E12. uPA and PAI-1 in primary breast cancer: from bench to bedside

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uPA and PAI-1: from the bench to the bedside

Invasion factors urokinase-type plasminogen activator (uPA) and its inhibitor, PAI-1, are the first novel biological prognostic factors validated at the highest level of evidence regarding their clinical utility in breast cancer. Their method of determination is standardised and quality-controlled. Thus, these two factors and their evaluation process (Fig. 1) may serve as an example on how to establish new markers for routine clinical use. For any new prognostic or predictive marker, the following requirements need to be fulfilled before they can be recommended for routine patient management:

1. Biological tumour model
2. Standardised and quality-controlled method of determination
3. Independent validation of significant prognostic/predictive impact
4. Highest level of evidence: prospective (therapy) trial/ meta-analysis
5. Clinical relevance of the result

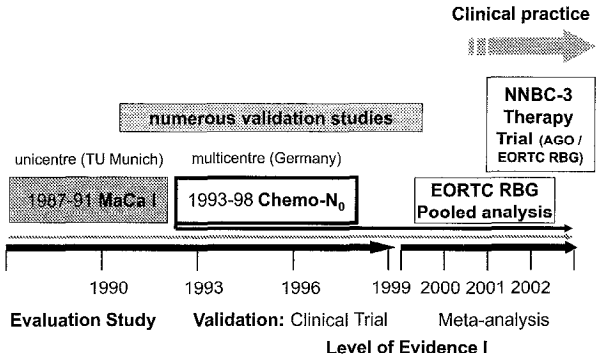

Fig. 1. Time line of the validation of the clinical utility of urokinase-type plasminogen activator (uPA) and its inhibitor, PAI-1, in primary breast cancer. EORTC, European Organisation for Research and Treatment of Cancer.

uPA and PAI-1 in primary breast cancer

The serine protease uPA and PAI-1 play a key role in tumour invasion and metastasis [1]. Antigen levels of both factors are determined using standardised enzyme-linked immunosorbent assays (ELISA) in primary tumour tissue extracts. The ELISAs for uPA and PAI-1 are robust enough for routine clinical use, and international quality assurance is guaranteed [2]. Extracts prepared from as little as 100 μg of tumour tissue corresponding to approximately 1 μg of protein extract are sufficient for testing. So far, no consistent clinically-relevant data have been generated using immunohistochemistry (IHC) or other techniques for the determination of uPA and PAI-1 protein expression in breast cancer tissue.

Since 1988, numerous studies have demonstrated that patients with low levels of uPA and PAI-1 have a significantly better survival than patients with high levels of either factor. A strong prognostic impact for uPA and PAI-1 on disease-free (DFS) and overall survival (OS) of primary breast cancer patients was shown in all published studies using biochemical assays [3]. The absence of any contradictory evidence on the prognostic impact of uPA and PAI-1 in breast cancer is quite unique for any biological tumour factor and is remarkable considering that published studies cover various demographic areas such as Europe, the United States of America (USA), and Japan. These unicentre data have been validated by an European Organisation for Research and Treatment of Cancer (EORTC) Receptor and Biomarker Group (RBG) pooled analysis comprising comprising 8377 patients with a median follow-up of 6.5 years [4], as well as by a multicentre, prospective, randomised therapy trial involving node-negative breast cancer patients ("Chemo N_0") [5]. The particular combination of both factors, uPA/PAI-1 (both low vs either or both factors high), outperforms the single factors, as well as other traditional prognostic factors, with regard to risk group assessment, particularly in node-negative breast cancer patients [6]. In risk groups as classified by uPA/PAI-1, HER2 status determined by fluorescent *in situ* hybridisation (FISH) or IHC, does not add significant prognostic information [7]. Node-negative breast cancer patients with low levels of uPA *and* PAI-1 have an excellent prognosis with an approximately 95% 5-year OS and may thus be candidates for being spared the burden of adjuvant chemotherapy. In contrast, node-negative patients with high uPA/PAI-1 levels are at substantially increased risk

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of relapse, comparable to that of patients with three or more involved axillary lymph nodes. First results from the "Chemo N_0" trial, as well as retrospective analyses [8], indicate that these high-risk patients benefit from adjuvant chemotherapy. Thus, combined determination of invasion factors uPA and PAI-1 in primary tumour extracts by ELISA supports risk-adapted individualised therapeutic strategies in patients with primary breast cancer, particularly those with node-negative breast cancer. In the Arbeitsgemeinschaft Gynäkologische Onkologie (AGO) evidence-based guidelines, the German AGO breast cancer expert panel recommends using uPA and PAI-1 for therapy decisions in node-negative breast cancer patients (www.ago-online.de).

High tumour tissue levels of uPA and/or PAI-1 correlate with tumour aggressiveness and poor patient outcome not only in breast cancer, but also in other malignancies such as ovarian, oesophageal, gastric, colorectal, lung or liver cancers [9]. In gastric cancer patients, preliminary clinical data suggests that the uPA-system plays an important role in early tumour cell dissemination [10]. So far, no Level I Evidence for the clinical utility of uPA and PAI-1 has been reported in other malignancies. Thus, immediate clinical consequences derived from the determination of uPA and PAI-1 are still limited to breast cancer.

Future directions

The uPA and PAI-1 ELISAs used in the "Chemo N_0" trial have been submitted to the Food and Drug Administration (FDA) for approval by the manufacturer American Diagnostica Inc. (Greenwich, CT, USA). A second multicentre trial (NNBC-3) has recently been started (principal investigator: Ch. Thomssen, Hamburg, Germany) in order to determine the optimal chemotherapy for high-risk node-negative patients with high uPA/PAI-1 levels. Moreover, the convincing experimental and clinical data indicating a key role for uPA and PAI-1 in tumour cell invasion and metastasis render these two factors promising targets for biological therapy against the tumour [9]. Novel therapeutic approaches involving the inhibition of interactions between uPA, PAI-1 and the uPA-R receptor, are currently being evaluated in preclinical models and early phase clinical trials.

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