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Cancer Diagnostics: Review

Urokinase Plasminogen Activator and Its Inhibitor, PAI-1, as Prognostic Markers in Breast Cancer: From Pilot to Level 1 Evidence Studies

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Background: **For optimum management of patients with cancer, accurate assessment of prognosis is essential. The primary determinant of outcome in malignancy is the formation of distant metastases. Urokinase plasminogen activator (uPA) is a serine protease causally involved in invasion and metastasis.**

Content: **Data from model systems show that uPA is unequivocally involved in cancer dissemination. Consistent with its role in metastasis, multiple independent groups have shown that high uPA concentrations in primary breast cancers correlate with poor prognosis. For determining outcome, the prognostic impact of uPA was both independent of traditionally used factors and prognostic in patients with axillary node-negative disease. Paradoxically, high concentrations of plasminogen activator inhibitor (PAI-1), an endogenous inhibitor of uPA, also correlate with poor prognosis in patients with breast cancer, including the subgroup with node-negative disease. The prognostic value of uPA/PAI-1 in axillary node-negative breast cancer patients was recently confirmed in both a prospective randomized trial and a pooled analysis, i.e., two different level 1 evidence (LOE-1) studies.**

Conclusions: **uPA and PAI-1 are among the first biological prognostic factors to have their clinical value validated using LOE-1 evidence studies. Determination of these analytes may help identify low-risk node-negative breast cancer patients for whom adjuvant chemotherapy is unnecessary.**

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The main cause of morbidity and mortality in patients with cancer is the formation of distant metastases. The process of metastasis is a multistep event involving local invasion, degradation of the extracellular matrix (ECM),[1] angiogenesis, intravasation (entry of malignant cells into the circulation), evasion of apoptosis and survival in the circulation, extravasation (exiting from bloodstream), and growth at a secondary site [for a review, see Ref. *(1)*]. One of the key mediators of this process is the serine protease urokinase plasminogen activator (uPA).

uPA is a 53-kDa trypsin-like protease that converts the zymogen plasminogen into active plasmin *(2)*. Although uPA is a relatively specific protease, plasmin acts on a wide variety of protein substrates. These include most components in the ECM, such as laminin, fibronectin, and fibrin *(2)*. Plasmin can also mediate ECM degradation indirectly by activation of certain latent matrix metalloproteases (MMPs) such as MMP-3, MMP-9, MMP-12, and MMP-13 *(3)*. The formation of the active MMPs allows further proteolysis of the ECM. Degradation of the ECM is a prerequisite for cancer invasion and metastasis.

In vivo, uPA catalytic activity can be inactivated by several inhibitors, including PAI-1, PAI-2 *(4)*, and maspin *(5)*. Of these three, PAI-1 is thought to be the primary inhibitor of uPA. PAI-1 inhibits uPA by forming a stable complex with a 1:1 stoichiometry. In addition to binding to uPA, PAI-1 can also attach itself to the ECM protein, vitronectin. Binding to vitronectin allows PAI-1 to modulate cellular adhesion and migration *(6)*. The ability of PAI-1 to control adhesion and migration appears to be independent of its protease inhibitory capacity *(6)*.

Role of uPA in Cancer Invasion and Metastasis

Results from experiments carried out on model systems show that uPA is causally involved in cancer invasion and

[1] Nonstandard abbreviations: ECM, extracellular matrix; uPA, urokinase plasminogen activator; MMP, matrix metalloprotease; and LOE-1, level 1 evidence.

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metastasis [for a review, see Ref. *(7)*]. Briefly, this evidence is as follows:

- Correlations exist between uPA concentrations and the metastatic potential of different cell lines;
- Antibodies and inhibitors against uPA prevent or reduce metastasis;
- Prevention of uPA from binding to the receptor uPAR decreases the formation of metastases;
- Transfection of nonmetastatic cells with cDNA for uPA enhances metastasis in recipient cells; and
- Neoplastic lesions in mice deficient in either plasminogen or uPA exhibit slower growth and show less progression than similar lesions in wild-type animals.

It was originally believed that uPA promoted cancer dissemination simply by degrading the ECM, thus allowing invasion and metastasis. Although controlled degradation is indeed likely to clear a path for the migration of malignant cells, it is now clear that uPA has additional activities that permit it to play a role in cancer spread. These other roles include its ability to stimulate angiogenesis, mitogenesis, and cell migration and to modulate cell adhesion [for a review, see Ref. *(2)*]. Recently, uPA was also shown to prevent apoptosis *(8)*. Inhibition of apoptosis could increase the survival potential of malignant cells during the metastatic process, thus increasing the possibility for the establishment of a secondary deposit. This multifunctional capability may explain why uPA is such a potent mediator of cancer spread.

Role of PAI-1 in Cancer Invasion and Metastasis

Because PAI-1 is an inhibitor of uPA, it might be expected to prevent invasion and metastasis. Indeed, in some model systems, overexpression of PAI-1 reduced the formation of metastases *(9, 10)*. Other studies, however, have shown that PAI-1 promotes, rather than inhibits, invasion and metastasis. For example, coexpression of uPA and PAI-1 was found to be necessary for optimal invasion of lung carcinoma cells through an artificial membrane *(11)*. In other studies, PAI-1 deficiency in mice decreased angiogenesis and prevented cancer cell invasion *(12)*. Possible mechanisms by which PAI-1 contributes to cancer dissemination include preventing excess degradation of the ECM, modulating cell adhesion *(6)*, playing a role in angiogenesis *(12)*, and stimulating cell proliferation *(13)*.

Prognostic Value of uPA in Different Cancers

BREAST CANCER

Because uPA is directly involved in metastasis, it is an ideal candidate for investigation as a prognostic marker *(14)*. In a pilot study carried out in the late 1980s, Duffy et al. *(15)* were the first to show that breast cancer patients with high uPA activity in their primary tumors had a worse disease-free interval than patients in whom uPA activity was low. The results presented in this preliminary report have now been confirmed by at least 20 independent groups [for reviews, see Refs. *(16, 17)*]. As a marker for breast cancer, the prognostic information provided by uPA is independent of the traditional prognostic factors for this disease, such as tumor size, tumor grade, axillary node status, and steroid receptor status; is stronger than most of these factors; and most important, is prognostic in axillary node-negative patients *(16, 17)*.

OTHER CANCERS

In addition to being a prognostic factor in breast cancers, high uPA concentrations have also been shown to correlate with aggressive disease in patients with gastric, colorectal, esophageal, bladder, ovarian, and endometrial cancers [for reviews, see Refs. *(16, 17)*].

Prognostic Value of PAI-1 in Different Cancers

In 1991, Janicke et al. *(18)* first reported that high concentrations of PAI-1 predicted an adverse outcome in patients with breast cancer. As with uPA, these early results have been confirmed by multiple investigators [for reviews, see Refs. *(2, 17)*]. Similar to uPA in breast cancer, PAI-1 is also an independent prognostic factor and predicts outcome in node-negative patients *(2, 17)*. Other malignancies in which PAI-1 was shown to correlate with outcome include gastric, ovarian, and endometrial cancers as well as neuroblastomas *(2, 17)*.

Preparing uPA and PAI-1 for Routine Clinical Use

The above-mentioned studies clearly show that high concentrations of both uPA and PAI-1 are predictive of a poor prognosis in multiple cancers, especially breast cancer. The question therefore arises as to whether uPA and PAI-1 should now enter routine clinical use. Two important criteria should be met before a new marker is used for clinical purposes: (*a*) assay validation, including evaluation in external quality assurance schemes, and (*b*) clinical validation. Clinical validation should be carried out using a level 1 evidence (LOE-1) study, i.e., in either a large randomized prospective trial in which evaluation of the marker is the primary objective of the study or a metaanalysis/pooled analysis of small-scale prospective or retrospective trials *(19)*. Both these validation requirements have now been met for uPA and PAI.

ASSAY VALIDATION

Although activity assays, immunohistochemistry, and ELISAs have all been used to measure uPA and PAI-1, it is the last type of assay that has undergone the most detailed evaluation. In 1996, Benraad et al. *(20)* evaluated six different ELISA systems for uPA determination. The main conclusions to emerge from this study were:

- All the assays developed for measuring uPA in tissue extracts had a lower limit of detection of <32 ng/L uPA and thus had adequate sensitivity for detecting uPA in breast cancer extracts;

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- Within-assay precision for all the assays investigated was satisfactory;
- All assays displayed an acceptable degree of parallelism after dilution of tissue extracts; and
- Although the absolute concentration of uPA detected varied with the different methods, in general, good correlations were found between the different assays.

Assays for uPA and PAI-1 have also been studied in external quality assurance trials *(21)*. For example, in a multicenter study involving six laboratories in Germany, the between-laboratory CV for uPA varied between 6.2% and 8.2%. For PAI-1, the CVs varied between 13.2% and 16.6%. It should be stated that in this study, all participating laboratories used commercially available ELISAs from the same supplier.

CLINICAL VALIDATION

As mentioned above, clinical validation can be performed using either a prospective randomized trial or a meta-analysis/pooled analysis of small-scale retrospective and prospective studies. Both types of studies have recently been carried out for uPA and PAI-1 in breast cancer.

The prospective randomized investigation was a multicenter study of almost 600 patients carried out in Germany *(22)*. In this trial, node-negative breast cancer patients with low uPA and PAI-1 concentrations did not receive any systemic adjuvant chemotherapy. On the other hand, patients with high concentrations of the protease and/or its inhibitor were randomized to receive adjuvant cyclophosphamide–methotrexate–fluorouracil treatment or to be observed. Following an interim analysis after a medium follow-up of 32 months, patients with low concentrations of both proteins had an estimated 3-year recurrence rate of 6.7%, whereas those with high concentrations of the protease and/or its inhibitor had a recurrence rate of 14.7% ($P = 0.006$). Multivariate analysis showed that the prognostic impact of uPA/PAI-1 was independent of tumor grade, tumor size, and steroid receptor status.

The second type of LOE-1 study that validated the prognostic value of uPA and PAI-1 in breast cancer involved a pooled analysis of 18 different data sets containing >8377 patients *(23)*. After a median follow-up of 79 months, both uPA and PAI-1 were found to be independent prognostic factors. Although less powerful than axillary nodal status, both uPA and PAI-1 were stronger predictors of outcome than tumor size, tumor grade, hormone receptor status, or patient age. As well as being prognostic in the total population of patients, high concentrations of the two markers also predicted adverse outcomes in both the node-positive and node-negative subgroups. Most important, both uPA and PAI-1 predicted outcome in the node-negative subgroup that did not receive any adjuvant treatment.

To my knowledge, uPA and PAI-1 are the first biological factors to have their prognostic value validated using either a prospective randomized trial or a pooled analysis of published and unpublished data. The results of these two studies suggest that node-negative breast cancer patients with low uPA and PAI-1 concentrations have a low risk of disease relapse. For these low-risk patients, adjuvant chemotherapy may be avoided, thus increasing the quality of life and reducing healthcare costs.

Conclusion

uPA and PAI-1 are among the first tumor markers to have their clinical value confirmed in LOE-1 studies. These markers can therefore be now considered for the routine assessment of prognosis in patients with newly diagnosed breast cancer. For determining prognosis, uPA and PAI-1 are likely to be most useful in the axillary node-negative subgroup. Approximately 70% of node-negative women are cured of their disease by surgery and radiotherapy, whereas ~30% relapse within 10 years. Current prognostic factors are not sufficiently sensitive to identify the subgroup of patients who are likely to develop recurrent disease. uPA and PAI-1 may therefore may be the first biological markers to assist in the differentiation of aggressive and indolent node-negative breast cancers. In this scenario, women with node-negative disease and high concentrations of uPA and PAI-1 could be given adjuvant chemotherapy, whereas those with low concentrations of both proteins could be spared the side effects and costs of this treatment. Finally, uPA and PAI-1 may be of value in selecting appropriate therapies for patients with breast cancer. Preliminary results suggest that patients with increased concentrations of either uPA or PAI-1 fail to respond to hormone therapy in advanced disease *(24)*. On the other hand, those with high concentrations of both uPA and PAI-1 appear to benefit from adjuvant chemotherapy *(22, 25)*. Clearly, assays for uPA and PAI-1 have the potential to lead to individualized treatment strategies in patients with breast cancer *(23)*.

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