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review

Clinical utility of serine proteases in breast cancer

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The serine protease uPA and its inhibitor PAI-1 are involved in the degradation of tumor stroma and basement membrane. The independent prognostic value of serine protease urokinase-type plasminogen activator uPA and its inhibitor PAI-1 in breast cancer has been almost uniformly confirmed in numerous individual studies as well as in a meta-analysis, including 18 data sets of more than 8,000 patients. According to these observations, the risk of relapse in node negative patients with low levels of uPA and PAI-1 is less then 10%; these patients could be spared from toxic adjuvant systemic therapy. Clinically relevant and even more important is the information that uPA and its inhibitor PAI-1 may also have a predictive value for response to either hormonal or cytotoxic therapy in early breast cancer. According to our data obtained from altogether 460 operable breast cancer patients, uPA and PAI-1 may have a predictive value for the response to hormone therapy, but not to chemotherapy. The high PAI-1 levels were associated with a higher risk of relapse in the patients without adjuvant systemic therapy (HR 2.14; C.I. 95%= 0.48-9.56; p=0.321) and in the patients treated with chemotherapy (RR 2.48; C.I. 95%= 1.35-4.57; p=0.003). However, in the patients treated with adjuvant hormone therapy, either alone or in combination with chemotherapy, the prognostic value of uPA and PAI-1 was diminished. Moreover, high levels of both uPA and PAI-1 were associated with a lower risk of relapse (HR 0.79; p=0.693 and HR 0.26 p= 0.204, respectively). On the basis of currently available evidence, serine protease uPA and its inhibitor PAI-1 are certainly the markers that improve a proper selection of candidates for adjuvant systemic therapy and may also be the markers that could facilitate treatment decision in each individual patient, which is of utmost importance.

Key words: breast neoplasms; urinary plsminogen activator; plsminogen activator inhibitor 1; prognosis

Introduction

Breast cancer is the most common malignancy in females all over the world; more than a million of women diagnosed with this disease each year. Tremendous improvements in the treatment of this disease have been made during the last decades and the survival rates improved from around 50% to more than 70% in operable disease. This improvement is mostly

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due to early detection of the disease and to the introduction of adjuvant systemic therapy.[1] According to the meta-analysis, in which data obtained from more than 50,000 patients participating in different studies were included, adjuvant systemic therapy was found to reduce the risk of death by approximately one third in all operable breast cancer patients, irrespective of their individual risk of death based on traditional prognosticators, such as lymph node involvement, tumor stage, tumor grade, etc.[2,3] The absolute benefit is the same in all patient sub-groups and only the relative benefit is much higher in the high risk subgroup of patients. According to the so-called classical clinico-pathological data, the majority of patients are categorized into average and high-risk groups and treated by some kind of adjuvant therapy, although it is well known that less than 50% of all patients and only 30% of patients with node- negative disease are to develop metastases without any systemic treatment. Therefore, there is an urgent need to identify new prognostic markers that will enable us to categorize patients according to their individual risk of relapse more precisely. On the other hand, there is a continuous search for new biological markers, which could not only prognosticate the faith of the disease, but would rather predict the response of each individual patient to the particular therapy. This approach ensures that both the individual patient and the society as a whole benefit, by minimizing the treatment-related side effects and maximizing cure rates. There are a lot of new, prospective prognostic and predictive factors under investigation and serine proteases are among them.[4]

Prognostic factors in breast cancer

A prognostic factor for breast cancer is defined as any measurement available at the time of diagnosis or surgery and associated with disease-free or overall survival in the absence of systemic adjuvant therapy. Based on the knowledge of prognostic factors, the risk of relapse for each individual patient treated solely by local therapy could be estimated. Nowadays, tumor size, lymph node status, histological type and grade, mitotic figure count, and hormone receptors are considered the standard prognostic factors according to which the adjuvant therapy is planned. The prognostic value of these, so-called standard prognosticators in early breast cancer, has been confirmed in multiple studies and their value as category I prognostic factors has been recognized at a 1999 Consensus Conference held under the auspices of the College of American Pathologists.[5] According to these factors, only about 10% of patients are considered to have minimal, less than 10% risk of relapse and are therefore not offered adjuvant systemic therapy.[6,7]

There are additional new prognostic factors under investigation, which may better separate patients according to their own risk of relapse and spare the patients with low risk of relapse from unnecessary toxic treatment. Undoubtedly serine protease, urokinase-type plasminogen activator uPA and its inhibitor PAI-1 are among them. The prognostic value of both uPA and PAI-1 has already been confirmed at the highest level I evidence, in the meta-analysis and in the prospective randomized trial.[8,9]

Prognostic value of uPA and PAI-1

The serine protease uPA and its inhibitor PAI-1 are involved in the degradation of the tumor stroma and basement membrane. A critical balance of uPA, its cell surface receptor uPA-R, and PAI-1 are the prerequisites for efficient focal proteolysis, adhesion, and migration, and hence, the subsequent tumor cell invasion and metastases.[10] In 1988, Duffy with co-workers was the first to report that high primary tumor enzymatic activity of uPA

is associated with poor survival of breast cancer patients.[11] Shortly, the same authors did not only confirm this finding in a larger study, but also demonstrated the independent prognostic impact of uPA on the disease-free survival and overall survival.[12] This information was confirmed and even strengthened by the finding of the German group that not only the enzymatic activity, but to an even greater extend also the tumor tissue antigen level of uPA is of prognostic relevance.[13] The prognostic impact of uPA has since been confirmed by several investigators[14-24]; and PAI-1 was also found to be an independent prognostic marker in breast cancer (Table 1).[14-16,18-25] Surprisingly, high levels of PAI-1 were found to be associated with a higher risk of recurrence and a shorter overall survival in breast cancer. The data from basic research may help to explain the finding that PAI-1 does not act as a true uPA inhibitor, but rather as a proteolitic factor. PAI-1 was found to be indispensable for optimal focal proteolysis, adhesion, and migration, and subsequent tumor cell.[10]

The independent prognostic value of serine protease uPA and its inhibitor PAI-1 in breast cancer has been confirmed in a meta-analysis, including 18 data sets of 8,377 patients with a median follow-up of 6.5 years.[8] In this meta-analysis, uPA, PAI-1 as well as the combination of both were found to be the strongest prognostic factors in breast cancer, next to lymph node involvement, irrespective of the type of surgery, year of surgery, publication status of data sets, as well as menopausal status and lymph node status.[26] The independent prognostic value of uPA and PAI-1 was also confirmed in a prospective randomized multicenter therapy trial in node-negative breast cancer ("Chemo N_0"), randomizing high-uPA/PAI-1 patients to adjuvant CMF or observation only, which was performed in 13 German academic centers and in our center in Slovenia between 1993-1999.[9] In this study, uPA and PAI-1 were prospectively determined in detergent extracts of primary tumor tissue using commercially available ELISA assays (American Diagnostica Inc., Greenwich, CT). The first scheduled interim analysis of this

Table 1. Prognostic impact of uPA and PAI-1 in early breast cancer – overview of selected references

Author (year)	Country	Factors analyzed	Patients (N0)		Follow up (median, months)	Reference
Duffy (1988)[11]	Ireland	uPA	52	(25)	17	Cancer 62:531
Jänicke (1993)[13]	Germany	uPA, PAI-1	247	(101)	30	BCRT 24:195
Grøhndahl-Hansen (1993)[15]	Denmark	uPA, PAI-1	119	(12)	102	Cancer Res 53: 2513
Foekens (1994)[16]	Netherlands	uPA, PAI-1	657	(273)	48	J Clin Oncol 12:1648
Fernö (1996)[17]	Sweden	uPA	688	(265)	42	Eur J Cancer 32:793
Kim (1998)[18]	Japan	uPA, PAI-1	130	(130)	53	Clin Cancer Res 4:177
Kute (1998)[19]	USA	uPA, PAI-1	168	(168)	58	BCRT 54:147
Knoop (1998)[20]	Denmark	uPA, PAI-1	429	(178)	61	Br J Cancer 77:932
Harbeck (1999) [21]	Germany	uPA, PAI-1	316	(147)	77	Breast Cancer Res Treat 54:147
Bouchet (1999)[22]	France	uPA, PAI-1	499	(233)	72	J Clin Oncol 17:3048
Foekens (2000)[23]	Netherlands	uPA, PAI-1	2780	(1405)	88	Cancer Res 60:636
Jänicke (2001)[9]	Germany	uPA, PAI-1	556	(556)	32	JNCI 93:913
Konecny (2001)[24]	USA / Germany	uPA, PAI-1	587	(283)	26	Clin Cancer Res 7:2448
Look (2002)[8]	Europe	uPA, PAI-1	8377	(4676)	79	JNCI 94:116

study confirmed the independent prognostic impact of both uPA/PAI-1 for the disease-free survival; within the frame of this study, the previously optimized cut-offs for uPA and PAI-1 used to distinguish between low and high uPA and PAI-1 were validated.[21] According to the data obtained in the frame of this prospective study, the low-risk group, identified by uPA/PAI, encompasses as much 50% of node-negative patients, which is much more than 10% of node-negative patients, categorized into the low-risk group according to the clinical-pathological criteria. The risk of relapse in the patients with low levels of uPA and PAI-1 was found to be only 6.7% at 3-years, and these patients could be spared from toxic adjuvant systemic therapy, especially chemotherapy.

The uniformly found prognostic value of uPA and PAI-1 in multiple individual studies as well as in a prospective randomized trial and meta-analysis is quite unique among prognostic markers. For most of the so-called established and very well recognized prognostic factors, such as tumor size, histological type and grade, hormone receptor status as well as mitotic index, the data are far from being so consistent. In addition, the standardization of the method of determining, interpreting, and reporting the uPA and PAI-1 levels was possible, which is a prerequisite for a clinically useful marker. The immunoenzymatic assays are standardized, international quality assurance of the kit is guaranteed, [27] and the optimal cut-off values have been validated.[21] In addition, the extracts, prepared from as little as 100 μg tumor tissue, corresponding to about 1 μg protein extract, suffice for testing. All these facts make a serine protease uPA and its inhibitor PAI-1 an ideal prognostic factor for routine clinical use.

Predictive factors in breast cancer

To optimize treatment approaches and to improve the results of treatment, new biological markers, which will help us not only to predict the course of disease, but will also be able to predict the response to specific therapy in each individual patient, the so-called predictive markers are urgently needed. A predictive factor is any measurement associated with response or lack of response to a particular therapy. If, in addition to prognostic value, a marker also has a predictive value for response to a particular therapy, its prognostic strength could be increased or diminished, which depends on the fact whether worse or better prognosis correlates with treatment efficacy.

After four decades of systemic treatment, we are still faced with only two established predictive factors in breast cancer: hormone receptor status for response to hormonal therapy and HER-2 status for response to HER-2 antibody trastuzumab. Thus, the patients whose tumors strongly express hormone receptors are likely to respond to tamoxifen or other hormonal manipulates, while the patients with receptor-negative disease do not benefit from hormonal therapy.[3] Similarly, HER-2 overexpression in primary tumors predict a better response to trastuzumab.[28] There are also some new, putative markers for the response to either hormonal therapy or different chemotherapeutic agents, such as HER-2, p53 and BCL-2, which are under investigation. However, the data on their predictive value are still insufficient and even contradictory[4], which enables us to use them as a guide in the selection of systemic therapy. According to our data,[29] as well as the data published by Munich and Rotterdam group,[30,31,32] serine protease uPA and its inhibitor PAI-1 may also have a predictive value for the response to either hormonal or cytotoxic chemotherapy in early breast cancer.

Predictive value of uPA and PAI-1

The latest observations, based on the data provided by the patients mostly treated by

some kind of adjuvant systemic treatment, showed a possible loss of the prognostic value of uPA and PAI-1[17,29-32], which indicates that the level of proteases in the primary tumor could also predict a response to systemic therapy. In addition, the data from meta-analysis show that the bad prognostic impact of high levels of either uPA or PAI-1 was most pronounced in the sub-groups of patients with node-negative disease and in the sub-groups of patients treated before nineties which are the sub-groups that did not receive adjuvant systemic treatment in such an extent as the sub-groups of patients with node-positive disease, and the sub-groups of patients treated in the last decade.

So far, findings on the predictive value of uPA and its inhibitors for response to hormonal therapy are limited and, to some extent, even contradictory. Preclinical data observations that estrogens as well as antiestrogens modulate the expression of uPA and tumor cell growth in vitro[33-35] suggested that the levels of serine proteases in the primary tumor could be predictive for the efficacy of hormonal manipulations in breast cancer. In a large group of 235 patients with metastatic breast cancer, high levels of both uPA and PAI-1 in primary tumor predicted a poor response to tamoxifen.[36] On the contrary, the observations made in the frame of the Munich group[30,31] and Swedish group[17] pointed out that high levels of uPA[17,30] and PAI-1[30] or the combination of uPA/PAI-1[31] in the primary tumor may predict a better response to the adjuvant tamoxifen treatment. However, in a large study conducted in another Swedish center, PAI-1 was not found to predict any benefit of adjuvant tamoxifen treatment.[37] Similarly, in the largest data set obtained from two different centers (Munich and Rotterdam), no significant interaction between the combination of uPA/PAI-1 and the efficacy of adjuvant tamoxifen was found in altogether 3,424 patients.[32] Our data, obtained from 460 early breast cancer patients, speak in favor of the predictive value of high uPA and PAI-1 for a good response to hormonal therapy in adjuvant setting.[29]

The data on the possible predictive value of serine proteases for the response to chemotherapy are even more scarce and maybe less contradictory. In the first published study no significant influence of PAI-1 levels on the response to the neoadjuvant anthracycline based chemotherapy in locally advanced breast cancer was found.[38] Recently, the results from Munich pointed out that high levels of PAI-1 alone[30] or the combination of uPA/ PAI-1[31] may predict for a better response to adjuvant chemotherapy. This observation was confirmed in a large data set obtained from two different centers (Munich and Rotterdam); in a collective of 3424 patients the benefit from the adjuvant chemotherapy was found to be significantly higher in the subgroup of patients with high levels of uPA/PAI-1.[32]

According to our data, obtained from 460 operable breast cancer patients, uPA and PAI-1 may have a predictive value for the response to hormone therapy, but not to chemotherapy.[29] In our study, the high uPA levels were found to be associated with a higher risk of relapse in the patients without any adjuvant systemic therapy and in the patients treated with adjuvant chemotherapy (HR 1.37 and HR 1.44, respectively; non-significant). The high PAI-1 levels were also associated with a higher risk of relapse in the patients without adjuvant systemic therapy (HR 2.14; C.I. 95%= 0.48-9.56; p=0.321) and in the patients treated with chemotherapy (RR 2.48; C.I. 95%= 1.35-4.57; p=0.003) (Table 2). However, in the patients treated with adjuvant hormone therapy, either alone or in combination with chemotherapy, the prognostic value of uPA and PAI-1 was diminished, even more, high levels of both uPA and/or PAI-1 were associated with a lower risk of relapse (HR 0.79; p=0.693 and HR 0.26 p= 0.204, respectively) (Table 2). The 3-

Table 2. Risk of relapse according to uPA and PAI-1 levels in sub-groups of patients with different adjuvant systemic therapies (adopted from reference 29).

Prognostic factor (in ng/mg protein)	Without ST (n=52)	HT (n=141)	HT or ChT&HT (n=252)	ChT (n=156)	ChT or ChT&HT (n=267)
uPA	p=0.71	p=0.693	p=0.914	p=0.381	p=0.194
(<3vs ≥3)	HR=1.37	HR=0.79	HR=1.04	HR=1.44	HR=1.50
	95%CI (0.27-7.1)	95%CI (0.24-2.56)	95%CI (0.50-2.18)	95%CI (0.64-3.25)	95%CI (0.81-2.77)
PAI-1	p=0.321	p=0.204	p=0.8294	p=0.003	p=0.002
(<14 vs ≥14)	HR=2.14	HR=0.26	HR=0.64	HR=2.48	HR=2.22
	95%CI (0.48-9.56)	95%CI (0.03-2.06)	95%CI (0.25-1.68)	95%CI (1.35-4.57)	95%CI (1.35-3.66)

ST=adjuvant systemic therapy; HT= adjuvant hormone therapy; ChT=adjuvant chemotherapy

year DFS rates were not found to be influenced by uPA and/or PAI-1 in the patients treated with hormonal therapy, whereas in the patients treated with adjuvant chemotherapy and in the small group of patients without any adjuvant therapy, the bad prognostic impact of high uPA or/and PAI-1 levels is obvious (Figure 1).

Unfortunately, the data on the predictive value of serine protease uPA and its inhibitor PAI-1 are not as univocal as are the data on the prognostic value of these two markers. According to their mechanism of action, it could be expected that uPA and PAI-1 does not play a major role in the prediction of the response to either hormonal therapy or chemotherapy in large tumors presented in the metastatic disease. However, serine proteases may have an impact on the growth and spread of micrometastatic disease under adjuvant systemic therapy. The above data, although obtained in the frame of the retrospective observations and contradictory in some way, are informative enough to strengthen our believes that serine protease uPA and its inhibitor PAI-1 may have predictive value for the response to either hormonal therapy or chemotherapy in early breast cancer. To make any firm conclusions on the predictive value of proteases, the data from a larger data sets need to be obtained and analyzed by means of different statistic tools, like interaction terms used, and the assessment of the predictive value of these markers in the frame of prospective clinical trial should be made. The ideal way to evaluate the predictive value of any marker is to set a prospective randomized study with no treatment arm. However, due to ethical reasons, such a study is not feasible in adjuvant breast cancer treatment any more.

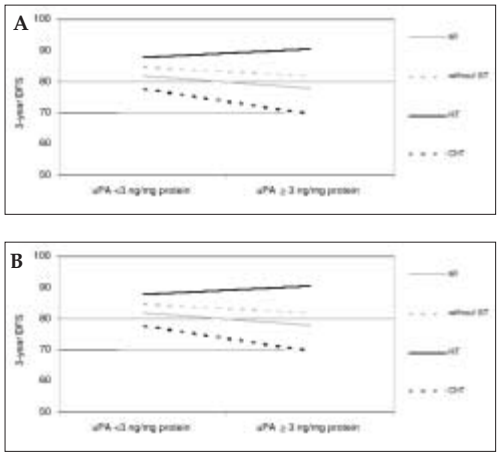

Figure 1. A: 3-year DFS of the patients with low and high values of uPA in the sub-groups of patients treated with different adjuvant systemic therapies (adopted from reference 29). B: 3-year DFS of the patients with low and high values of PAI-1 in the sub-groups of patients treated with different adjuvant systemic therapies (adopted from reference 29).

Conclusion

A part of breast cancer research is still focused on finding prognostic markers which

would help us to identify better the patients who could be spared adjuvant systemic therapy, but there is also an even more urgent need to identify new predictive markers that would help us to understand better which treatment option may be of benefit to each individual patient. On the basis of currently available data, serine protease uPA and its inhibitor PAI-1 are certainly the markers that improve a proper selection of patients for adjuvant systemic therapy and could be used as a new prognostic tool that will reduce the risk of over-treatment with better identification of patients who need adjuvant systemic therapy. In addition, according to the encouraging preliminary data, serine protease uPA and its inhibitor PAI-1 might also be the markers that will improve the treatment decision in each individual patient in the near future.

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