The Breast 46 (2019) 101–107

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

Stromal coexpression of uPA/PAI-1 protein predicts poor disease outcome in endocrine-treated postmenopausal patients with receptor-positive early breast cancer

C.F. Singer [a, *], M. Filipits [b], S.W. Jahn [c], L. Abete [c], R. Jakesz [d], R. Greil [e], T. Bauernhofer [f], W. Kwasny [g], M. Seifert [a], F. Fitzal [h], M. Kastner [a], M. Schmitt [i], F. Moinfar [j], M. Gnant [k]

[a] Department of OB/GYN, Comprehensive Cancer Center, Medical University of Vienna, Austria
[b] Institute of Cancer Research, Medical University of Vienna, Vienna, Austria
[c] Institute of Pathology, Medical University of Graz, Graz, Austria
[d] Department of Surgery, Medical University of Vienna, Vienna, Austria
[e] Department of Medicine III, Paracelsus Medical University Salzburg, Salzburg, Austria
[f] Department of Medicine, Medical University of Graz, Graz, Austria
[g] Department of Surgery, LKH Wiener Neustadt, Wiener Neustadt, Austria
[h] Department of Surgery, Krankenhaus der Barmherzigen Schwestern, Linz, Austria
[i] Department of OB/GYN, Technical University of Munich, Munich, Germany
[j] Institute for Clinical Pathology, Krankenhaus der Barmherzigen Schwestern, Linz, Austria
[k] MD for the Austrian Breast and Colorectal Cancer Study Group. Department of Surgery, Medical University of Vienna, Vienna, Austria

ARTICLE INFO

Article history:
Received 9 January 2019
Received in revised form
29 April 2019
Accepted 2 May 2019
Available online 3 May 2019

Keywords:
uPA
PAI-1
Prognosis
Breast cancer
Immunohistochemistry

ABSTRACT (198 WORDS)

Background: To evaluate whether uPA/PAI-1 protein in hormone receptor−positive (HR+) breast tumor can predict prognosis in early breast cancer (BC).
Methods: 606 women with HR + BC who had ≥5 years of endocrine therapy and in whom tumor tissue was available were included in this analysis. Stromal uPA/PAI-1 protein expression was evaluated by immunohistochemistry and correlated with distant recurrence-free survival (DRFS) and overall survival (OS).
Results: Stromal uPA was detected in 292/538 tumors (54.3%) while 269/505 samples (53.3%) exhibited stromal PAI-1. Co-expression of both proteins was found in 163/437 (37.3%) samples. Stromal uPA/PAI-1 co-expression was not associated with tumor size, age, nodal status, grading, or receptor status. Tumor stroma with both uPA and PAI-1 protein expression were more likely to have a shorter DRFS (HR: 1.87; 95%CI 1.18−2.96; p = 0.007) and OS (HR: 1.29; 95%CI 0.93−1.80; p = 0.129) than women without uPA/PAI-1 co-expression. After a median follow-up of 10 years, women with uPA/PAI-1-positive tumors experienced a significantly shorter DRFS (86.5% vs 72.4%; p < 0.001) and OS (70.4% vs 58.9%; p = 0.020) compared to women with uPA/PAI-1 negative tumors.
Conclusion: Stromal co-expression of uPA and PAI-1 in breast cancer predicts poor DRFS and OS in postmenopausal women with HR + early-stage BC who receive endocrine therapy.

Introduction

The plasminogen activator system plays an important role in tumor invasion and metastasis. Not surprisingly, intratumoral levels of urokinase-type plasminogen activator (uPA) and its inhibitor plasminogen activator inhibitor type 1 (PAI-1) have a prognostic significance in early breast cancer. Over the years, several retrospective studies have consistently shown that patients with high intratumoral levels of uPA and/or PAI-1 experience a

* Corresponding author. Clinical-Translational Gynecological Oncology Head, Center for Familial Breast- and Ovarian Cancer Head, Center for Breast Health Medical University of Vienna / General Hospital Vienna, Department of Gynaecology Waehringer Guertel 18-20, 1090, Vienna, Austria..
E-mail address: christian.singer@meduniwien.ac.at (C.F. Singer).
URL: http://www.brustgesundheitszentrumwien.at

https://doi.org/10.1016/j.breast.2019.05.007
0960-9776/

significantly shorter disease-free survival (DFS) and overall survival (OS) than patients with low uPA/PAI-1 concentration. The American Society of Clinical Oncology 2007 Update of Recommendations for the Use of Tumor Markers in Breast Cancer has therefore suggested the use of the invasion markers uPA and its inhibitor PAI-1 for risk assessment [1]. More recently, a prospectively designed randomized phase III study in nodal-negative early breast cancer ("Chemo-N0 trial") has demonstrated that, in the absence of chemotherapy, the actuarial 10-year recurrence rate for high-uPA/PAI-1 expressing tumors is 23% vs. 13% in tumors with low levels of uPA/PAI-1 expression. Patients with high-uPA/PAI-1 expressing tumors who received CMF had a 26% lower recurrence rate, and a significantly longer DFS than women who did not. Chemo-N0 is thus the first prospective biomarker-based therapy trial demonstrating that women with low intratumoral uPA/PAI-1 expression achieve a good long-term DFS without adjuvant systemic chemotherapy, whereas tumors with high-uPA/PAI-1 expression derive a significant benefit from the addition of adjuvant CMF [2].

Despite the convincing level-1 evidence on the validity of uPA/PAI1 prognostication now available, the clinical utility of ELISA-based uPA/PAI1 analysis has been greatly limited by the requirement of fresh tumor tissue extracts [3]. In addition, the currently used analytical assay requires relatively large amounts of tumor tissue, thereby further confining its clinical applicability. ELISA-based uPA and PAI-1 measurements are therefore still infrequently used, particularly since gene expression-based assays now allow for a reproducible risk assessment in archived tumor tissues [4]. To overcome these assay-inherent limitations, immunohistochemical analysis of intratumoral uPA and PAI-1 protein expression has been suggested for the identification of women with poor prognosis. While uPA and PAI-1 can both be immunodetected in malignant breast epithelium and in surrounding stroma alike, *in situ* hybridization studies of invasive ductal breast cancer tissue have shown that uPA and PAI-1 mRNA expression is confined to the stromal myofibroblasts immediately surrounding invasive cancer cells [5–8]. We have therefore investigated the stromal expression of uPA and PAI-1 in FFPE-based tumor samples from a prospectively designed phase III study in hormone receptor-positive (HR+) postmenopausal women treated with endocrine therapy (ABCSG-06).

Methods

The present investigation is part of the Austrian Breast & Colorectal cancer Study Group (ABCSG) translational research program (https://www.abcsg.com/). HR + postmenopausal women included in this study had been randomized into the prospectively randomized adjuvant endocrine ABCSG-06 trial between 1990 and 1995, and had received 5 years of adjuvant tamoxifen with or without aminoglutethimide for the first 2 years of treatment. Approximately 50% of participating patients were subsequently re-randomized to receive 2 years of extended anastrozole, or to observation alone (ABCSG-06a). Patients re-randomized into the anastrozole arm of ABCSG-06a were censored once they received AI treatment to ensure that all analyzed patients had 5 years of endocrine treatment. A REMARK diagram detailing the study cohort is shown in Fig. 1. None of the patients had received adjuvant chemotherapy or HER2-directed therapy. Trial design, inclusion criteria and the main clinical results of these trials have been reported previously [9]. FFPE tumor blocks from HR + postmenopausal patients were collected from participating centers at the time of surgery and were stored at room temperature.

uPA and PAI1 immunohistochemistry

For uPA and PAI-1 analysis, consecutive FFPE tissue sections (3–5 μm) were deparaffined with EZPrep (Ventana Inc) and endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide. After a short rinse in tap water and TBS-solution, tissue sections were coated with 10% goat serum in TBS as blocking solution. The anti-uPA monoclonal antibody (Sekisui Diagnostics, clone #3689) was used at a dilution of 1:300 and incubated for 30 min at 37 °C. It recognizes free and receptor bound, single and two chain (HMW) urokinase and the B-chain (33 kD) fragment. The antibody is directed against a B-chain epitope of human urokinase, near the catalytic site [10]. The anti-PAI1 monoclonal antibody (Sekisui Diagnostics, clone #ADG3786) was used at a dilution of 1:35 and slides were incubated for 30 min at 37 °C. It recognizes both active and inactive forms of PAI-1, and in addition it recognizes PAI-1 complexed with urokinase, tissue-type plasminogen activators, or vitronectin. It is specific for human PAI-1 and no cross-reactivity was observed against other members of the plasminogen activator system (PAI-2, uPA, or tPA) [11,12]. Samples were then subjected to biotinylated goat anti-mouse immunoglobulin for 30 min, and consecutively incubated with streptavidin-HRP complex for another 30 min at room temperature, before DAB substrate was added for 10 min (Dako Inc). Sections were washed using Ultra Wash (Ventana Inc), counterstained with hematoxylin for 1–4 min, and immersed in tap water. Slides were then rinsed in detergent-containing tap water, dehydrated, and mounted with Aquatex (Merck, Germany). Tumors were evaluated for the presence of uPA and PAI1 reactivity of the tumor stroma by two pathologists (S.J. and L.A.), who were blinded to the patient's clinical outcome. Immunohistochemical uPA and PAI1 expression in more than 10% of the tumor stroma was scored as "positive" for the respective marker. The range of staining intensities for both antibodies was assessed, and the 10% cut off for tumor stroma uPA and PAI-1 positivity was defined, after the first 50 consecutive slides had been stained and analyzed. This 10% cut off value for uPA and PAI-1 expression was then employed on the remaining tissue sections. Evaluation was based on assessment of 4x and 10x magnification of tumor areas. This study was approved by the Ethics Committee of the Medial University of Vienna in accordance with the Declaration of Helsinki. Informed consent was also obtained from all participants prior to the start of this study.

Statistical analysis

The primary endpoints of the statistical analyses were distant recurrence-free survival (DRFS) and overall survival (OS). DRFS was defined as the interval between date of surgery and first evidence of relapse at any distant site. Because of a median age of 65 at trial initiation and the long-term follow up, patients were censored if they had, in the absence of breast cancer recurrence, died from of confirmed reasons unrelated to their malignancy. Baseline data, dichotomized according to the stromal uPA/PAI-1 co-expression status, were compared in univariate analyses using the Chi-square and in a multiple logistic model. Survival rates were estimated using the Kaplan-Meier method. The prognostic value of stromal uPA/PAI-1 co-expression was studied using univariate and multiple Cox models. All p values are shown as the results of two-sided tests. $P \leq 0.05$ was considered statistically significant. All statistical analyses were done using SPSS software version 15.0 (SPSS, Inc.)

Results

Of the 2.021 women who had entered the ABCSG 6 trial, evaluable HR + FFPE tumor samples and clinical data were available in a subset of 606 patients. This subset was representative of the overall study population (Supplementary Table 1). Intratumoral stromal uPA expression was assessable in 538 of 606 cases (88.8%),

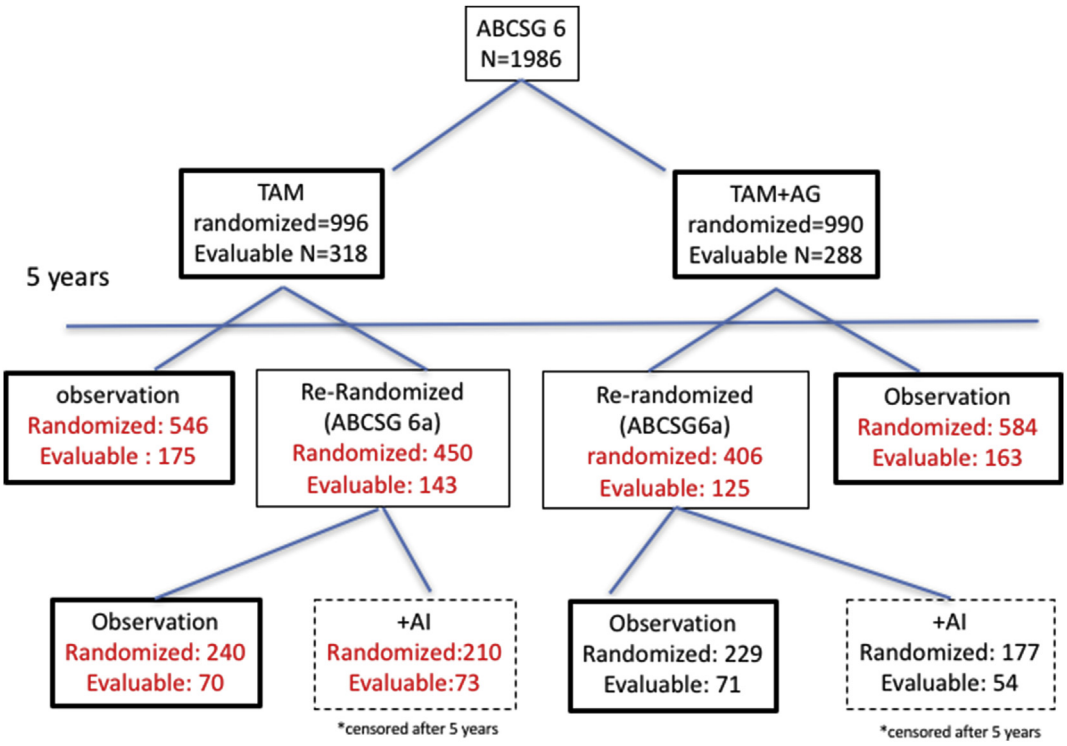

Fig. 1. REMARK diagram describing the study cohort.

and PAI-1 expression in 505 of 606 (83.3%) cases. Expression of both parameters in a given tumor was measurable in 437 cases (72.1%). Overall, stromal expression of uPA was detected in 292 of 538 tumors (54.3%; Fig. 2A), and stromal PAI1 expression was detected in 269 out of 505 samples (53.3%; Fig. 2C). Heterogeneous staining was frequently observed, with a propensity of positive staining for both antibodies in centrally located fibrotic tumor areas as well as in tumor areas rich in fibroblasts. Stromal uPA and PAI-1 co-expression was, however, only observed in 163 of 437 (37.3%) of cases.

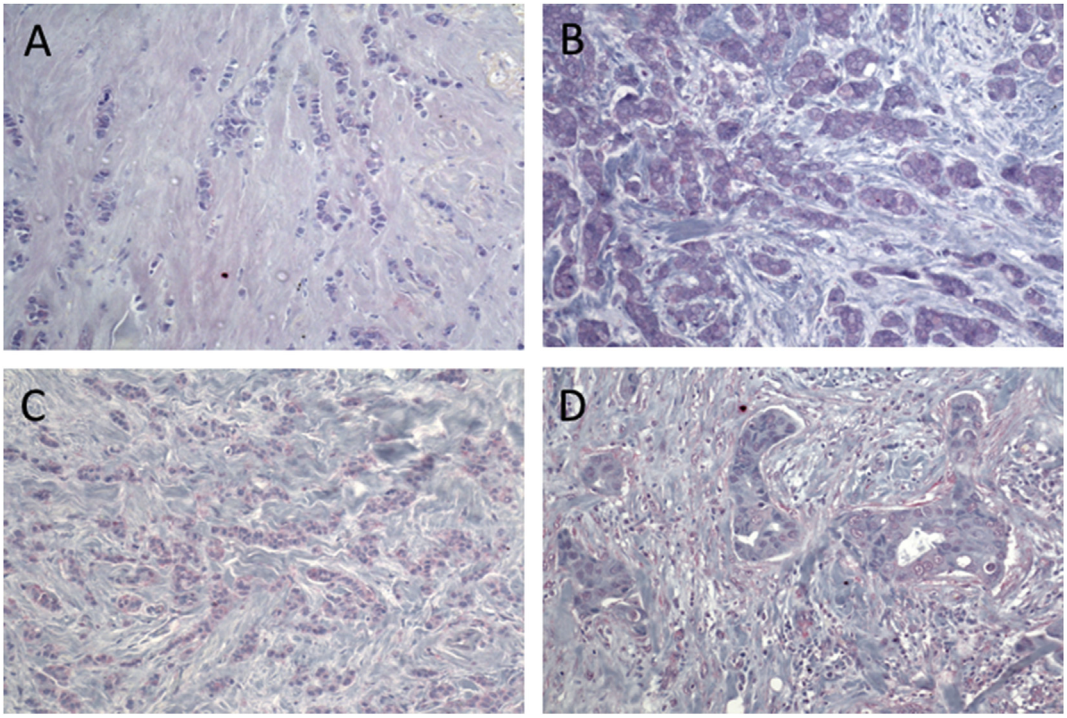

Fig. 2. Immunohistochemical detection of uPA and PAI-1. Representative invasive breast cancer samples with A) and without B) stromal uPA, and with C) and without D) stromal PAI-1 protein expression.

The clinical and tumor characteristics according to the stromal uPA/PAI-1 co-expression status are shown in Table 1: while co-expression of uPA and PAI-1 was not associated with age, tumor size, grading, or receptor status, we did observe a trend towards a significant correlation with nodal status ($p = 0.054$, Chi Square test).

At a median follow-up of 10 years, 82 of 437 (18.8%) patients, in whom both uPA and PAI1 immunohistochemistry was evaluable, had experienced distant relapses (45 patients with intratumoral uPA and PAI-1 co-expression vs 37 patients with uPA or PAI-1 expression only, or none of the two parameters). 148 of 437 (33.9%) patients had died (67 with intratumoral co-expression vs 81 patients without). Stromal expression of uPA (hazard ratio (HR) for relapse, 1.86; 95% C.I. 1.20–2.88, $p = 0.005$), and stromal co-expression of uPA and PAI1 (HR for relapse 2.21; 95% C.I. 1.43–3.41, $p < 0.001$), were both significantly associated with a worse distant recurrence-free survival in univariate analyses, and there was a trend for stromal PAI1 expression for a decreased DRFS (HR for relapse 1.49; 95% C.I. 0.99–2.26, $p = 0.057$). Stromal expression of uPA (HR 1.44; 95% C.I. 1.05–1.96, $p = 0.023$) and stromal co-expression of uPA and PAI1 (HR 1.47; 95% C.I. 1.06–2.03, $p = 0.020$) were both also significantly associated with OS in univariate analysis, while no such correlation was seen for stromal PAI1 staining ($p = 0.545$; Table 2).

The Kaplan Meier curves for breast cancer patients with and without intratumoral uPA expression describe a significantly different DRFS (log rank $p = 0.005$) and are shown in Fig. 3A and D. The corresponding curves for PAI1 (log rank $p = 0.055$) and for the co-expression of uPA and PAI1 (log rank $p < 0.001$) are shown in Fig. 3B and C, respectively, and in Figure D. A similar, albeit less pronounced difference in the Kaplan Meier curves was seen for overall survival in women with or without stromal PAI1 expression (log rank $p = 0.022$; Fig. 4A), and with or without stromal uPA and PAI1 co-expression (log rank $p = 0.020$; Fig. 4C), whereas no significant difference was observed when only PAI1 expression was evaluated (log rank $p = 0.545$; Fig. 4B).

The independent effect of stromal uPA and PAI-1 co-expression on DRFS and OS was assessed by multivariate Cox proportional hazard models adjusted for age, tumor size, nodal status, tumor grade, as well as ER and PR expression. In multivariate analyses, the expression of both proteins in the tumor stroma remained significantly associated with prolonged DRFS (adjusted HR for distant relapse $p = 1.870$; 95% CI 1.184–2.955; $p = 0.007$ Cox regression analysis) showed a trend towards an improved OS (adjusted HR for death $p = 1.291$; 95% CI 0.928–1.795; $p = 0.129$) when compared to women whose tumors did not co-express uPA and PAI-1. While stromal uPA expression alone remained prognostic for DRFS (HR 1.64; 95% C.I. 1.04–2.57, $p = 0.032$) and showed a trend for OS (HR 1.36; 95% C.I. 0.99–1.86, $p = 0.058$) in multivariate analysis, stromal PAI1 staining was not associated with either DRFS ($p = 0.264$) or OS ($p = 0.930$) in this model (Table 2).

Table 1
Patient characteristics.

Characteristics	No stromal uPA/ PAI1 co-expression $N = 274$ (%)	Stromal uPA/PAI1 co-expression $N = 163$ (%)	*P*
Age			
<51	13 (4.7%)	5 (3.1%)	
51–60	82 (29.9%)	46 (28.2%)	
61–70	94 (34.3%)	53 (32.5%)	
>70	85 (31.0%)	59 (36.2%)	0.631
Tumor size			
T1	145 (52.9%)	78 (47.9%)	
T2	120 (43.8%)	77 (47.2%)	
T3	9 (3.3%)	8 (4.9%)	0.478
Nodal status			
N0	169 (61.7%)	85 (52.1%)	
1 - 3 positive nodes	79 (28.8%)	51 (31.3%)	
4 - 10 positive nodes	22 (8.0%)	19 (11.7%)	
>10 positive nodes	4 (1.5%)	8 (4.9%)	0.054
Tumor grade			
G1	43 (15.7%)	31 (19.0%)	
G2	166 (60.6%)	87 (53.4%)	
G3	43 (15.7%)	29 (17.8%)	
GX	22 (8.0%)	16 (9.8%)	0.525
Estrogen Receptor			
0	2 (0.7%)	2 (1.2%)	
+	65 (24.0%)	34 (20.9%)	
++	96 (35.4%)	60 (36.8%)	
+++	108 (39.9%)	67 (41.1%)	0.851
Progesterone Receptor			
0	75 (27.9%)	46 (28.4%)	
+	63 (23.4%)	35 (21.6%)	
++	55 (20.4%)	43 (26.5%)	
+++	76 (28.3%)	38 (23.5%)	0.442
Adjuvant therapy			
Tamoxifen	146 (53.3%)	87 (53.4%)	
Tamoxifen + aminoglutethimide	128 (46.7%)	76 (46.6%)	0.986

Conclusions

Despite being uniformly endorsed for risk assessment in early breast cancer by both national and international guidelines, the requirement for fresh tissue and the need for a sufficiently large tumor volume has greatly limited the implementation of uPA/PAI ELISA-based assays in routine clinical practice [13–15]. Therefore,

Table 2
Cox proportional hazard models for DRFS and OS.

Variable	HR for distant recurrence	95% CI	*P*	HR for death	95% CI	*P*
Univariate						
Stromal uPA	1.86	1.20–2.88	0.005	1.44	1.05–1.96	0.023
Stromal PAI1	1.49	0.99–2.26	0.057	1.10	0.80–1.52	0.545
Stromal uPA/PAI1	2.21	1.43–3.41	<0.001	1.47	1.06–2.03	0.020
Multivariate						
Age	0.84	0.64–1.08	0.172	1.61	1.32–1.96	<0.0001
Tumor size	1.71	1.18–2.50	0.005	1.36	1.03.-1.81	0.031
Nodal status	2.21	1.70–2.88	<0.0001	1.61	1.31–1.97	<0.0001
Tumor grade	0.98	0.73–1.30	0.863	1.05	0.86–1.29	0.618
ER	0.75	0.56–1.02	0.067	0.80	0.64–0.99	0.038
PR	0.81	0.66–0.99	0.037	1.02	0.88–1.18	0.803
Adjuvant therapy	0.85	0.54–1.34	0.480	1.08	0.78–1.51	0.630
Stromal uPA	1.64	1.04–2.57	0.032	1.36	0.99–1.86	0.058
Stromal PAI1	1.28	0.83–1.98	0.264	0.99	0.71–1.36	0.930
Stromal uPA/PAI1	1.87	1.18–2.96	0.007	1.29	0.93–0.80	0.129

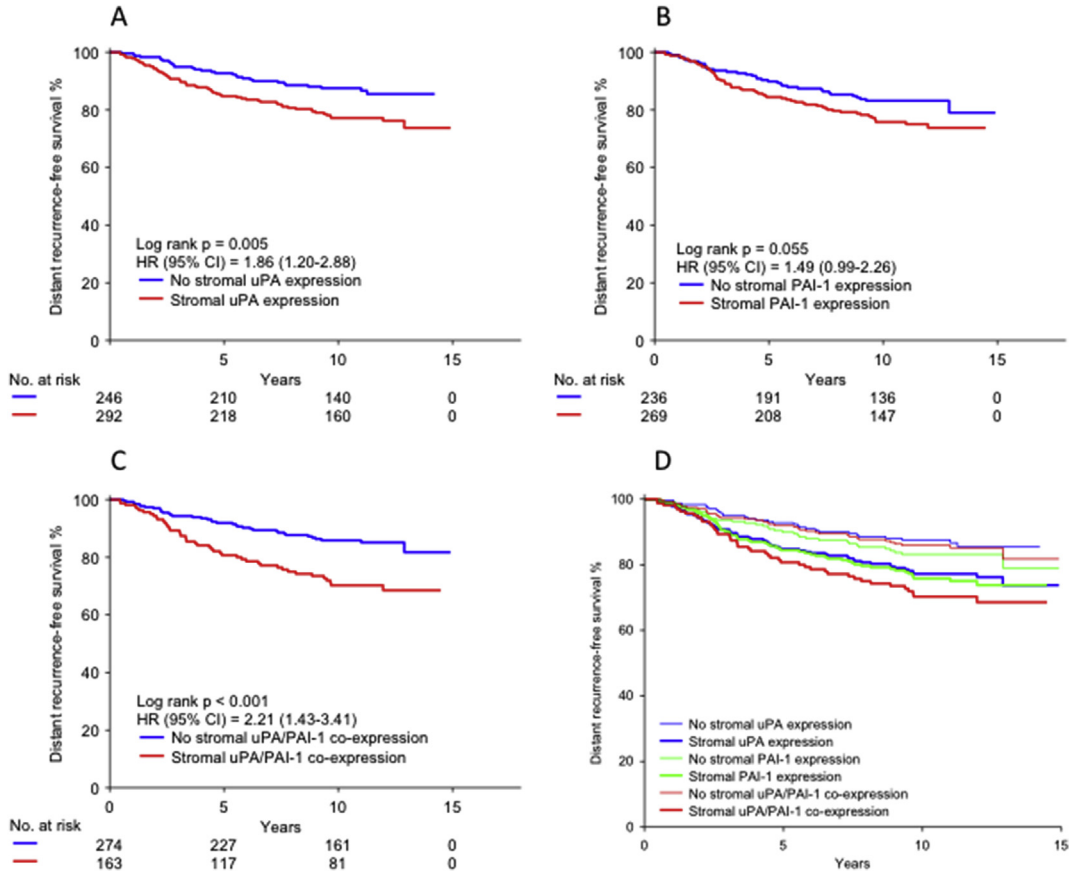

Fig. 3. DRFS in postmenopausal breast cancer patients with endocrine-responsive tumors according to stromal uPA protein expression (A), stromal PAI1 protein expression (B), and stromal co-expression of uPA and PAI1 (C). Stromal uPA and PAI1 expression, and uPA/PAI1 co-expression (D).

IHC analysis of uPA and PAI-1 protein expression may be a better and less expensive way of investigation, in order to include uPA/PAI-1 as routine prognostic factor at the diagnosis of HR + BC. Over the years, several groups have thus investigated alternative methods to measure intratumoral uPA and PAI-1. Among them, quantitative RT-PCR-based mRNA expression analysis has been evaluated most extensively. In breast cancer cell lines mRNA levels have been shown to be highly correlated with their respective uPA and PAI-1 concentrations (each: $r = 0.95$; $p < 0.001$) as measured by ELISA. However, in surgical breast cancer samples the correlation between uPA/PAI-1 mRNA and protein levels were considerably weaker, or even not significant at all [16].

IHC is another reliable method that can be performed in fixed and archived samples, and which only requires small amounts of tumor tissue. A recent comparison between uPA/PAI-1, when analyzed using an optimized immunohistochemistry protocol, and the corresponding uPA/PAI-1 ELISA concentrations resulted in an overall concordance of 78%, and in a concordance of 94% in the high-risk patient group [17]. In another cross-platform concordance study uPA and PAI-1 immunohistochemistry was performed together with ELISA-based protein measurements in laser capture microdissected tumor tissue, to investigate the spatial expression of the two proteins. The authors found that uPA and PAI-1 in tumor stroma, in tumor cells, and in un-separated tumor tissue did not exhibit any significant differences in protein-levels determined by ELISA. Cox regression analysis showed that patients with high uPA and/or high PAI-1-levels, as compared to patients with low levels of either factor, experienced a significantly shorter relapse-free survival and overall survival. These results suggest that a strong expression of uPA and PAI-1 in the tumor stroma, as well as in tumor cells, have the same impact on the clinical behavior of breast cancer [18]. Nevertheless, there are also reports that do not support a prognostic role for uPA or PAI-1 when measured by immunohistochemistry [19–21]. The reason for the apparent lack of correlation in these studies could lie in the fact that epithelial rather than stromal protein expression was evaluated, but could also be explained by poor antibody specificity, choice of a wrong cut-off, or simply by too small sample sizes.

While most studies investigating the prognostic role of uPA and PAI1 have therefore used ELISA-based assays, the value of immunohistochemical analysis of uPA and PAI-1 in predicting DRFS and OS has never been evaluated in a prospective trial population.

We here report that, by using immunohistochemistry from archived tumor tissues, stromal uPA protein expression was detected in 54.3% and PAI-1 expression in 53.3% of cases. While uPA and PAI-1 were detectable in malignant epithelium and surrounding stroma alike, neither epithelial uPA nor PAI-1 expression was found to be predictive of outcome (data not shown). Our results are in line with growing evidence suggesting that stromal rather than epithelial uPA and PAI-1 protein expression is associated with malignant behavior and consecutively poor outcome in breast cancer: Dublin et al. demonstrated that stromal, but not epithelial uPA and PAI-1 expression, correlated with local invasion and tumor size. Furthermore, only fibroblastic uPA expression was associated with relapse-free survival in this study [22].

Our findings further support an increasing number of publications that highlight the potential of stromal biomarkers in predicting outcome in breast cancer patients: Characterization of the

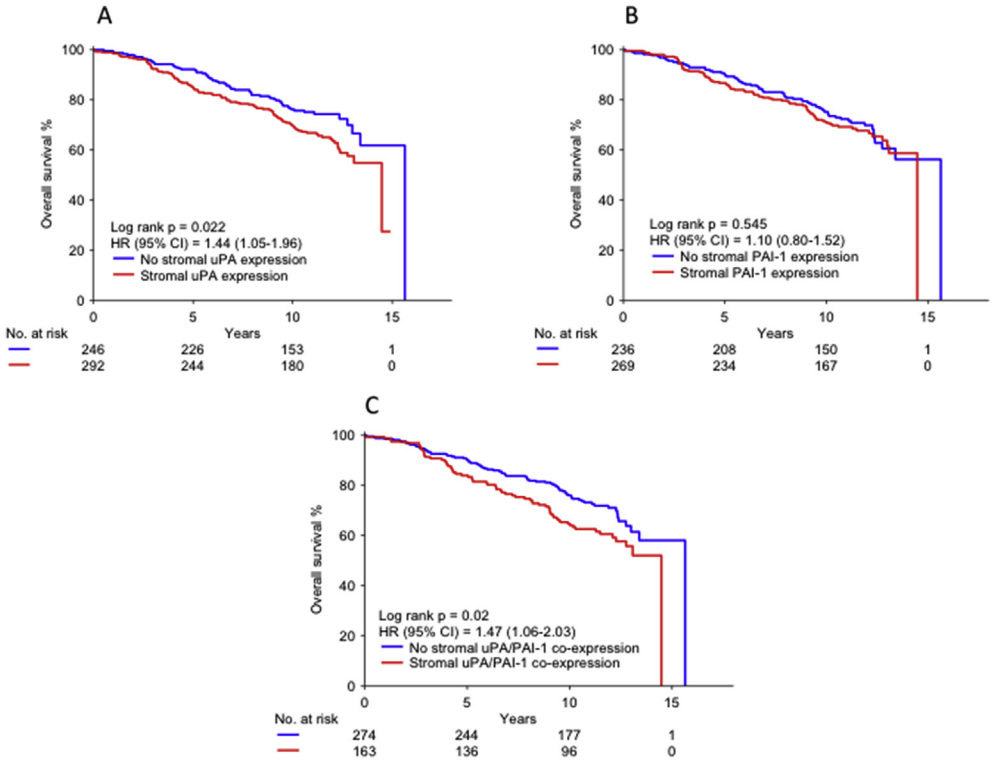

Fig. 4. OS in postmenopausal breast cancer patients with endocrine-responsive tumors according to stromal uPA protein expression (A), stromal PAI1 protein expression (B), and stromal co-expression of uPA and PAI1 (C).

intratumoral lymphocytic infiltrate [23–25], as well as stromal vascularization [26,27] have been shown to be of prognostic/predictive value in breast cancer. Also, the CoIXallpha1 protein expression in stromal fibroblasts has recently been reported to be predictive of response to neoadjuvant chemotherapy in ER positive/HER2 positive breast cancer [28], and immunohistochemical expression of S100A9 in myeloid cells of the tumor stroma correlated with decreased overall survival in ER-/PR-breast cancers [25].

Due to the retrospective nature of our analysis, our study has several limitations: ABCSG-06 patients were recruited between 1990 and 1995, at a time when uPA/PAI-1 ELISA assays were not yet available, and thus no direct comparison between ELISA and IHC results is possible. However, the DRFS rates that were observed in our high and low risk groups – when stratified by immunohistochemical uPA and PAI-1 expression - are strikingly similar to the 10-year recurrence rates observed in the respective ELISA-based high and low risk groups in the "Chemo-N0 trial". Furthermore, the long follow-up of our study and the ensuing long FFPE storage periods as well as variations in tissue fixation conditions between the contributing centers might well explain why several samples could not be analyzed due to technical reasons in our study. In order to obtain level 1b evidence, our results now need to be replicated and/or validated in other prospectively designed phase 3 trials such as ABCSG12, ABCSG8, ATAC, or BIG-1-98, which have also been performed in pre- and in postmenopausal, endocrine responsive breast cancer [29–31].

Also, a direct comparison between ELISA-based and immunohistological detection strategies in a prospectively designed trial (such as the phase 3 "Chemo-N0 study" – where the uPA and PAI-1 ELISA data as well as clinical outcome are already available) would be highly interesting [2]. A comparison of gene expression prognostic information vs uPA/PAI-1 ELISA and IHC based assays, as well as cost benefit analyses should also be considered in future studies in order to contribute to the decision-making.

The pivotal tumor-promoting role of uPA, and the recent development of low-molecular weight uPA inhibitors, have now also rendered uPA an attractive therapeutic target. Two synthetic inhibitors have already undergone clinical evaluation in phase I, and the orally available upamostat has been studied in a phase II trial, in which a combination of upamostat and capecitabine was compared to capecitabine monotherapy in 132 women with advanced breast cancer. The addition of upamostat to capecitabine resulted in a median PFS of 8.3 months (95% C.I. 5.6–9.6) as compared to 7.5 months (95% CI 4.2 to 12.8) in the monotherapy arm. Interestingly, in patients who had already received prior adjuvant chemotherapy, the addition of upamostat increased the PFS from 4.3 months (95% CI 2.6 to 9.7) to 8.3 months (95% CI 5.6 to 10.9) [32]. Unfortunately, uPA protein assessment was not part of the trial protocol. However, given the limited expression of uPA protein, it is fair to speculate that, had upamostat therapy been restricted to those patients whose tumors express uPA protein, response rates would have been higher.

Taken together, we have analyzed the intratumoral uPA and PAI-1 protein expression in endocrine-responsive early stage breast cancers from HR + postmenopausal women who had been enrolled into a prospectively-designed phase III study (ABCSG-6). We have demonstrated that stromal co-expression of uPA and PAI-1 is detectable in 37.3% of breast cancer samples and is associated with poor DRFS and OS. In addition to its prognostic role, immunohistochemical detection of uPA might also potentially become a clinically useful biomarker assay for the prediction of response to uPA-targeted therapies.

Funding

This work was supported by an unrestricted grant from Novartis.

Role of the funding source

The sponsor had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Ethics approval and consent to participate

Ethics approval was obtained from Institutional Review Board of Medical University of Vienna and consent was obtained from all participants.

Consent for publication

No identifiable data used - not applicable.

Availability of data and material

The datasets used and/or analyzed for the study are available from the corresponding author upon request.

Conflicts of interest

None.

Acknowledgements

We thank Claudia Olenik (American Diagnostica) for the provision of antibodies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2019.05.007.

References

[1] Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25(33):5287–312.
[2] Harbeck N, Schmitt M, Meisner C, et al. Ten-year analysis of the prospective multicentre Chemo-N0 trial validates American Society of Clinical Oncology (ASCO)-recommended biomarkers uPA and PAI-1 for therapy decision making in node-negative breast cancer patients. Eur J Cancer 2013;49(8):1825–35.
[3] Duffy MJ, McGowan PM, Harbeck N, Thomssen C, Schmitt M. uPA and PAI-1 as biomarkers in breast cancer: validated for clinical use in level-of-evidence-1 studies. Breast Cancer Res 2014;16(4):428.
[4] Zanotti L, Bottini A, Rossi C, Generali D, Cappelletti MR. Diagnostic tests based on gene expression profile in breast cancer: from background to clinical use. Tumour Biol 2014;35(9):8461–70.
[5] Nielsen BS, Sehested M, Duun S, et al. Urokinase plasminogen activator is localized in stromal cells in ductal breast cancer. Lab Invest 2001;81(11):1485–501.
[6] Offersen BV, Nielsen BS, Hoyer-Hansen G, et al. The myofibroblast is the predominant plasminogen activator inhibitor-1-expressing cell type in human breast carcinomas. Am J Pathol 2003;163(5):1887–99.
[7] Nielsen BS, Rank F, Illemann M, Lund LR, Dano K. Stromal cells associated with early invasive foci in human mammary ductal carcinoma in situ coexpress urokinase and urokinase receptor. Int J Cancer 2007;120(10):2086–95.
[8] Castello R, Landete JM, Espana F, et al. Expression of plasminogen activator inhibitors type 1 and type 3 and urokinase plasminogen activator protein and mRNA in breast cancer. Thromb Res 2007;120(5):753–62.
[9] Jakesz R, Greil R, Gnant M, et al. Extended adjuvant therapy with anastrozole among postmenopausal breast cancer patients: results from the randomized Austrian Breast and Colorectal Cancer Study Group Trial 6a. J Natl Cancer Inst 2007;99(24):1845–53.
[10] Kramer MD, Vettel U, Schmitt M, Reinartz J, Brunner G, Meissauer A. Monoclonal-antibodies against plasminogen activators and plasmin(ogen). Fibrinolysis 1992;6:103–11.
[11] Foekens JA, Schmitt M, Vanputten WLJ, et al. Plasminogen-activator inhibitor-1 and prognosis in primary breast-cancer. J Clin Oncol 1994;12(8):1648–58.
[12] Schmitt M, Graeff H, Kindermann Gn. Prospects in diagnosis and treatment of breast cancer : proceedings of the joint international symposium on prospects in diagnosis and treatment of breast cancer, 10-11 November 1993. Munich, Germany. Amsterdam ; New York: Excerpta Medica; 1994.
[13] Senkus E, Kyriakides S, Penault-Llorca F, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24(Suppl 6):vi7–23.
[14] Sturgeon CM, Duffy MJ, Stenman UH, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. Clin Chem 2008;54(12):e11–79.
[15] Schneeweis A, et al. Diagnosis and treatment of patients with primary and metastatic breast cancer Prognostic and predictive factors. 2010. retrieved from, https://www.ago-online.de/fileadmin/downloads/leitlinien/mamma/oktober2012/g_mamma_10_1_1_18_prognostic_predictive_factors.pdf.
[16] Biermann JC, Holzscheiter L, Kotzsch M, et al. Quantitative RT-PCR assays for the determination of urokinase-type plasminogen activator and plasminogen activator inhibitor type 1 mRNA in primary tumor tissue of breast cancer patients: comparison to antigen quantification by ELISA. Int J Mol Med 2008;21(2):251–9.
[17] Lang DS, Heilenkotter U, Schumm W, et al. Optimized immunohistochemistry in combination with image analysis: a reliable alternative to quantitative ELISA determination of uPA and PAI-1 for routine risk group discrimination in breast cancer. Breast 2013;22(5):736–43.
[18] Hildenbrand R, Schaaf A, Dorn-Beineke A, et al. Tumor stroma is the predominant uPA-, uPAR-, PAI-1-expressing tissue in human breast cancer: prognostic impact. Histol Histopathol 2009;24(7):869–77.
[19] Gohring UJ, Scharl A, Thelen U, Ahr A, Crombach G. Comparative prognostic value of cathepsin D and urokinase plasminogen activator, detected by immunohistochemistry, in primary breast carcinoma. Anticancer Res 1996;16(2):1011–8.
[20] Reilly D, Andreasen P, Duffy MJ. Urokinase-type plasminogen-activator and its inhibitor pai-1 in breast-cancer - studies at both protein and messenger-rna level. Biochem Soc Trans 1991;19(1). S44-S44.
[21] Costantini V, Sidoni A, Deveglia R, et al. Combined overexpression of urokinase, urokinase receptor, and plasminogen activator inhibitor-1 is associated with breast cancer progression - an immunohistochemical comparison of normal, benign, and malignant breast tissues. Cancer 1996;77(6):1079–88.
[22] Dublin E, Hanby A, Patel NK, Liebman R, Barnes D. Immunohistochemical expression of uPA, uPAR, and PAI-1 in breast carcinoma. Fibroblastic expression has strong associations with tumor pathology. Am J Pathol 2000;157(4):1219–27.
[23] Loi S, Sirtaine N, Piette F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol 2013;31(7):860–7.
[24] Adams S, Gray RJ, Demaria S, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol 2014;32(27):2959–66.
[25] Bergenfelz C, Gaber A, Allaoui R, et al. S100A9 expressed in ER(-)PgR(-) breast cancers induces inflammatory cytokines and is associated with an impaired overall survival. Br J Canc 2015;113(8):1234–43.
[26] Kraby MR, Kruger K, Opdahl S, Vatten LJ, Akslen LA, Bofin AM. Microvascular proliferation in luminal A and basal-like breast cancer subtypes. J Clin Pathol 2015;68(11):891–7.
[27] Li Y, Wei X, Zhang S, Zhang J. Prognosis of invasive breast cancer after adjuvant therapy evaluated with VEGF microvessel density and microvascular imaging. Tumour Biol 2015;36(11):8755–60.
[28] Brodsky AS, Xiong J, Yang D, et al. Identification of stromal ColXalpha1 and tumor-infiltrating lymphocytes as putative predictive markers of neoadjuvant therapy in estrogen receptor-positive/HER2-positive breast cancer. BMC Canc 2016;16(1):274.
[29] Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst 2009;101(21):1446–52.
[30] Early Breast Cancer Trialists' Collaborative G, Dowsett M, Forbes JF, et al. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 2015;386(10001):1341–52.
[31] Gnant M, Mlineritsch B, Schippinger W, et al. Endocrine therapy plus zoledronic acid in premenopausal breast cancer. N Engl J Med 2009;360(7):679–91.
[32] Goldstein LJ, Oliveira CT, Heinrich B, et al. A randomized double-blind phase II study of the combination of oral WX-671 plus capecitabine versus capecitabine manotherapy in first-line HER2-negative metastatic breast cancer (MBC). J Clin Oncol 2013;31(15).