Ned Tijdschr Klin Chem 1995; 20: 305-311

Biochemical assessment of tissue prognostic factors in breast cancer

M. A. BLANKENSTEIN

Breast cancer is a multistage disease and multiple markers may be required to evaluate the behaviour of this malignancy in different stages. Although lymph node status, tumour size, grade and stage either alone or combined to an index may be used to distinguish between groups of patients with different prognosis, the need for other factors is clearly felt (1).

Biochemical parameters assessed in tumour tissues have been studied in this respect for a long time and an overwhelming amount of literature is available (2,3). It is the purpose of the present paper to discuss the current status of assessment of biochemical prognostic factors and the role of the clinical chemistry laboratory.

The first question we need to ask is whether we indeed need prognostic factors for breast cancer in view of the unmistaken trend toward the general use of adjuvant treatment schedules, irrespective of the outcome of assessment of biochemical prognostic factors. This issue has been addressed by Gasparini et al (3) and Clark (4). Clark sees at least three situations in which prognostic factors could be helpful, i.e. 1] to identify patients whose prognosis is so good that it would not be cost-effective to install adjuvant therapy following local surgery; 2] to identify patients with such a poor prognosis that a more aggressive adjuvant approach is warranted; and 3] to identify patients who are likely to respond to or resist certain forms of treatment. Table 1 lists a number of cellular components which have been studied for their prognostic value, some successfully, others unsuccessfully.

Steroid receptors

The oestrogen and progestin receptor (ER and PR) are the most widely known tissue prognostic factors. The simultaneous presence of ER and PR in a primary tumour specimen indicates a 68% chance of success of endocrine therapy. The absence of both receptors is associated with only a 9% chance of response to such treatment and the presence of either one receptor indicates an intermediary chance (5). It has been standard practice for quite some time to assess the ER and PR concentration in cytosols from every breast cancer specimen resected (6). The trend toward general use of adjuvant treatment and the development of histochemical techniques for the assessment of receptors have caused many hospitals to refrain completely from receptor assessment or to abandon quantitative steroid receptor assessment. This trend is clearly illustrated in figure 1. As reviewed earlier (7), assessment of steroid receptor concentrations in primary breast cancer may provide information on the progress of dedifferentiation, the growth rate of the tumour, the effect of adjuvant endocrine and chemotherapy, the probability of responding to first line endocrine therapy, the pattern of metastases and survival time. Similarly, receptor assessment in metastatic lesions can provide information on the progress of dedifferentiation, the growth rate, effect of endocrine treatment and survival time (7). In some patient groups, such as young patients with rapidly growing tumours, or postmenopausal patients with slowly growing tumours, prognosis is

Table 1. Incomplete summary of tissue components evaluated as prognostic factors in human breast cancer

Oestrogen Receptor (ER)
Progestin receptor (PR)
Epidermal growth factor receptor (EGF-R)
Somatostatin receptor
Insulin-like growth factor receptor
LHRH Receptor
Cathepsin-D
PS-2
Heat shock proteins hsp27, hsp70, hsp90
Tyrosine kinase
Thymidine Kinase
Urokinase-type plasminogen activator (uPA)
Plasminogen activator inhibitors (PAI-1 and PAI-2)
Her2/neu oncogen, protein
Tumor suppressor genes, like P53
Oncogenes, like Retinoblastoma gene (RB-1)
Parathyroid hormone-related protein (PTHrP)
Prostate-specific antigen (PSA)

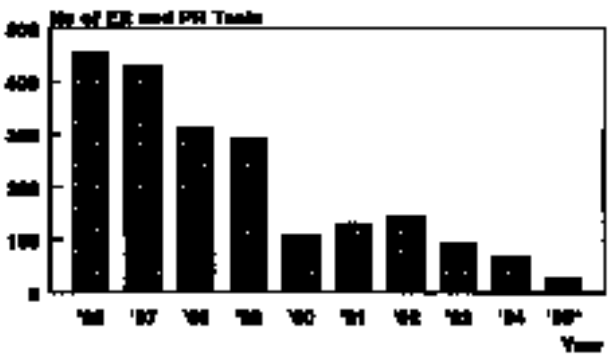

Figure 1. Number of quantitative ER and PR tests in breast cancer cytosol performed in the area serviced by the Academic Hospital Utrecht in the last decade. *The value for 1995 is an optimistic extrapolation based on the number of requests during the first six months.

Laboratorium Endocrinologie, Academisch Ziekenhuis Utrecht

Address correspondence to: Dr. M.A. Blankenstein, Laboratorium Endocrinologie, Academisch Ziekenhuis Utrecht G02.625, Postbus 85500, 3508 GA Utrecht.

Table 2. Properties of techniques for assessment of steroid receptors in breast cancer

Characteristic	LBA*	EIA	ICA
Quantitative Results	Yes	Yes	Semi
Affinity of Binding (K_d)	Yes	No	No
Minimum sample size	50 mg	20 mg	2-10 sections
Application to fine needle aspirates	No	No	Yes
Information of localization of receptors	No	No	Yes
Availability of EORTC quality control	Yes	Yes	ID
Cost of labour*	+	–	–
Cost of reagents*	–	+	+
Expensive equipment required	Yes	Yes	No
Use of radioactivity	Yes	No	No
Use of hazardous chemicals	No	Yes	Yes

*: Abbreviations and symbols: LBA: Ligand Binding Assay with Scatchard Plot Analysis; EIA: Enzyme ImmunoAssay; ICA: Immuno Cytochemical Assay; ID: In Development; +: more than average; –: less than average.

determined predominantly by clinical factors and the choice of treatment and/or follow-up scheme would not be influenced by receptor assessment. The relatively large group of patients in which the picture is clinically not so clear may benefit from receptor assessment.

Initially, steroid receptors were assessed in breast cancer cytosol with a ligand binding assay. The EORTC Receptor Study Group has standardized this assay and runs a quality assessment programme (8-10 and references therein). Techniques for receptor assessment have diversified, however, with the advent of monoclonal antibodies against the receptors. Immunoenzymatic and immunohistochemical methods have appeared on the scene. Unfortunately, this has not led to a greater consistency among laboratories (10). Properties of the various receptor assays are summarized in table 2. It is clear from this table that the biochemical procedures (LBA en EIA) and the cytochemical (ICA) assay are to be considered complementary rather than mutually exchangeable. It is, therefore, to be regretted that many hospitals use only one of the two modalities. It should be noted that in some instances application of immunohistochemical methods is the only option. Examples of such instances are fine needle aspirates of metastatic lesions which do not lend themselves to biopsy, and primary tumours on which, for whatever reason, no receptor assay has been performed and for which only paraffin-embedded tissue remains. Different properties may weigh more heavily in some hospitals than in others. The alleged disadvantage that expensive equipment, like an ultracentrifuge and a scintillation counter, is necessary for performance of the LBA, is often outweighed by the fact that such equipment is present anyway in the dedicated laboratories which perform such assays. Similarly, the use of radioactivity in the LBA may outbalance the presence of hazardous chemicals in the immunochemical methods. Tissue heterogeneity is an issue which is not easily dealt with. In the quantitative biochemical procedures the receptor concentration is measured in cytosols prepared from a representative portion of the tumour and, the result of such assays being an average, information on distribution of the tissue is lost. In principle, this problem should easily be overcome by using the immunocytochemical staining. However, results of this technique are also influenced by the heterogeneous distribution of receptor sites over the tissue. Charpin et al (11) have shown that variation in results of staining procedures is considerably larger when random sections of a tumour are processed, rather than when serial sections are used. This leads to the recommendation to use multiple and random sections for the immunocytochemical assay. It is questionable if this is routinely done.

Early in 1993, validation of the immunohistochemical techniques for steroid receptor measurement was still considered incomplete, both clinically and technically (12). Nevertheless, it was hoped that the remaining problems, which were related to consensus about the scoring system, definition of positive and negative, and the use of appropriate control materials could soon be resolved (12). Up to now, however, this has not been the case but hospitals continue to switch from biochemical procedures to staining for the determination of what is often called hormone receptor "status" (13). In a well- designed study, Molino et al (14) recently reported on the comparison of immunohistochemical and biochemical methods. As in other studies (15), a significant correlation was found, but there was still discrepancy. These authors found a staining percentage of 45% to be optimal to predict the result of the ligand binding assay, which was subjected to regular external quality control. At this staining percentage, where 441/699 tumours were found to be positive as compared to 495/699 for the ligand binding assay, sensitivity and specificity to predict the ligand binding result were 0.810 and 0.804, respectively.

Reporting receptor results

The use of the term receptor "status", which appears in the vast majority of papers dealing with the subject, implies that criteria are available to assign a certain status to a particular cytosol specimen. It is discouraging to see how the literature essentially lacks such information. In papers describing clinical studies on breast cancer, in which receptor "status" is being used to classify patients or in relation to other prognostic factors, one would expect to find information on tissue collection, preservation and processing, together with information on the type of receptor assay used, its coefficient of variation, and the assay and standard used for quantification of the reference parameter (protein) and the cut-off value used to assign the receptor status.

Table 3. Summary of the presence of information on methods used for steroid receptor assays in an arbitrary selection of journals in the first six months of 1995

Journal	No of papers*	Specification of				
		Receptor Assay**	Protein Assay	Definition Cut-Off	QC Data	External QC
BCRT***	12	6(4)	0	9	0	1
Br J Cancer	5	3(2)	1	5	0	1
Cancer	6	1(1)	0	0	0	0
Cancer Res	3	3(1)	1	3	0	0
J Clin Oncol	12	3(1)	1	6	0	1
All	38	16(9)	3	20	0	3

*: Papers describing receptor data in the results section; **: The number of reports with incomplete information is given in parentheses; ***: Breast Cancer Res Treat.

In complete agreement with an earlier observation (10), recent publications are also devoid of such data. The summary of a limited screening of the literature in the first six months of 1995 is shown in table 3. Receptor methodology is specified in only 16 out of 38 studies. In 9 out of these 16 studies, the specification is insufficient to determine exactly what method was used, i.e. whether or not a Scatchard plot analysis was done in the case of ligand binding assays. The worst examples in my opinion are two papers in which data from patients whose receptor "status" was determined with an unspecified ligand binding assay were simply mixed with those from patients in whose tumours an equally unspecified immunohistochemical assay was used, and any information on the comparison of the two methods and cut-off levels used to define receptor positivity was lacking (16,17).

Cut-off values used to differentiate between receptor-rich and receptor-poor specimens are available in 20 out of 38 studies. Five different values are used, i.e. 3, 10, 15, 20 and 25 fmol/mg protein, but the method for protein estimation and the standard used are not specified, rendering the data incomparable. Also for immunohistochemical procedures great differences occur in scoring systems. Receptor positivity may be derived from "any staining" to "45% stained cells" (12,14).

The lack of information on performance of the receptor assays should not be taken as an indication that laboratories generally do not perform adequately. However, it precludes comparisons between different papers, and does not allow proper evaluation of the merit of some studies. Admittedly, journals should pay more attention to proper and complete description of methodologies.

Breast cancer, a changing disease?

It has been noted that the percentage of tumours found receptor positive as well as receptor values, assessed in a single laboratory, have increased in the last two decades (18). In a multivariate analysis including age, tumour size and number of positive nodes, Pujol et al found that the median receptor level of primary breast cancer tissues increased from 14 fmol/mg protein in 1973 to 58 fmol/mg protein in 1992. The positivity rate increased significantly from 73% to 78 % in the same period. Given the fact that the changes observed could not be attributed to technical improvements or changes in tumour size, age of the patients or number of lymph nodes affected, they concluded that the rising ER level may indicate a change in breast cancer biology and in hormonal events that influence genesis and growth of breast cancer (18). This appears to be corroborated by the results of Potter et al (19) who found that the expression of ER and PR is associated with different epidemiological risk factors. Receptor status would thus define fundamental types of breast cancer with potentially different etiologies. It is tempting to spectulate that changes in exposure to risk factors are associated with genesis of different tumours.

Validation of prognostic factors

Prior to their general introduction, new prognostic factor assays should be properly validated, with respect to both clinical value and analytical performance. With respect to the clinical value of new markers, results obtained with a group of patients should be verified with another, independent patient group (4). This will sometimes lead to dismissal of the marker, as in the case of heat shock proteins hsp27 and hsp90 (20). Assays for markers which seem to have potential value need to be standardized. Enthusiasm about new markers may lead to an explosion of assay methods which, not unexpectedly and by virtue of differences in their design and materials used, produce different results. When application of different techniques leads to different outcomes, it should not be surprising that interest in the new marker fades away. Probably the best example of this is the epidermal growth factor receptor (EGF-R). Work on this prognosticator has been reviewed by Klijn et al (21,22). In more than 50 studies totalling over 8000 patients, almost half the number of specimens was found to be EGF-R positive. Individual studies, however, ranged from 14% to 91% in this respect. This difference is attributed to differences in techniques, cut-off points etc. and emphasizes the need for rigourous analytical validation of assays for new prognosticators.

A similar example, although not yet studied as extensively as EGF-R, is Cathepsin D, a proteolytic enzyme with a potential role in tumour invasiveness and cell proliferation. As with the EGF-R, CathD has been studied with different techniques and antibodies, leading to different results (23). This has led Clark (4) to conclude that clinical application of CathD must await further definition based on standardized methodologies.
One problem encountered in assessing prognosticators is that they tend to be correlated with each other and with other characteristics of the patient or the tumour. True validation of a prognosticator thus requires demonstration of independent prognostic values for the new factor. In addition, ways to deal with combined information provided by several independent markers need to be defined (2).

Standardization of assay techniques
As mentioned above, the EORTC Receptor Study Group has designed a standard assay protocol for assessment of steroid receptors (8). A standard protocol is also available for EGF-R (24) and methodology for other prognosticators is currently being evaluated. In view of the expansion of activities, the name of the group has recently been changed to EORTC Receptor and Biomarker Study Group. As laboratories are free to choose techniques, general adoption of recommended techniques solely depends on the commitment each individual laboratory is willing to make. Nowadays many laboratories seem to prefer commercially available assays. This calls for external quality assessment, as approval by government agencies such as the FDA does not guarantee that a diagnostic device will perform properly (25). Since steroid receptor assays have been available for such a long time, one might expect that all requirements with respect to validation and standardization have been fulfilled by now. In spite of all the efforts of EORTC and other working parties on this subject, this is not yet the case. There are discrepancies between results of different techniques, and intra- and interlaboratory variations are not ideal. Improvement of both types of variation are being dealt with in the EORTC Receptor and Biomarker Study Group. It is clear that CVs drop considerably with prolonged participation in the QC programme. Interlaboratory variation can be further reduced by the use of a common reference preparation for calibration of systematic differences which remain between laboratories. As general goals, the interlaboratory and intralaboratory coefficients of variation might be set at 10% and 15%, respectively. It should be borne in mind that these values relate to the analytical performance of the receptor assays, performed on a proper quality control specimen. Obviously, the variation in the outcome of actual samples may be greater, depending on variations in tissue handling procedures (26). Clinicians, and especially coordinators of multi-center clinical trials, should demand proof of proper performance of the laboratories prior to and at regular intervals after the start of the trials. It goes without saying that the general principles discussed here for steroid receptor assays are equally valid for other biochemical procedures.

Selection of prognostic factors
When one considers the vast number of prognosis-associated markers currently under study and the impact of population screening on the availability of tissue, it is evident that a selection must be made in the repertoire of biochemical prognosticators. A distinction should be made between tissue markers with established prognostic significance and those which are currently still under investigation. Assessment of markers associated with the success of treatment should be considered prior to the start of that treatment.
A number of questions related to this topic are:
- Who requests the assessment of prognostic factors?
- Who is or will be using the results of such tests for evaluation of prognosis and/or therapeutic decisions?
- Who determines which parameters are to be measured and by what method(s)?
- What is the quality of these methods and who checks this quality?
- What is the priority if there is not enough tissue for all tests requested/desired?
- Who "owns" the tissue anyway?

The answers to these questions are not always easy to obtain. When tissue is sent from the operating theatre to the prognostic factor laboratory, the surgeon may be considered to be the formal requestor of the biochemical investigation, although the result does not influence the surgical treatment. Alternatively, the pathologist may be considered to be the requestor of the test since the specimen to be investigated should be representative of the entire tumour and only a qualified pathologist can provide this. The medical oncologist who treats the patient for advanced disease, preferably a long time after the operation, may find the receptor data very useful, but has to rely on the surgeon and/or the pathologist to get the tissue to the laboratory. Decisions about the assessment of clinically established prognostic factors should not be influenced by the structure of budgetary systems.
It is most rewarding for the prognostic factor laboratory when the results produced are being used for the benefit of the patient. When selecting biochemical parameters, it should be taken into consideration that patients may be eligible for entering in multi-centre clinical trials. Eligibility will not always be known at the time of surgery, which is a plea for providing a basic prognostic factor panel and maintenance of an ultra-deep frozen specimen collection. Selection of properly evaluated methodology and participation in external quality control programmes are integral parts of the establishment and maintenance of a prognostic factor facility. The composition of such a basic prognostic factor panel is still a matter of debate. An example of such a panel is given in table 4.

New prognostic factors
As knowledge about the factors which control the proliferation of tumours increases, more and more tissue components may be proposed as possible pro-

Table 4. Composition of a tentative basic prognostic factor panel to be assessed in all breast cancer specimens

- *Basic and clinical markers* Type, stage, menopausal status, age and other relevant routine clinical information
- *A marker reflecting growth potential* Thymidine Kinase Activity or S-Phase Fraction
- *A marker reflecting invasive potential* PAI-1; uPA; CathD
- *Markers reflecting probability of responce to:* - Endocrine Treatment: Oestrogen and Progestin Receptors; pS2 - Chemotherapy: EGF-R; erbB2; Multi Drug Resistance parameters

Table 5. Stepwise multi-centre evaluation of new prognostic indicators as proposed by the EORTC Receptor and Biomarker Study Group (formerly the EORTC Receptor Study Group)

- Proposal of Potential New Marker by "Scouts"
- Testing by selected laboratories and comparison with possible other methods. Topics to be included are: feasibility, reproducibility, robustness, sensitivity
- Establishement of the 1st generation of External Quality Control
- Establishment of chosen methodology in other laboratories and evaluation of feasibility of QC procedure.
- Evaluation on international scale of clinical and biological value of the marker
- Acceptation of marker which passes all five steps for clinical practice and multicentre-studies

gnostic factors. No single laboratory can perform all the tasks associated with accepting a tissue component as a new prognostic factor. A stepwise evaluation of potentially new markers proposed by the EORTC Biomarker Study Group is depicted in table 5. If this scheme were uniformly accepted, only properly validated prognostic factors would be introduced in clinical practice. It is imperative that laboratories dedicated to prognostic factors should be able to pursue their investigations on identification of possible better markers, method standardization and establishment of quality control procedures. This means that surgeons and pathologists should be stimulated to continue to make tissues available to these laboratories.

Interesting new factors include the components of the urokinase system, i.e. urokinase type plaminogen activator, uPA, its inhibitors PAI-1 and PAI-2, and its receptor (27-30) mutant forms of the oestrogen receptor with constitutive, i.e. hormone-independent activity (31,32); tyrosine kinase activity (33) and prostate-specific antigen (34).

The rationale behind the study of components of the plasminogen activator system is that proteases can help tumour cells to invade into the surrounding tissue. uPA can activate plasmin, which can degrade several tissue components and also activates type-IV collagenase, which in turn attacks collagen and basement membrane proteins. As uPA and plasmin are in feed-back equilibrium and plasminogen activator inhibitors can act at different sites in the activation cascade, it is critically important to determine which components of the system play the key roles in the activation of tissue degradation. Standardization of methodology and first line quality control are currently being established and results of multi-center studies should become available in the near future.

The occurrence of ER negative/PR positive tumours has been an intriguing phenomenon, which has often been attributed to laboratory errors, since in normal oestrogen target tissues PR synthesis is under oestrogenic regulation. It is now clear that such a tumour phenotype indeed exists. The ER negativity is due to the presence of an aberrant mRNA which lacks Exon 5 and codes for a protein which is truncated to such an extent that it lacks its ligand binding domain and epitopes for the antibodies used in immunochemical assays. This protein, which is undetectable in ER assays, has retained its transactivation properties and is therefore assumed to be capable of activating PR synthesis (35). The Exon 5 deleted mRNA now appears to have prognostic value (32). Presence of this aberrant mRNA per se need not be sufficient to fully explain the ER–/PR+ phenotypes, since it is also found in ER–/PR– tumours (31).

Enzymatic activation of tyrosine-specific protein kinase seems to be a common and major event in the action of many growth factors. Tyrosine kinase, first reported by Hennipman et al (36), has independent prognostic value in a model with patient age, menopausal status, tumour size, number of positive lymph nodes, steroid receptors and uPA (33).

At first glance, evaluation of breast cancer prognosis by assessment of prostate-specific antigen does not seem to make much sense. Since it has turned out that PSA is not prostate-specific as was initially thought, and around 30% of breast cancers was found to express this androgen regulated protease, a study was conducted to evaluate the relationship between PSA in cytosol and prognosis. PSA expression was related with an early stage, small tumours and ER positivity and turned out to have independent prognostic value in ER and node positive patients (34).

It thus appears that TK, mutated ER forms and PSA have successfully passed step 1 in the stepwise evaluation scheme for new prognostic markers (table 5) and further investigation of these markers seems justified.

Conclusion

A great variety of biochemical prognostic factors for breast cancer has become available and many more are still to come. Selection of the best possible marker panel will require a multidisciplinary approach. Methods to be used for the different makers will include a variety of biochemical, histochemical and molecular biological techniques at protein, RNA and

DNA level. Ideally, standardized procedures should be available for all these techniques, and their performance should be monitored at regular intervals. The complexity of the prognostic factor area and the trend toward consolidation of diagnostic laboratories may be taken as arguments to perform prognostic factor assessment in a number of specialized and dedicated laboratories willing to commit themselves to guidelines emerging from national and international cooperative efforts to provide the best possible laboratory care for patients with breast cancer.

Literature

1. Balslev I, Axelsson CK, Zedeler K, Rasmussen BB, Carstensen B, Mouridsen HT. The Nottingham Prognostic Index applied to 9,149 patients from the studies of the Danish Breast Cancer Cooperative Group (DBCG). Breast Cancer Res Treat 1994; 32: 281-290.
2. Mansour EG, Ravdin PM, Dressler L. Prognostic factors in early breast carcinoma. Cancer 1994; 74 (Suppl): 381-400.
3. Gasparini G, Pozza F, Harris AL. Evaluating the potential usefulness of new prognostic and predicitve indicators in node-negative breast cancer patients. J Natl Cancer Inst 1993; 85: 1206-1219.
4. Clark GM. Do we really need prognostic factors for breast cancer? Breast Cancer Res Treat 1994; 30: 117-126.
5. Sunderland MC, McGuire WL. Hormones and breast cancer. Trends Endocrinol Metab 1991; 2: 72-76.
6. Alexieva-Figusch J, Putten WLJ van, Blankenstein MA, Blonk-van der Wijst J, Klijn JGM. The prognostic value and relationships of patient characteristics, estrogen and progestin receptors and site of relapse in primary breast cancer. Cancer 1988; 15: 758-768.
7. Beex LVAM, Peterse JL, Nortier JWR, Veelen H van, Blankenstein MA. Receptoren voor steroidhormonen en mammacarcinoom. Ned Tijdschr Geneeskd 1992; 136: 2056-2060.
8. EORTC Breast Cancer Cooperative Group. Revision of standards for the assessment of hormone receptors in human breast cancer. Eur J Cancer 1980; 16: 1513-1516.
9. Koenders A, Thorpe SM (on behalf of the EORTC Receptor Group). Standardization of steroid receptor assays in human breast cancer-IV. Long-term within and between laboratory variation of estrogen and progesterone receptor assays. Eur J Cancer Clin Oncol 1986; 22: 945-952.
10. Blankenstein MA, Benraad TJ. Multilaboratory assessment of tissue prognostic factors in breast cancer: the EORTC Receptor Study Group experience. Can J Oncol 1993; 3 (Suppl 2): 58-64.
11. Charpin C, Martin PM, De Victor B, Lavaut MN, Habib MC, Andrac L, Toga M. Multiparametric study (SAMBA 200) of estrogen receptor immunocytochemical assay in 400 human breast carcinomas: analysis of oestrogen receptor distribution heterogeneity in tissues and correlations with dextran coated charcoal assay and morphological data. Cancer Res 1988; 48: 1578-1586.
12. Aldred DC. Should immunohistochemical examination replace biochemical hormone receptor assays in breast cancer? Am J Clin Pathol 1993; 99: 1-3.
13. Tesch M, Shwamma A, Henderson R. Immunohistochemical determination of estrogen and progesterone receptor status in breast cancer. Am J Clin Pathol 1993; 99: 8-12.
14. Molino A, Micciolo R, Turazza M, Bonetti F, Piubello Q, Corgnati A, Sperotto L, Martignoni G, Bonetti A, Nortilli R,Castelli P, Rodella S, Capelli P, Manfrin E, Pelosi G, Cetto GL. Estrogen receptors in 699 primary breast cancers: a comparison of immunohistochemical and biochemical methods. Breast Cancer Res Treat 1995; 34: 221-228.
15. Parl FF, Posey, YF. Discrepancies of biochemical and immunohistochemical estrogen receptor assays in breast cancer. Human Pathol 1988; 19: 960-966.
16. Orr RK, Fraher KM. Parity is associated with axillary nodal involvement in operable breast cancer. Breast Cancer Res Treat 34: 71-76.
17. Seidman JD, Schnaper LA, Aisner SC. Relationship of the invasive component of the primary breast carcinoma to axillary lymph node metastatsis. Cancer 1995; 75: 65-71.
18. Pujol P, Hilsenbeck SG, Chamness GC, Elledge RM. Rising levels of estrogen receptor in breast cancer over 2 decades. Cancer 1994; 74: 1601-1606.
19. Potter JD, Cerhan JR, Sellers TA, McGovern PG, Drinkard C, Kushi LR, Folson AR. Progesterone and estrogen receptor and mammary neoplasia in the Iowa's health study: how many kinds of breast cancer are there? Cancer Epidem Biomarkers Prev 1995; 4: 319-326.
20. Ciocca DR, Clark GM, Tandon AK, Fuqua SAW, Welch WJ, McGuire WL. Heat shock protein hsp70 with axillary lymph node-negative breast cancer patients: prognostic implications. J Natl Cancer Inst 1993; 85: 570-574.
21. Klijn JGM, Berns PMJJ, Schmitz PIM, Foekens JA. The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: a review on 5232 patients. Endocr Rev 1992; 13: 3-17.
22. Klijn JGM, Berns PMJJ, Schmitz PIM, Foekens JA. Epidermal growth factor receptor (EGF-R) in clinical breast cancer: Update 1993. Endocr Rev Monographs 1993; 1: 171-174.
23. Ravdin PM. Evaluation of Cathepsin D as a prognostic factor in breast cancer. Breast Cancer Res Treat 1993; 24: 219-226.
24. Benraad TJ, Foekens JA. Hydroxyapatite assay to measure epidermal growth factor receptor in primary human breast tumours. Ann Clin Biochem 1990; 27: 272-273.
25. Tietz NW. Accuracy in clinical chemistry - Does anybody care? Clin Chem 1994; 40: 859-861.
26. Blankenstein MA. Comparison of ligand binding assay and enzyme immunoassay of oestrogen receptor in human breast cancer cytosols - Experience of the EORTC Receptor Group. Breast Cancer Res Treat 1990; 17: 91-98.
27. Jänicke F, Schmitt M, Pache L, Ulm K, Harbeck N, Höfler H, Graeff H. Urokinase (uPA) and its inhibitor PAI-1 are strong and independent prognostic factors in node negative breast cancer. Breast Cancer Res Treat 1993; 24: 195-208.
28. Foekens JA, Schmitt M, Putten WLJ van, Peters HA, Kramer MD, Jaenicke F, Klijn JGM. Plasminogen activator inhibitor-1 and prognosis in primary breast cancer. J Clin Oncol 1994; 12: 1648-1658.
29. Foekens JA, Look MP, Peters HA, Putten WLJ van, Portengen H, Klijn JGM. Urokinase-type plasminogen activator and its inhibitor PAI-1: Predictors of poor response to tamoxifen therapy in recurrent breast cancer. J Natl Cancer Inst 1995; 87: 751-756.
30. Foekens JA, Buessecker F, Peters HA, Krainick U, Putten WLJ van, Look MP, Klijn JGM, Kramer MD. Plasminogen activator inhibitor-2: Prognostic relevance in 1012 patients with primary breast cancer. Cancer Res 1995; 55: 1423-1427.
31. Blankenstein MA, Koehorst SGA, Kallen CJH van ver, Jacobs HM, Spriel AB van, Donker GH, Van't Verlaat, Blaauw G, Thijssen JHH. Oestrogen receptor independent expression of progestin receptors in human meningioma - a review. J Steroid Biochem Molec Biol 1995; 53: 361-365.
32. Daffada AAI, Johnston SRD, Smith IE, Detre S, King N, Dowsett M. Exon 5 deletion variant estrogen receptor messenger RNA expression in relation to tamoxifen resistance and progesterone receptor/pS2 status. Cancer Res 1995; 55: 288-293.

33. Romain S, Chinot O, Klijn JGM, Putten WLJ van, Guirou O, Look M, Martin PM, Foekens JA. Prognostic value of cytosolic tyrosine kinase activity in 249 node-positive breast cancer patients. Br J Cancer 1994; 70: 304-308.
34. Yu H, Giai M, Diamandis EP, Katsaros D, Sutherland DJA, Levesque MA, Roagna R, Ponzone R, Sismondi P. Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. Cancer Res 1995; 55: 2104-2110.
35. Fuqua SA, Fitzgerald SD, Chamness GC, Tandon AK, McDonell DP, Nawaz Z, O'Malley BW, McGuire WL. Variant human breast cancer estrogen receptor with constitutive transcriptional activity. Cancer Res 1991; 51: 105-109.
36. Hennipman A, Oirschot BA van, Smits J, Rijksen G, Staal GEJ. Tyrosine kinase activity in breast cancer, benign breast disease and normal breast tissue. Cancer Res 1989; 49: 516-521.

Summary

Biochemical assessment of tissue prognostic factors in breast cancer. Blankenstein MA. Ned Tijdschr Klin Chem 1995; 20: 305-311.

Information on prognosis and probability of response to treatment of breast cancer patients can be derived in part from an assessment of prognostic factors in the tumour tissue. Oestrogen and progestin receptors were among the first and still remain important. The generalized use of adjuvant treatments and the diversification of techniques for receptor assays have caused many hospitals to abandon quantitative receptor assay or to refrain from receptor assays completely. New prognostic factors are being proposed more and more frequently, but prior to their generalized acceptance, they should be validated both analytically and clinically to a greater extent than is usually done. Standardization of techniques and establishment of external quality control procedures are part of the technical validation. Among these potentially useful new prognostic factors are the components of the urokinase system, like urokinase type plasminogen activator and its inhibitors, thymidine kinase, oestrogen receptor variants with constitutive, i.e. hormone- independent, transactivation properties, and (even?) prostate specific antigen. Techniques for the assessment of new prognostic factors are at the protein, RNA and DNA level and thus require specialized laboratories with dedicated staff, willing to commit themselves to guidelines emerging from international cooperative efforts to provide the best possible laboratory care to the breast cancer patient. To maintain the momentum in the research of such laboratories, it is imperative that they are provided with tissue specimens whenever tumour size allows for it.

Key-words: breast cancer prognostic factors, oestrogen receptors, progestin receptors.

Ned Tijdschr Klin Chem 1995; 20: 311-313

Molecular biological tools in the diagnosis and prognosis of mammary carcinoma

J. ten KATE

Breast cancer has received a great deal of attention in the last few decades in which much progress has been made in characterising the alterations at the DNA level in the different types of breast cancer. These developments offer new hope for patients having breast cancer and for members of families showing a predisposition for this disease.

Neoplastic transformation in general has been shown to be caused by multiple mutations in oncogenes (dominant) and suppressor genes (recessive). The accumulation of mutations leads to the development of invasive, metastasising cancers (1). 40% of patients suffering from breast cancer finally die of the disease. Breast cancer research aims at reducing this figure. One important issue is to develop markers which make it possible to recognise the group with bad prognosis at the time of diagnosis, so that special therapeutical regimens can be developed for this group of patients.

Breast cancers can be subdivided into 4 categories: (1) hereditary, (2) familial, (3) sporadic, and (4) as part of a predisposing syndrome (2,3). This paper will deal with the cancers of categories 1 and 3.

Department of Clinical Chemistry, De Wever Hospital, Heerlen, the Netherlands

Address correspondence to: Dr. J. ten Kate, Dept. of Clinical Chemistry, De Wever Hospital, P.O. Box 4446, 6401CX Heerlen, the Netherlands.

Hereditary breast cancer

Three publications in Science are a milestone in the field of hereditary breast/ovarian cancer (4-6). These papers present the research concerning the discovery of BRCA-genes (Breast Cancer-genes). BRCA1 was located on chromosome 17 and was found to be mutated in 50% of the hereditay breast cancer families (4). Four mutations are described, of which three inactivate the gene and one is an amino acid mutation. The product of this gene was described to be expressed in breast as well as in ovarian epithelial cells. The mutations in this gene did not cause tumors in breast cancer only, but also predispose for the development of ovarian cancer. In sporadic cases, however, these mutations were not observed (5). In contrast to the findings in colon cancer, the mutations in the hereditary form of breast/ovarian cancer do not occur in the sporadic cases.

The third paper in this series describes the BRCA2-gene, which was located on chromosome 13 (6). Mutations in this gene were predisposing for the development of breast cancer, also in males. Predisposition for ovarian cancer seems to be less pronounced in comparison with mutations in the BRCA1-gene.