymph node involvement, SLN positive: metastatic sentinel lymph node involvement present, SLN negative: metastatic sentinel lymph node involvement absent*

	SLN positive	SLN negative
uPA ≥ 3 ng/ml, *i.e.* positive	7	85
uPA< 3 ng/ml, *i.e.* negative	4	88

Sensitivity: 63.3%
Specificity: 50.9%
Positive predictive value: 7.6%
Negative predictive value: 95.6%
Diagnostic accuracy: 51.6%

Table IV. *Contingency table (2 by 2 table) comparing absolute patient numbers for positive and negative PAI-1 values with metastatic sentinel lymph node involvement, SLN positive: metastatic sentinel lymph node involvement present, SLN negative: metastatic sentinel lymph node involvement absent*

	SLN positive	SLN negative
PAI-1 ≥14 ng/ml, *i.e.* positive	4	82
PAI-1 <14 ng/ml, *i.e.* negative	7	91

Sensitivity: 36%
Specificity: 52.6%
Positive predictive value: 4.7%
Negative predictive value: 92.9%
Diagnostic accuracy: 51.6%

Table V. *Contingency table (2 by 2 table) comparing absolute patient numbers for uPA or PAI-1 or both positive versus uPA and PAI-1 negative with metastatic sentinel lymph node involvement, SLN positive: metastatic sentinel lymph node involvement present, SLN negative: metastatic sentinel lymph node involvement absent.*

	SLN positive	SLN negative
uPA or PAI-1 or both positive	8	102
uPA and PAI-1 negative	3	71

Sensitivity: 72.7%
Specificity: 41.0%
Positive predictive value: 7.3%
Negative predictive value: 95.9%
Diagnostic accuracy: 42.9%

half of the node-negative breast cancer patients, have a rather low risk of disease recurrence and can, therefore, be spared the burden of adjuvant systemic chemotherapy. However, the optimal chemotherapy for high-risk patients according to their high uPA and/or PAI-1 levels, who are at risk to develop metastasis, still needs to be determined (14). The recently published 10-year analysis data of the Chemo N0 breast cancer trial confirmed the clinical significance of uPA and PAI-1 as prognostic markers: High-risk patients randomised to cyclophosphamide- methotrexate-5-fluorouracil (CMF) therapy had a 26.0% lower estimated probability of disease recurrence than those randomised for observation (intention-to-treat-analysis: hazard ratio 0.74 (0.44-1.27); p=0.28). Thus, the Chemo-N0 trial was the first prospective biomarker-based therapy trial in early breast cancer defining patients reaching good long-term disease-free survival without adjuvant systemic therapy. Using a standardised uPA/PAI-1 ELISA, almost half of N0-patients could be spared chemotherapy, while high-risk patients benefit from adjuvant chemotherapy. These 10-year results validate the long-term prognostic impact of uPA/PAI-1 and the benefit from adjuvant chemotherapy in the high-uPA/PAI-1 group at the highest level of evidence. This study supported the guideline-based routine use of uPA/PAI-1 for risk-adapted individualised adjuvant chemotherapy indications in node-negative breast cancer (26).

The Node-Negative Breast Cancer-3 (NNBC-3) Europe trial, a prospective multi-centre phase III therapy trial, had two primary objectives: i. to compare risk assessment and clinical outcome based on the tumour-biological factors uPA/PAI-1 to those based on established, clinical and pathomorphological factors and ii. to optimise adjuvant chemo therapy for high-risk node-negative breast cancer patients. Especially for the latter trial extensive quality measurements were made with respect to the measurement of uPA and PAI-1.

A large meta-analysis conducted by the European Organisation for Research and Treatment of Cancer (EORTC) Receptor and Bio- marker Group, comprising 8377 breast cancer patients from 18 independent collectives, validated the prognostic (19) and predictive impact of uPA/PAI-1 (20), thus achieving the highest level of evidence for clinical utility of a cancer-associated biomarker, according to the American Society of Clinical Oncology (ASCO) tumour marker utility grading system (TMUGS) (29).

The present study did not analyze outcome data such as disease-free or overall survival due to limitations associated with the retrospective data analysis but focused on the question of metastatic lymph node involvement. Interestingly, uPA and PAI-1 were shown to have prognostic relevance in the aforementioned studies. However, as these proteases are associated with tumor spread one would expect an increase in nodal metastatic involvement. As this could not be shown these proteases seem to have more impact on distant metastatic spread than on development of metastases in axillary nodes.

Urokinase-type plasminogen activator and PAI-1 are predictors for distant metastasis. Their prognostic impact with regard to locoregional recurrence is still under discussion and needs further investigation. Whereas Knoop *et al.* (30) did not observe any significant impact of uPA and PAI-1 on

locoregional relapse, Cufer *et al*. (31) found PAI-1 levels above median in primary tumor tissue to be significantly associated with an increased risk for locoregional relapse. Both uPA and PAI-1 are only weakly-correlated with other traditional prognostic factors (32). The prognostic impact of uPA and PAI-1 is independent of established prognostic factors such as tumor size, tumor grade, hormone receptor status, or menopausal status (33). In node-positive patients, the prognostic impact of uPA/PAI-1 is only surpassed by that of nodal status, whereas in node-negative patients, uPA/PAI-1 is the strongest prognostic factor (15). Node-negative patients with low uPA and PAI-1 have a rather low risk of disease recurrence (30) and are thus candidates for being spared the burden of adjuvant chemotherapy. In contrast, high uPA/PAI-1 node-negative patients have a considerably increased risk of relapse, comparable to that of patients with ≥ 3 involved axillary lymph nodes (15, 34). Hence, for these patients, adjuvant systemic therapy, particularly chemotherapy, is clearly indicated (33). More recent studies confirmed the prognostic importance of uPA and PAI-1 (35, 36).

Limitations of the present study are associated with the retrospective nature of data analysis. The main disadvantage is that from all patients with a primary diagnosis of breast cancer within the study period only a fraction were analyzed with respect to uPA and PAI-1, *i.e.* 184/1,035 patients (18%). However, clinical parameters did not differ between groups.

In conclusion, despite their established prognostic value in patients with primary breast cancer uPA and PAI-1 had only a low positive predictive value for metastatic sentinel lymph node involvement and about one third of patients with metastatic sentinel nodes were negative for uPA and PAI-1, indicating that uPA and PAI-1 do not allow to select patients who do not need sentinel lymph node excision.

Conflicts of Interest

None.

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