ONKOLOGIE

Focus: Gynecological Oncology · Review Article

Onkologie 2003;26:438–445
DOI: 10.1159/000072976

Clinical Relevance of Prognostic Factors in Axillary Node-Negative Breast Cancer

C. Thomssen[a] F. Jänicke[a] N. Harbeck[b]

[a] Klinik und Poliklinik für Gynäkologie, Universitäts-Klinikum Eppendorf, Hamburg,
[b] Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, Klinikum rechts der Isar, Technische Universität München, Germany

Key Words
Breast cancer, prognostic factors · Evidence-based medicine · Urokinase · PAI-1

Summary
In node-negative breast cancer, advices for adjuvant therapy are based on traditional factors like age, tumour size, grade of differentiation, and steroid hormone receptor status. Several new factors that may better describe tumour behaviour, like proliferation rate (determined by thymidine labelling index, S-phase fraction, mitotic index, or Ki-67), presence of disseminated tumour cells, as well as expression of invasion factors (urokinase-type plasminogen activator uPA and its inhibitor PAI-1) and of cell cycle genes (cyclin E), as well as gene expression patterns ('genomic profiling') are currently discussed as future methods of risk assessment and also as tools for prediction of response to specific therapy modalities. Recommendations for routine use should be based on criteria of evidence-based medicine and on their impact on clinical decision making. Among the aforementioned factors, only the invasion factors uPA and PAI-1 have reached the highest levels of evidence and are mature enough to be transferred into clinical routine: their prognostic impact has been shown in several retrospective and prospective studies and in a pooled analysis of almost 3,500 node-negative patients. Their clinical impact was demonstrated in a prospective therapy trial. In addition, a predictive value with regard to chemotherapy efficacy has recently been supposed. Thus, in order to correctly assess the individual risk and to design an adequate adjuvant treatment plan for node-negative breast cancer patients, we recommend to use uPA and PAI-1 as additional criteria together with grading and age.

Schlüsselwörter
Mammakarzinom, prognostische Faktoren · Evidenz-basierte Medizin · Urokinase · PAI-1

Zusammenfassung
Beim nodal-negativen Mammakarzinom wird die Indikation zur adjuvanten Therapie immer noch anhand der traditionellen Prognosefaktoren Alter, Tumorgröße, Differenzierungsgrad und Hormonrezeptorstatus gestellt. Eine Reihe neuer Faktoren, die die Art des Tumors vielleicht besser beschreiben würden, wie Proliferationsrate (bestimmt als Thymidin-Labeling Index, S-Phase-Fraktion, Mitose-Index oder Ki-67), Nachweis disseminierter Tumorzellen, Expression der Invasionsfaktoren uPA (Plasminogenaktivator vom Urokinase-Typ) und seines Inhibitors PAI-1, Expression des Zellzyklusproteins Cyclin E und die Beschreibung von Genexpressionsmustern («genomic profiling»), werden als zukünftige Methoden der Risikoabschätzung und auch als prädiktive Faktoren für den Therapieerfolg diskutiert. Die Empfehlungen, neue Faktoren in der Routine einzusetzen, sollten durch ausreichende «Evidenz» abgesichert sein und den Einfluss auf die klinische Therapieentscheidungen berücksichtigen. Unter den zuvor genannten Faktoren haben nur die Invasionsfaktoren uPA und PAI-1 das höchste Evidenzniveau erreicht. Nur diese sind damit reif genug, um in die klinische Routine überführt zu werden. Ihre prognostische Bedeutung ist in mehreren unabhängigen retrospektiven und prospektiven Studien gezeigt worden und in einer Metaanalyse an fast 3500 Patientinnen bestätigt worden. Die Bedeutung für die Therapieentscheidung ist in einer prospektiven multizentrischen Studie gezeigt worden. Zusätzlich konnte kürzlich in einer weiteren Untersuchung die prädiktive Bedeutung für den Erfolg einer adjuvanten Chemotherapie abgeschätzt werden. Zusammenfassend würden wir daher empfehlen, uPA und PAI-1 zusammen mit Differenzierungsgrad und Alter als Entscheidungshilfe mit heranzuziehen, um das individuelle Risiko bestmöglich abzuschätzen und eine adäquate adjuvante Therapie festzulegen.

KARGER

Fax +49 761 4 52 07 14
E-mail Information@Karger.de
www.karger.com

Accessible online at:
www.karger.com/onk

Prof. Dr. Christoph Thomssen
Klinik und Poliklinik für Gynäkologie
Universitäts-Klinikum Eppendorf
Martinistrasse 52, D-20246 Hamburg
Tel. +49 40 42803-3510, Fax -4355
E-mail thomssen@uke.uni-hamburg.de

Introduction

Adjuvant therapy in breast cancer can reduce the mortality rate by 50% [1], but adjuvant therapy may also produce substantial toxicity. Thus, in order to individualize therapy strategies, prognostic and predictive factors are required. To date, more than 100 factors with a potential prognostic impact on survival or predictive impact on therapy response have been discussed. However, only for a few factors consistent evidence and clinical relevance have been demonstrated, such that clinical recommendations can be derived.

On the panel at the St. Gallen Early Breast Cancer Conference 2003 several factors were discussed: patient characteristics (age, menopausal status, race), tumour characteristics (size, nodal status, grade of differentiation) and biomarkers (ER/PgR, HER-2/neu, uPA/PAI-1, and others). Several questions were raised, e.g. about the significance of micrometastases, the value of tumour size in node-negative breast cancer and the impact of the expression levels of steroid hormone receptors. However, for none of the new factors a final conclusion was drawn. Validation according to principles of evidence-based medicine was not attempted (table 1) [2].

With regard to the new biological factor HER-2/neu, but also the steroid hormone receptor, the surprisingly high number of false positives and false negatives was noted and quality control of laboratory tests was demanded. In addition, HER-2/neu was considered rather as predictive factor for therapy response than as prognostic factor. The invasion markers uPA and PAI-1 were acknowledged as validated markers that might give useful information for some patients. It was stated that long term 10-year survival data from prospective trials are still missing and that the old data of the pooled analysis cannot be extrapolated for current guidelines [3].

Validation of New Prognostic Factors

New prognostic factors should be validated with regard to current criteria for development of prognostic factors, the criteria of evidence based medicine (tumour marker utility grading system, TMUGS; Oxford Levels of Evidence), and the clinical relevance [4–7]. Promising data exist for the following proposed prognostic factors: disseminated tumour cells ('micrometastases'), proliferation rate determined by thymidine labelling index ('TLI'), the invasion markers urokinase-type plasminogen activator ('uPA') and its inhibitor 'PAI-1', the cell cycle protein 'cyclin E' and the gene profile as determined by RNA-microarray methods ('genomic profiling').

For this overview, PUBMED was screened with regard to the above-mentioned markers. In addition, results recently published in abstracts were included in this overview.

Disseminated Tumour Cells

Disseminated cells detected in bone marrow or peripheral blood may represent the very cells from which future metastases could arise. However, the real fate of these cells remains unclear. The presence of the majority of disseminated cells may be a temporary phenomenon. Characterisation of the few cells that survive and their potential to grow out to manifest metastases is an important issue of current research. Data suggest that markers of aggressive cell behaviour can be detected on the disseminated cells (e.g. uPA-R, HER-2/neu, etc.) [8–12].

Representative and reproducible detection of disseminated tumour cells requires bone marrow aspirates from at least two anatomical sites usually obtained at the time of primary tumour excision. By cell enrichment and immunocytochemistry, single epithelial cells or cell clusters can be detected in the range of 1–25 epithelial cells per one million mononuclear cells. Specimen evaluation is greatly facilitated using automated detection systems. However, clinical data cannot be easily reproduced due to different enrichment methods, different antibodies for immunocytochemistry, or different detection methods used [12–13]. Methodological standardisation and quality control still have to be established.

For more than 15 years, researchers have reported prospective single-centre studies that were designed to evaluate the prognostic impact of disseminated tumour cells [8, 13–15]. The majority of these trials showed a significant prognostic impact. Disseminated tumour cells are detected in about 30% of all node-negative breast cancer patients. Presence or absence of disseminated tumour cells is associated with a substantial difference in risk of relapse (hazard ratio (HR): 6.1) [11]. The therapeutic impact was tested in a trial that randomly assigned patients with disseminated cells in their bone marrow to adjuvant bisphosphonate treatment or observation only [16]. A prospective trial on adjuvant chemotherapy has completed patient recruitment, but no results have been published so far [17]. Recently, a pooled analysis comprising more than 3,448 patients and demonstrating prognostic impact has been presented [18]. However, interpretation of the results of this analysis is hampered by the fact that the underlying methodology for detection of disseminated cells is not standardised.

In summary, detection of disseminated tumour cells has not fulfilled all requirements for being acknowledged as a valid prognostic marker (table 2). Single well-powered studies and a recent metaanalysis suggest clinical utility of this marker

Table 1. Risk assessment in node-negative breast cancer patients according to the consensus of St. Gallen 2003 [2]

Factor	Minimal risk	'Average risk'
Tumour size	≤ 2 cm	> 2 cm
Steroid hormone receptor status	positive	negative
Tumour grade	1	2 or 3
Age, years	≥ 35	< 35

Table 2. Requirements in development of prognostic factors (modified from [4, 5])

	BM	TLI	uPA/PAI	Cyclin E	Genome
Biological Model	+	+	+	+	(+)
Simple and robust method	(–)	(–)	+	(–)	(+)
Prospective evaluation in a pilot study	+	+	+	(+)	(+)
Validation in a second patient cohort	+	+	+	–	+
Prospective therapy trial	–	+	+	–	–
Metaanalysis	(+)	–	+	–	–
LoE	1b(–)	1b	1a	2b	2b
Clinical relevance	+/–	+	+	–	–

BM = Disseminated tumour cells in bone marrow aspirates, uPA = urokinase-type plasminogen activator, PAI-1 = plasminogen activator inhibitor 1, TLI = thymidine labelling index, Genome = genomic signature / profiling, LoE = Levels of evidence according to Oxford system.

(TMUGS I +/–) (table 3). But it does not reach the highest level of evidence (LoE) due to heterogeneous study results (Oxford 1b⁻ D). Nonetheless, detection of disseminated tumour cells is considered to be one of the most important candidates to be generally used as prognostic marker. Next to its prognostic value, detection of disseminated tumour cells may be even more important as a monitoring tool during systemic therapy or as a method for phenotyping residual tumour cells as potential targets for subsequent therapies [12, 19]. However, particularly lack of a standardised, quality controlled methodology and heterogeneous full-paper publications presently do not allow recommendation of this marker for routine use.

Thymidine Labelling Index

Proliferation rate can be determined by thymidine labelling index (TLI), S-phase fraction, mitotic index, or Ki-67 (MIB 1) immunohistochemistry. Thymidine labelling index describes the rate of proliferation of an individual tumour. Highly proliferating tumours may be more malignant and more aggressive than those with a low proliferation rate [20–23]. The proliferation rate is used as selection principle for cytotoxic chemotherapy [24].

Determination of proliferation rate by TLI requires fresh tissue that will be cultivated in the presence of radioisotope-labelled thymidine [25–26]. Quantification of thymidine uptake is performed by evaluating an autoradiograph. Although the results obtained by this method seem to be rather stable and are subject to an organized quality control [27], the method was not well accepted as it is time-consuming, labour-intense and involves handling of radioactive isotopes.

A well planned, large prospective study on 1,800 node-negative breast cancer patients demonstrated the prognostic impact of TLI [26]. Patients with highly proliferating tumours (TLI > 3%) had a significant worse prognosis than those with slowly proliferating tumours (TLI ≤ 3%; 8-year-relapse rate 43% vs. 30%, HR 1.9; $p < 0.001$). In this study, 38.2% of patients were assigned to the low risk group. In a number of follow-up studies, these results have been validated [28–30]. In two smaller studies the independent prognostic impact was not confirmed [31–32]. In at least three prospective trials the beneficial impact of chemotherapy in patients with highly proliferating tumours was studied. In two trials, by adjuvant chemotherapy a significant reduction of risk could be achieved in tumours with high TLI: 83% 5-year recurrence rate with CMF (cyclophosphamide, methotrexate, 5-fluorouracil) versus 72% without CMF [33]; 78.4% 5-year recurrence rate with FEC (5-fluorouracil, epirubicin, cyclophosphamide) versus 67.8% without FEC [34]. It was also demonstrated that adding doxorubicin to CMF is of significant benefit in tumours with high proliferation rates [35]. Such valid clinical data do not exist for other proliferation markers such as S-phase fraction or Ki-67. The prognostic impact of S-phase fraction was demonstrated in a large monocentre trial from the San Antonio group [36], yet lack of methodological standardisation and clinical consequences have restricted this factor to the research setting.

In conclusion, TLI may cause a change of the treatment. Patients with low TLI may be spared adjuvant chemotherapy. Patients with high TLI have an elevated risk of relapse despite their negative axillary lymph node status and should receive adjuvant anthracycline containing chemotherapy. TLI reaches a high level of evidence (Oxford 1b B). The published data are strong enough to use TLI in distinct clinical situations as a decision criterion (TMUGS I ++) (table 3). However, neither a prospective *multicentre* trial with regard to prognostic impact of TLI nor a meta-analysis or systematic review have yet been performed.

uPA/PAI-1

Proteolytic systems enable cancer cells to invade the extracellular matrix and to form metastases at distant sites. One of these proteolytic systems comprises the serine protease urokinase-type plasminogen activator (uPA), its receptor (uPAR, CD87), and its main inhibitor (PAI-1). In a variety of human cancers, including breast cancer, uPA, uPA-R, and PAI-1 are present at increased levels in malignant tissue [37–39]. High

Table 3. Scoring of prognostic factors according to TMUGS and Oxford Levels of Evidence (LoE) [5–7]

	TMUGS LoE	Grades of recommendation	Oxford LoE	Grade of recommendation
Micrometastasis (detection of disseminated tumour cells)	I (single high powered prospective study designed to test marker utility)	+/– (data are *suggestive* for utility)	1b(–) (individual cohort study; validation in a single population) [(-) means heterogeneous results]	D (inconsistent or inconclusive studies)
Thymidine labelling index (TLI)	I (single high powered prospective study designed to test marker utility)	++ (marker can be used in selected situations only)	1b (individual cohort study; validation in a single population)	B (extrapolations from level 1 studies)
Urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1	I (single high powered prospective study designed to test marker utility and meta-analysis)	++ (marker can be used in selected situations only)	1a (systematic review of cohort studies; validation in different populations)	A (consistent level 1 studies)
Cyclin E (by Western blot)	III (large retrospective studies)	+/– (data are *suggestive* for utility)	2b (retrospective cohort study)	C (extrapolations from level 2 or 3 studies)
Genomic signature (genomic profiling)	III (large retrospective studies)	+/– (data are *suggestive* for utility)	2b (retrospective cohort study)	C (extrapolations from level 2 or 3 studies)

antigen levels of uPA and of PAI-1 in primary breast tumours were associated with poor prognosis [40–41]. The initially surprising finding of the relationship between a protease inhibitor and unfavourable prognosis was subsequently explained by the role of PAI-1 in tumour cell adhesion, cell migration, and angiogenesis [39, 42–43].

Tumour tissue concentrations of invasion markers uPA and PAI-1 are measured using a commercially available ELISA similar to the previously used steroid hormone receptor determination. For preparing tumour extracts, snap-frozen material is pulverized and then suspended in buffer containing a non-ionic detergent. After centrifugation, the solution can be used for ELISA [41, 44]. High reproducibility with low coefficients of variance was demonstrated in the prospective evaluation of a quality assurance program [44]. The need for fresh tissue was often stated to be the major obstacle to use these factors more broadly. However, micromethods for quantification of tumour tissue concentrations of these factors are currently in development and validation. Thus, it can be expected that determination of these factors even in very small amounts of tissue e.g. in core needle biopsies or kryostat sections will provide valid results.

In several retrospective and prospective studies the prognostic value of uPA activity [40], uPA antigen content [41], and of PAI-1 antigen content [41] was described. In multivariate analyses, both markers had independent and strong impact on disease-free survival and overall survival, particularly in node-negative breast cancer patients [41]. Only weak correlations with known markers were found [45]. More detailed statistical analysis also showed variation of the prognostic significance with the time of observation [46–47].

For uPA, 3 ng/mg protein, and for PAI-1, 14 ng/mg protein, respectively, was determined as optimal cut-off value with the highest likelihood of a significant difference with regard to disease-free survival [48]. These cut-off levels were validated after longer follow-up periods [45] as well as by the confirmatory therapy trial 'Chemo-N0' [49]. According to this cut-off, 55% of node-negative breast cancer patients were assigned to the low-risk group (5-year risk of recurrence <5%), 45% were identified to be at high risk of recurrence (>30% 5-year risk of recurrence) [41].

In the prospective, multicentre confirmatory trial 'Chemo-N0' [49], 689 node-negative breast cancer patients were included. Patients at high risk of relapse as identified by elevated uPA and/or PAI-1 tumour levels were randomly assigned to either adjuvant chemotherapy (6 courses CMF) or observation only; all other patients (low uPA *and* PAI-1) were observed only. The prognostic impact of uPA and PAI-1 was confirmed. Patients with low uPA *and* PAI-1 tumour levels had only 6.7% recurrences after a short median follow-up of 32 months, compared to 14.7% for patients with high levels of uPA and/or PAI-1 (HR 2.83; $p = 0.008$). Tumour grading, the invasion markers uPA and PAI-1 and age were the only independent and strong prognostic factors. In addition, it was demonstrated that in the high-risk group, adjuvant CMF treatment resulted in a substantial reduction of risk of relapse by 42%.

Table 4. Multivariate stratified analysis for relapse-free survival in lymph node subgroups of patients. Data adapted from the pooled analysis [3]

Factor (df)	χ^2	HR	95%	CI p-value
All lymph node-negative patients (n = 3483, 970 events)				
Base model (11)	129.3	*1.00*		< 0.001
Combination of uPA and PAI-1 (2)	116.8			< 0.001
uPA		2.37	1.78–3.16	
PAI-1		1.90	1.45–2.49	
Lymph node-negative patients without adjuvant therapy (n = 2864, 829 events)				
Base model (11)	105.5	*1.00*		< 0.001
Combination uPA and PAI-1 (2)	94.0			< 0.001
uPA		2.34	1.71–3.21	
PAI-1		1.86	1.39–2.48	

The prognostic impact of uPA and PAI-1 was also evaluated by a pooled analysis performed by the European Organization for Research and Treatment of Cancer Receptor and Biomarker Group (EORTC RBG) and comprising 18 prospective and retrospective monocentre studies in different European countries with a total of 3,483 node-negative breast cancer patients and a median follow-up of 79 months [3]. A separate analysis was performed in patients who did not receive systemic adjuvant therapy (n = 2,864). Table 4 shows the results of adding uPA and PAI-1 to the base model for relapse-free survival. In the base model, the traditional factors age, tumour size, and grading were included. High uPA and/or PAI-1 tumour levels were statistically significant associated with poor relapse-free survival and overall survival when added to the base model (all $p < 0.001$). It is remarkable that the increase in significance ($\Delta\chi^2$) associated with the addition of uPA and PAI-1 had a magnitude similar to the baseline significance (χ^2) with the traditional prognostic factors.

In summary, for uPA and PAI-1 the standard requirements for development of new prognostic factors are fulfilled (table 2, 5) and clinical relevance has been shown at least for selected situations (TMUGS 1 ++). A high level of evidence (Oxford 1a A) can be given since an individual randomised controlled trial with narrow confidence intervals was performed and a clinical decision rule was validated (table 3). For being supported by consistent level 1 studies, recommendation level A might be adequate. Since grading and age interact independently with the risk of recurrence, uPA and PAI-1 should be used particularly for patients with an intermediate grade of differentiation.

Thus, the following clinical decision rules can be suggested:

– No adjuvant chemotherapy in patients with well-differentiated tumours (G1), or tumours of intermediate differentiation (G2) and low levels of both, uPA and PAI.
– Adjuvant chemotherapy is indicated for patients with high-grade tumours (G3), for patients with age below 35 years, and for patients with tumours of intermediate differentiation (G2) and high level of either or both, uPA and PAI-1.

Cyclin E

In normal cells, cyclin E accelerates the transition from G 1 to S phase [50–51]. The cyclin E gene is amplified and full-length cyclin E protein as well as low-molecular-weight isoforms often are expressed in breast-cancer cell lines [52–53]. These isoforms are hyperactive in inducing progression from G 1 to S phase [54]. Constitutive overexpression of cyclin E has been shown to cause chromosomal instability [55]. In about 10% of transgenic mice cyclin E has oncogenic potential [56]. This spectrum of biologic activity suggests that cyclin E may have multiple roles in the development and outcome of breast cancer.

Several evaluations of cyclin E as a prognostic factor in breast cancer with contradictory results have been published. Semiquantitative data obtained by Western blot analysis [50] showed a strong and independent prognostic impact for the total cyclin E tumour concentration and the level of the low molecular weight isoform of cyclin E. The Western blot technique may be adequate for research purposes, however, it was not designed and validated as a robust high-throughput technique for routine diagnostics.

In a recently published study [50], a retrospective random sample of 395 tumours was tested and evaluated for prediction of overall survival. Nearly 68% of all patients had low cyclin E levels. In patients with stage I/II disease and low total cyclin E levels, the death rate was less then 5% after 5 years. Less than 30% of patients expressing high total cyclin E levels survived. With regard to risk of death from cancer in this study for cyclin E, a strong, independent, statistically significant and clinically relevant impact on prognosis has been demonstrated (HR 13.3). Although these results are derived from retrospective data, and in other studies partially contradictory results were found when immunohistochemistry instead of Western blotting was used, quantification of cyclin E should be considered a future candidate for a prognostic marker [57–58].

In summary, it is too early to derive clinical recommendations from these data. It is the first study showing a prognostic effect, confirmatory prospective studies have yet to be performed (TMUGS III +/–; Oxford 2b C) (table 3). Moreover,

Table 5. Criteria for development of prognostic markers (standard evaluation rules) as proposed earlier [4, 5] and corresponding work-up for the invasion markers uPA and PAI-1. For the first time, level 1 of evidence could be reached by a consequent and successful work-up of these criteria [62]

Standard evaluation rules	Work-up for the invasion markers uPA and PAI-1
Biological model and hypothesis	tumour-associated proteolysis, invasion, migration, adhesion, angiogenesis [37–39, 42–43]
Simple, robust, and valid determination method, quality assurance of testing	ELISA similar to previously routinely performed steroid receptor analysis (snap-frozen material required), high reproducibility with low coefficients of variance demonstrated in multicentre studies [41–44]
Statistical evaluation prospectively planned (factors are primary objectives)	retrospective and prospective explorative studies performed including analyses of correlations and prognostic impact [40, 41, 45–49]
Cut-off optimisation	cut-off optimisation performed and later validated [45, 49]
Validation of the clinical relevance according to criteria of evidence based medicine (prospective pilot and confirmatory trials, meta-analysis)	concordant results in prospective and retrospective monocentre explorative studies, in – multicentre confirmatory therapy trial, and in pooled analysis published [3, 49]
Clinical relevance for treatment decisions	clinical relevance for treatment decisions demonstrated by prospective multicentre therapy trial [49]

the authors [50] do not mention the type of treatment and no separate analysis of node-negative patients was done. Therefore specific conclusions for this important subgroup of breast cancer patients cannot be drawn.

Genomic Profiling

More than one hundred biological markers are suggested to be correlated with breast cancer prognosis, but only a few are discussed to have clinical impact. By looking into the expression of ten thousands genes simultaneously, microarrays can provide information about distinct patterns of gene expression at the RNA-level, that may correlate with prognosis or response to therapy. Thus, *a posteriori,* biological models may be derived from such 'genomic profiling' or 'genomic signatures'. However, *a priori,* it does not represent a distinct biological model or hypothesis.

Standardised RNA-microarrays for routine diagnostic use can only be produced by specialized companies. As testing material, snap-frozen tumour samples have to be used. Sample preparation is time consuming and labour intensive. However, in one experiment ten thousands of genes can be quantified with regard to enhanced or decreased expression. Evaluation requires highly sophisticated statistics in order to find significant clusters in the huge amount of data. Once distinct gene patterns with a prognostic impact have been established, data quantity may be substantially reduced. However, the method is still under development and mainly used for research purposes. Quality assurance or standardisation of sample preparation or assay procedure have not yet been established. In general, RNA determination techniques are highly susceptible to methodological variations which are an everyday problem in clinical routine.

In a pivotal study, it was described how to select genes of interest (70 genes out of 25,000) in order to predict outcome in 78 node-negative breast cancer patients [59]. These results were then confirmed using an independent second cohort of 151 node-negative breast cancer patients [60]. Node-negative patients with a 'good-prognosis signature' had 93.4% probability of distant disease-free survival, those with a 'poor-prognosis signature' 56.2% (HR 15.0). No further confirmatory studies have been published to date (TMUGS III +/–; Oxford 2b C) (table 3). Currently, the group is designing a multicentre prospective trial that focuses on the impact on therapy.

These remarkable data may lead to a better identification of low-risk and high-risk node-negative breast cancer patients than other methods of risk assessment. However, before transfer into daily practice or clinical routine, the methods of tissue preparation, test procedure and evaluation as well as the economical questions that arise with methods so sophisticated have to be solved.

Conclusion

For the first time, with uPA and PAI-1, prognostic markers have been consequently and successfully developed according to standard evaluation rules. Data suggesting clinical routine use are supported by highest levels of evidence according to TMUGS criteria (LoE 1 ++) as well as to Oxford criteria (LoE 1a A) [5–7, 61].

Among the other markers discussed above, only TLI reaches high levels of evidence with regard to its prognostic impact. However, the need of fresh tissue and radioactivity assay prevent a broad use of this technique. Genomic profiling, cyclin E, and disseminated tumour cells are promising markers, but still away from clinical routine use (table 2).

These considerations are the basis of the recommendations of the German Gynaecologic Working Group (AGO) with regard to diagnosis and treatment of breast cancer [6].

References

1 Peto R, Boreham J, Clarke M, Davies C, Beral V: UK and USA breast cancer deaths down 25% in year 2000 at ages 20 – 69 years. Lancet 355;1822: 2000.

2 Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thurlimann B, Senn HJ: Meeting Highlights: Updated International Expert Consensus on the Primary Therapy of Early Breast Cancer. J Clin Oncol 2003;21:3357–3365.

3 Look MP, van Putten WL, Duffy MJ, Harbeck N, Christensen IJ, Thomssen C, Kates R, Spyratos F, Ferno M, Eppenberger-Castori S, Sweep CG, Ulm K, Peyrat JP, Martin PM, Magdelenat H, Brunner N, Duggan C, Lisboa BW, Bendahl PO, Quillien V, Daver A, Ricolleau G, Meijer-Van Gelder ME, Manders P, Fiets WE, Blankenstein MA, Broet P, Romain S, Daxenbichler G, Windbichler G, Cufer T, Borstnar S, Kueng W, Beex LV, Klijn JG, O'Higgins N, Eppenberger U, Janicke F, Schmitt M, Foekens JA: Pooled Analysis of Prognostic Impact of Urokinase-Type Plasminogen Activator and Its Inhibitor PAI-1 in 8377 Breast Cancer Patients. J Natl Cancer Inst 2002;294:116–128.

4 McGuire WL, Tandon AK, Alred DC, Chamness GC, Clark GM: Commentaries. How to use prognostic factors in axillary node-negative breast cancer patients. J Nat Cancer Inst 1990;82:1006–1015.

5 Hayes DF, Bast R, Desch CE, Fritsche Jr H, Kemeny NE, Jessup JM, Locker GY, Macdonald JS, Mennel RG, Norton L, Ravdin P, Taube S, Winn RJ: A tumor marker utility grading system (TMUGS): A framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996;88: 1456–1466.

6 von Minckwitz G, Brunnert K, Costa SD, Friedrichs K, Jackisch C, Gerber B, Harbeck N, Junkermann H, Möbus V, Nitz U, Schaller G, Scharl A, Thomssen C, Untch C für die Organkommission 'Mamma' der Arbeitsgemeinschaft Gynäkologische Onkologie (AGO): Evidenz-basierte Empfehlungen zur Primärbehandlung von Mammakarzinomen. Der Konsens der AGO-Organkommission 2003. www.ago-online.de.

7 Oxford Centre for Evidence-based Medicine Levels of Evidence (May 2001). Produced by Bob Phillips, Chris Ball, Dave Sackett, Doug Badenoch, Sharon Straus, Brian Haynes, Martin Dawes since November 1998. http://cebm.jr2.ox.ac.uk/docs/levels.html.

8 Mansi JL, Berger U, McDonnell T, Pople A, Rayter Z, Gazet JC, Coombes RC: The fate of bone marrow micrometastases in patients with primary breast cancer. J Clin Oncol 1989;7:445–449.

9 Harbeck N, Untch M, Pache L, Eiermann W: Tumour cell detection in the bone marrow of breast cancer patients at primary therapy: Results of a 3-year median follow-up. Br J Cancer 1994;69:566–571.

10 Heiss MM, Allgayer H, Gruetzner KU, Funke I, Babic R, Jauch KW, Schildberg FW: Individual development and uPA-receptor expression of disseminated tumour cells in bone marrow: A reference to early systemic disease in solid cancer. Nat Med 1995;1:1035–1039

11 Braun S, Pantel K, Muller P, Janni W, Hepp F, Kentenich CR, Gastroph S, Wischnik A, Dimpfl T, Kindermann G, Riethmuller G, Schlimok G: Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. N Engl J Med 2000;342:525–533.

12 Roggel F, Hocke S, Lindemann K, Sinz S, Welk A, Bosl M, Pabst M, Nusser N, Braun S, Schmitt M, Harbeck N: Minimal residual disease in breast cancer and gynecological malignancies: Phenotype and clinical relevance. Recent Results Cancer Res 2003; 162:89–100.

13 Funke I, Schraut W: Meta-analysis of studies on bone marrow micrometastases: An independent prognostic impact remains to be substantiated. J Clin Oncology 1998;16:557–566.

14 Diel IJ, Kaufmann M, Costa SD, Holle R, von Minckwitz G, Solomayer EF, Kaul S, Bastert G: Micrometastatic breast cancer cells in bone marrow at primary surgery: Prognostic value in comparison with nodal status. J Natl Cancer Inst 1996;88:1652–1658.

15 Pantel K, Otte M: Occult micrometastasis: Enrichment, identification and characterization of single disseminated tumour cells. Semin Cancer Biol 2001; 11:327–337.

16 Diel IJ, Solomayer EF, Costa SD, Gollan C, Goerner R, Wallwiener D, Kaufmann M, Bastert G: Reduction in new metastases in breast cancer with adjuvant clodronate treatment. N Engl J Med 1998; 339:357–363.

17 Funke I, Fries S, Jauch KW: Tumorzell-Nachweis im Knochenmark: Entscheidungshilfe zur adjuvanten Therapie bei nodal-negativen Patientinnen mit Mammakarzinom. Chirurg 1991;62:805–809.

18 Braun S, Vogl F, Schlimok G, Diel IJ, Coombes CR, Gerber B, Janni W, Gebauer G, Pantel K: Pooled analysis of prognostic impact of occult metastatic cells (OMC) in bone marrow: 10-year survival of 3448 breast cancer patients. Proc Am Soc Clin Oncol 2003;Abstr. 3402.

19 Braun S, Kentenich C, Janni W, Hepp F, deWaal J, Willgeroth F, Sommer H, Pantel K: Lack of effect of adjuvant chemotherapy on the elimination of single dormant tumor cells in bone marrow of high-risk breast cancer patients. J Clin Oncol 2000;18: 80–86.

20 Clayton F: Pathologic correlates of survival in 378 lymph node-negative infiltrating ductal breast carcinomas. Mitotic count is the best single predictor. Cancer 1991;68:1309–1317.

21 Sigurdsson H, Baldetorp B, Borg A, Dalberg M, Ferno M, Killander D, Olsson H: Indicators of prognosis in node-negative breast cancer. N Engl J Med 1990;322:1045–1053.

22 Brown RW, Allred CD, Clark GM, Osborne CK, Hilsenbeck SG: Prognostic value of Ki-67 compared to S-phase fraction in axillary node-negative breast cancer. Clin Cancer Res 1996;2:585–592.

23 Dettmar P, Harbeck N, Thomssen C, Pache L, Zifer P, Fizi K, Jänicke F, Nathrath W, Schmitt M, Graeff H, Höfler H: Prognostic impact of the proliferation associated factors Ki-67 and S-phase in node-negative breast cancer using MIB1 immunostaining and flowcytofluorometric S-phase determination. Br J Cancer 1997;75:1525–1533.

24 Skipper HE: Kinetics of mammary tumor cell growth and implications for therapy. Cancer 1971;28: 1479.

25 Silvestrini R, Daidone MG, Gasparini G: Cell kinetics as a prognostic marker in node-negative breast cancer. Cancer 1985;56:1982–1987.

26 Silvestrini R, Daidone MG, Luisi A, Boracchi P, Mezzetti M, DiFronzo G, Andreola S, Salvadori B, Veronesi U: Biologic and clinicopathologic factors as indicators of specific relapse types in node-negative breast cancer. J Clin Oncol 1995:13:697–704.

27 Paradiso A, Volpe S, Iacobacci A, Marubini E, Verderio P, Costa A, Daidone MG, Marchetti A, Mottolese M, Amadori D, De Paola F, Saragoni L, Medri L, Nenci I, Querzoli P, Gion M, Dittadi R, Plebani M, Orlando C, Bevilacqua G, Silvestrini R; Italian Network for Quality Assessment of Tumor Biomarkers: Quality control for biomarker determination in oncology: The experience of the Italian Network for Quality Assessment of Tumor Biomarkers (INQAT). Int J Biol Markers 2002;17:201–214.

28 Silvestrini R, Daidone MG, Luisi A, Mastore M, Leutner M, Salvadori B: Cell proliferation in 3,800 node-negative breast cancers: Consistency over time of biological and clinical information provided by 3H-thymidine labelling index. Int J Cancer 1997; 74:122–127.

29 Volpi A, De Paola F, Nanni O, Granato AM, Bajorko P, Becciolini A, Scarpi E, Riccobon A, Balzi M, Amadori D: Prognostic significance of biologic markers in node-negative breast cancer patients: A prospective study. Breast Cancer Res Treat 2000;63: 181–192.

30 Tubiana M, Pejovic MH, Koscielny S, Chavaudra N, Malaise E: Growth rate, kinetics of tumor cell proliferation and long-term outcome in human breast cancer. Int J Cancer 1989;44:17–22.

31 Courdi A, Hery M, Dahan E, Gioanni J, Abbes M, Monticelli J, Ettore F, Moll JL, Namer M: Factors affecting relapse in node-negative breast cancer. A multivariate analysis including the labeling index. Eur J Cancer Clin Oncol 1989;25:351–356.

32 Cooke TG, Stanton PD, Winstanley J, Murray GD, Croton R, Holt S, George WD: Long-term prognostic significance of thymidine labelling index in primary breast cancer. Eur J Cancer 1992;28:424–426.

33 Amadori D, Nanni O, Marangolo M, Pacini P, Ravaioli A, Rossi A, Gambi A, Catalano G, Perroni D, Scarpi E, Giunchi DC, Tienghi A, Becciolini A, Volpi A: Disease-free survival advantage of adjuvant cyclophosphamide, methotrexate, and fluorouracil in patients with node-negative, rapidly proliferating breast cancer: A randomized multicenter study. J Clin Oncol 2000;18:3125–3134.

34 Paradiso A, Schittulli F, Cellamare G, Mangia A, Marzullo F, Lorusso V, De Lena M: Randomized clinical trial of adjuvant fluorouracil, epirubicin, and cyclophosphamide chemotherapy for patients with fast-proliferating, node-negative breast cancer. J Clin Oncol 2001;19:3929–3937.

35 Daidone MG, Veneroni S, Benini E, Tomasic G, Coradini D, Mastore M, Brambilla C, Ferrari L, Silvestrini R: Biological markers as indicators of response to primary and adjuvant chemotherapy in breast cancer. Int J Cancer 1999;84:580–586.

36 Wenger CR, Clark GM: S-phase fraction and breast cancer – a decade of experience. Breast Cancer Res Treat 1998;51:255–265.

37 Andreasen PA, Kjoller L, Christensen L, Duffy MJ: The urokinase-type plasminogen activator system in cancer metastasis: A review. Int J Cancer 1997; 72:1–22.

38 Schmitt M, Harbeck N, Thomssen C, Wilhelm O, Magdolen V, Reuning U, Ulm K, Hofler H, Janicke F, Graeff H: Clinical impact of the plasminogen activation system in tumor invasion and metastasis: Prognostic relevance and target for therapy. Thromb Haemost 1997;78:285–296.

39 Rosenberg S: Modulators of the urokinase-type plasminogen activation system for cancer. Exp Opin Ther Patents 2000;10:1843–1852.

40 Duffy MJ, O'Grady P, Devaney D, O'Siorain L, Fennelly JJ, Lijnen HJ: Urokinase-plasminogen activator, a marker for aggressive breast carcinomas: Preliminary report. Cancer 1988;62:531–533.
41 Jänicke F, Schmitt M, Pache L: Urokinase (uPA) and its inhibitor PAI-1 are strong and independent prognostic factors in node-negative breast cancer. Breast Cancer Res Treatm 1993;24:195–208.
42 Bajou K, Noel A, Gerard RD, Masson V, Brunner N, Holst-Hansen C, Skobe M, Fusenig NE, Carmeliet P, Collen D, Foidart JM: Absence of host plasminogen activator inhibitor 1 prevents cancer invasion and vascularization. Nat Med 1998;4:923–928.
43 Stefansson S, Lawrence DA: The serpin PAI-1 inhibits cell migration by blocking integrin $\alpha_v\beta_3$ binding to vitronectin. Nature 1996;383:441–443.
44 Sweep CG, Geurts-Moespot J, Grebenschikov N, de Witte JH, Heuvel JJ, Schmitt M, Duffy MJ, Janicke F, Kramer MD, Foekens JA, Brunner N, Brugal G, Pedersen AN, Benraad TJ: External quality assessment of trans-European multicentre antigen determinations (ELISA) of urokinase-type plasminogen activator (uPA) and its type-1 inhibitor (PAI-1) in human breast cancer tissue extracts. Br J Cancer 1998;78:1434–1441.
45 Harbeck N, Thomssen C, Berger U, Ulm K, Kates RE, Hofler H, Janicke F, Graeff H, Schmitt M: Invasion marker PAI-1 remains a strong prognostic factor after long-term follow-up both for primary breast cancer and following first relapse. Breast Cancer Res Treat 1999;54:147–157.
46 Schmitt M, Thomssen C, Ulm K, Seiderer A, Harbeck N, Höfler H, Jänicke F, Graeff H: Time-varying prognostic impact of tumour biological factors urokinase (uPA), PAI-1 and steroid hormone receptor status in primary breast cancer. Br J Cancer 1997;76:306–311.
47 Harbeck N, Schmitt M, Kates RE, Kiechle M, Zemzoum I, Janicke F, Thomssen C: Clinical utility of urokinase-type plasminogen activator and plasminogen activator inhibitor-1 determination in primary breast cancer tissue for individualized therapy concepts. Clin Breast Cancer 2002;3:196–200.
48 Jänicke F, Pache L, Schmitt M, Ulm K, Thomssen C, Prechtl A, Graeff H: Both the cytosols and detergent extracts of breast cancer tissues are suited to evaluate the prognostic impact of the urokinase-type plasminogen activator and its inhibitor, plasminogen activator inhibitor type 1. Cancer Res 1994;54:2527–2530.
49 Jänicke F, Prechtl A, Thomssen C, Harbeck N, Meisner C, Untch M, Sweep CG, Selbmann HK, Graeff H, Schmitt M: Randomized adjuvant chemotherapy trial in high-risk, lymph node-negative breast cancer patients identified by urokinase-type plasminogen activator and plasminogen activator inhibitor type 1. J Natl Cancer Inst 2001:93:913–920.
50 Keyomarsi K, Tucker SL, Buchholz TA, Callister M, Ding Y, Hortobagyi GN, Bedrosian I, Knickerbocker C, Toyofuku W, Lowe M, Herliczek TW, Bacus SS: Cyclin E and survival in patients with breast cancer. New Engl J Med 2002;347:1566–1575.
51 Ohtsubo M, Theodoras AM, Schumacher J, Roberts JM, Pagano M: Human cyclin E, a nuclear protein essential for the G1-to-S phase transition. Mol Cell Biol 1995;15:2612–2624.
52 Keyomarsi K, Pardee AB: Redundant cyclin overexpression and gene amplification in breast cancer cells. Proc Natl Acad Sci U S A 1993;90:1112–1116.
53 Keyomarsi K, Herliczek TW: The role of cyclin E in cell proliferation, development and cancer; in Meijer L, Guidet S, Philippe M (eds): Progress in Cell Cycle Research. Vol. 3. New York, Plenum Press, 1997, pp 171–191.
54 Porter DC, Zhang N, Danes C, McGahren MJ, Harwell RM, Faruki S, Keyomarsi K: Tumor-specific proteolytic processing of cyclin E generates hyperactive lower-molecular-weight forms. Mol Cell Biol 2001;21:6254–6269.
55 Spruck CH, Won KA, Reed SI: Deregulated cyclin E induces chromosome instability. Nature 1999;401: 297–300.
56 Bortner DM, Rosenberg MP: Induction of mammary gland hyperplasia and carcinomas in transgenic mice expressing human cyclin E. Mol Cell Biol 1997;17:453–459.
57 Donnellan R, Kleinschmidt I, Chetty R: Cyclin E immunoexpression in breast ductal carcinoma: Pathologic correlations and prognostic implications. Hum Pathol 2001;32:89–94.
58 Kuhling H, Alm P, Olsson H, Ferno M, Baldetorp B, Parwaresch R, Rudolph P: Expression of cyclins E, A, and B, and prognosis in lymph node-negative breast cancer. J Pathol 2003;199:424–431.
59 van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH: Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530–536.
60 van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, Parrish M, Atsma D, Witteveen A, Glas A, Delahaye L, van der Velde T, Bartelink H, Rodenhuis S, Rutgers ET, Friend SH, Bernards R: A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999–2009.
61 Hayes DF: Current controversies in cancer: Do we need better prognostic factors in node-negative breast cancer? Arbiter. Eur J Cancer 2000;36:302–306.
62 Thomssen C, Harbeck N: Urokinase-type plasminogen activator and its inhibitor PAI-1: The first prognostic markers in node-negative breast cancer patients validated at the level-1 of evidence are ready for clinical routine use in breast cancer. Ligandassay 2002;7:103–110.