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Tumor Markers in Breast Cancer – European Group on Tumor Markers Recommendations

Rafael Molina[a] Vivian Barak[b] Arie van Dalen[c] Michael J. Duffy[e]
Roland Einarsson[f] Massimo Gion[h] Helena Goike[g] Rolf Lamerz[i]
Marius Nap[d] György Sölétormos[k] Petra Stieber[j]

[a]Laboratory of Biochemistry, Hospital Clinic, Medical School, Barcelona, Spain; [b]Immunology and Tumordiagnostics Laboratory, Hadassah University Jerusalem, Jerusalem, Israel; [c]Institute of Tumor Marker Oncology, Gouda, [d]Pathology Department, Atrium Medical Center Heerlen, Herleen, The Netherlands; [e]Department of Nuclear Medicine, St Vincent's University Hospital, Dublin, Ireland; [f]CanAg Diagnostics AB, Gothenburg, [g]IDL Biotech, Bromma, Sweden; [h]Centre for the Study of Biological Markers of Malignancy, General Regional Hospital Azienda ULSS 12, Venice, Italy; [i]Medizinische Klinik II and [j]Institut für Klinische Chemie, Klinikum Grosshadern, München, Germany; [k]Department of Clinical Biochemistry, Hillerød Hospital, Hillerød, Denmark

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Abstract
Recommendations are presented for the routine clinical use of serum and tissue-based markers in the diagnosis and management of patients with breast cancer. Their low sensitivity and specificity preclude the use of serum markers such as the MUC-1 mucin glycoproteins (CA 15.3, BR 27.29) and carcinoembryonic antigen in the diagnosis of early breast cancer. However, serial measurement of these markers can result in the early detection of recurrent disease as well as indicate the efficacy of therapy. Of the tissue-based markers, measurement of estrogen and progesterone receptors is mandatory in the selection of patients for treatment with hormone therapy, while HER-2 is essential in selecting patients with advanced breast cancer for treatment with Herceptin (trastuzumab). Urokinase plasminogen activator and plasminogen activator inhibitor 1 are recently validated prognostic markers for lymph node-negative breast cancer patients and thus may be of value in selecting node-negative patients that do not require adjuvant chemotherapy.

Introduction

Breast cancer accounts for approximately 35% of all cancers in Western females, with half the cases occurring in women less than 55 years old [1]. Multiple factors are associated with increased breast cancer risk [2–8]. While breast cancer incidence has increased over the last 30–40 years, mortality has remained stable, probably reflecting earlier diagnosis as well as improved treatment options [9, 10]. The latter include surgery, radiotherapy, chemotherapy, hormonal therapy and immunotherapy. The initial treatment of localized primary breast cancer is given with curative intent and usually includes surgery and/or

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Dr. Rafael Molina
Laboratory of Biochemistry
Hospital Clinic, Medical School
ES–08036 Barcelona (Spain)
Tel./Fax +34 93 451 22 03, E-Mail rmolina@clinic.ub.es

radiotherapy. In recent years, the prognosis for patients with breast cancer has improved due to the administration of adjuvant hormonal therapy and adjuvant chemotherapy [11, 12]. Currently, in the developed world, over 70% of patients with a diagnosis of breast cancer survive 5 years or more [13, 14].

Rational administration of these expensive and frequently unpleasant treatments requires identification both of those patients with localized disease at most risk of recurrence and those who have distant metastases or micrometastases which are unlikely to respond to local therapies [15, 16]. Therefore, objective methods for assessing response to treatment in patients receiving such therapies are highly desirable. In this article, we consider how the measurement of tumor markers in serum and tissue can best contribute to this assessment, presenting the recommendations of the European Group on Tumor Markers (EGTM) (table 1), and where appropriate comparing these markers with those of other expert panels [17, 18].

Serum Tumor Markers

Numerous serum tumor markers have been described for breast cancer, including members of the MUC-1 family of mucin glycoproteins (e.g., CA 15.3, BR 27.29, MCA, CA 549), carcinoembryonic antigen (CEA), oncoproteins (e.g., HER-2/c-erbB-2) and cytokeratins (e.g., tissue polypeptide antigen and tissue polypeptide-specific antigen) [19–24]. Members of the MUC-1 family are the most widely used serum tumor markers in breast cancer, but as they have similar diagnostic sensitivities and specificities, the use of more than one MUC-1 antigen is unlikely to confer any advantage [19, 20, 25]. However, CEA measurement can provide additional complementary information. For this reason, the combination of one MUC-1 marker and CEA is the recommended serum marker panel in patients with breast cancer.

Screening and Diagnosis

Their lack of organ and tumor specificity and low sensitivity, particularly in early-stage disease, generally invalidate the use of tumor markers for either screening or early diagnosis [17, 22]. Low levels of tumor markers in patients with suspected breast cancer never exclude the presence of malignancy. Nevertheless, tumor marker determination may complement patient staging: high levels of CA 15.3 (e.g. >50 U/ml) and/or CEA (e.g. >20 ng/ml)

Table 1. Serum markers in breast cancer: EGTM recommendations

Serum markers

The most useful serum markers for breast cancer are CA 15.3 (or BR 27.29) and CEA, but due to their low sensitivity, they cannot be recommended for screening or early diagnosis, but serial levels may be useful in the early diagnosis of distant metastases.

Prognosis: Preoperatively elevated levels of either CA 15.3 or CEA are associated with adverse outcome in patients with breast cancer; their use in combination with established prognostic factors is recommended.

Early diagnosis of recurrence: Serial CA 15.3 and CEA serum determinations are recommended for the early detection of recurrence in patients with breast cancer and no evidence of disease, if the detection of recurrent or metastatic disease would alter clinical management. The impact of this lead time information on patient outcome is not clear.

Therapy monitoring: Markers should be measured prior to every chemotherapy course and at least every 3 months for patients receiving hormone therapy.

The EGTM regards an increase in tumor marker concentration of at least 25% of the previous value – with the second value above the reference interval – to be significant. It is recommended that such an increase be confirmed with a second specimen obtained within a month. If the continued increase is confirmed, this provides evidence of progressive disease. Similarly, confirmed decreases in serum levels of more than 50% are consistent with tumor response.

Technical aspects: Well-documented and relevant IQC and EQA procedures must be in place and should be followed whenever tumor markers are measured. If it is necessary to change method during serial monitoring, this must be undertaken with considerable care.

Tissue markers

ER and PR should be assayed on all newly diagnosed breast cancer patients. The primary use of steroid receptors is for selecting patients for treatment with hormone therapy.

HER-2 should be used for selecting patients with advanced breast cancer for treatment with Herceptin and for identifying patients with early breast cancer for participation in clinical trials involving adjuvant Herceptin. HER-2 may also be of use for identifying patients who are particularly likely to benefit from adjuvant anthracycline-based chemotherapy.

HER-2 should not be used to predict response to either hormone, CMF or taxane therapy.

For determining HER-2 status, immunohistochemistry (calibrated against FISH) should be employed. For samples with an immunohistochemistry score of 2+, confirmation by FISH should be carried out.

uPA and PAI-1, determined by a validated ELISA, may be used for determining prognosis, especially in patients with lymph node-negative disease.

in patients thought to have localized disease suggest the presence of unsuspected metastatic disease.

The sensitivity of tumor markers is significantly higher in patients with advanced disease [21, 26–38] and is related to the site of recurrence [18, 30, 39]. CA 15.3 and CEA are not useful in the early diagnosis of locoregional recurrence, for which clinical examination is superior. However, abnormal CEA and CA 15.3 levels are found in 40–50 and 50–70% of patients with distant metastases [40–43]. Simultaneous use of both markers allows early diagnosis of metastases (mainly in bone and liver) in up to 60–80% of patients with breast cancer. By using combinations of several markers (e.g., CA 15.3, CEA and cytokeratins), it is possible to increase the sensitivity to at least 90% in patients with distant metastases [24, 33, 34, 44–46].

EGTM Recommendation

The most useful serum markers for breast cancer are CA 15.3 (or BR 27.29) and CEA, but due to their low sensitivity, they cannot be recommended for screening or early diagnosis.

Prognosis

Serum levels of CA 15.3 (and other MUC-1 glycoproteins) and CEA are related to tumor burden, with significantly higher values in patients with nodal involvement or large tumors [18, 26–28, 47]. Several studies have demonstrated shorter disease-free survival and overall survival in patients with high preoperative levels of these markers, while others reported conflicting data [18–20, 27–29, 48–52]. Most studies with large patient groups and long follow-up times conclude that preoperative serum CA 15.3 and CEA concentrations are independent prognostic factors [28, 29, 50–55]. However, it has not yet been demonstrated if the use of tumor markers as indicators of recurrence can lead to improvement in either patient disease-free survival or overall survival.

EGTM Recommendation

Preoperatively elevated levels of either CA 15.3 or CEA are associated with adverse outcome in patients with breast cancer, and the EGTM recommends their measurement in combination with established prognostic factors.

Early Detection of Recurrence

The main reasons for monitoring patients following treatment for primary breast cancer are to enable the early detection of new primary or locally recurrent cancers that may be cured by early intervention, as well as the early detection of metastatic disease. The sensitivity of tumor markers for detecting recurrent disease is clearly related to the site of recurrence [40–43]. As previously described, low sensitivity precludes the use of tumor markers in the early diagnosis of locoregional recurrence, but serial tumor marker measurements can contribute usefully to early diagnosis of distant metastatic disease, especially in liver and bone. Increases in MUC-1 serum markers reportedly provide the first indication of recurrence, prior to clinical or radiological indication (e.g., by chest X-ray, liver ultrasonography, bone scans), in 40–55% of treated patients [40–42, 56]. Additional CEA measurement can increase the sensitivity in the early detection of recurrence obtained with CA 15.3 by up to 5–25% of the patients [40]. Reported lead times vary from 2 to 18 months (mean 5.2) [30–32, 40–43, 55–62].

The specificity of markers for the detection of recurrence in the follow-up of patients with no clinical evidence of disease is related to the marker cut-off points used [30–32, 40–43, 56–58, 60–64]. In one study, the proportion of false-positive results (abnormal values without recurrence) was 5% for CEA and 6.5% for CA 15.3 using cut-off values of 5 μg/l and 35 U/ml, respectively [40]. Using higher cut-off values (CEA 10 μg/l, CA 15.3 60 U/ml) and at least two serial increases (>15%), specificity increased to almost 100% [41, 58]. Using the latter criteria, sensitivities of 45 and 30% have been reported for CA 15.3 and CEA, respectively, with an increase in either tumor marker providing the first sign of recurrence in 60–70% of patients [41, 42].

Early detection of metastasis has two different aims: one is the diagnosis, and the second, the possibility to earlier initiate systemic treatment. There is a controversy as to whether intensive screening incurs extra expenses and whether it unnecessarily increases anxiety; additionally, its value is uncertain regarding ultimate outcome [64, 65]. Other diagnostic tools such as chest radiography, bone scans and liver ultrasonograms are useful in the diagnosis of recurrent or metastatic disease but do not seem to increase survival [66–68]. In spite of the previously reported studies, the basic hypothesis that early diagnosis of recurrences may prolong survival is supported by the observation that smaller breast tumors generally are more likely to respond to therapy than larger tumors. The inverse relationship between tumor mass and chemotherapy response means that in more advanced metastatic disease, response to treatment is generally shorter and less likely [69].

EGTM Recommendation

The EGTM recommends serial CA 15.3 and CEA serum determinations for the early detection of recurrence in patients with breast cancer and no evidence of disease, if the detection of recurrent or metastatic disease would alter clinical management, although the impact of this lead time information on patient outcome is not clear. Currently, there are no data available regarding the optimum frequency for the measurement of serum tumor markers in the early diagnosis of recurrent disease. However, the EGTM panel suggests the following approach during the follow-up of asymptomatic women: tumor markers should be determined every 2–4 months (according to the risk of recurrence) during the initial 5 years after diagnosis, then every 6 months during the next 3 years and at yearly intervals thereafter.

Therapy Monitoring

The most important clinical application of tumor markers in metastatic breast cancer lies in monitoring response to therapy. Patients in remission usually have decreasing marker levels, while those with progressive disease generally have increasing levels [33, 35, 37, 38, 44, 45, 70–72]. Studies comparing tumor marker monitoring with conventional International Unit against Cancer criteria vary considerably – both in terms of the disease status of included patients and the criteria for marker assessment – but most authors conclude that the measurement of tumor markers provides an objective means of guiding therapy [33–36, 57–63, 70–76].

Frequency of Measurement

It depends on the treatment how frequently markers should be measured. The EGTM recommends that tumor markers in patients treated with chemotherapy should be determined before every chemotherapy course. In patients treated with hormone therapy, they should be measured at least every 3 months.

Defining Significant Changes

There is little agreement in the literature about what constitutes a clinically significant change in marker level. The EGTM regards an increase in tumor marker concentration of at least 25% of the previous value – with the second value above the reference interval – to be significant, recommending that such an increase be confirmed in a second specimen obtained within a month. If the continued increase is confirmed, this provides evidence of progressive disease. Similarly, confirmed decreases in serum levels of more than 50% are consistent with tumor response, to avoid the intraindiviual as well as the interassay variability [33–35, 46, 77–79]. Certain treatments may cause transient increases in serum marker levels, so that increases observed shortly after treatment must always be confirmed.

Advantages of Tumor Marker Monitoring

Monitoring with tumor markers has been shown to be superior to monitoring by conventional International Unit against Cancer criteria in a number of studies [33–35, 46, 77–83]. Biochemical changes often precede clinical or radiological signs of response or progression, potentially enabling earlier treatment decisions regarding continuation of effective therapy, discontinuation of ineffective therapy, change of therapy or more effective palliation. It has been suggested that biochemical assessment may result in cost savings of at least 50% when compared with assessment by clinical or radiological criteria, which often require expensive imaging techniques such as computer tomography scans [82]. However, whether this monitoring leads to enhanced survival or better quality of life remains to be determined [83, 84].

EGTM Recommendation

Markers should be measured prior to every chemotherapy course and at least every 3 months for patients receiving hormone therapy. Objective criteria for assessing changes in markers should be in place and increases or decreases confirmed appropriately.

Measurement of Serum Markers

Analytical requirements for tumor markers are similar to those for most other clinical analytes: the correct and appropriate specimen should be analyzed by a method which meets defined quality requirements for both internal quality control (IQC) and external quality assessment (EQA) [85].

Tumor marker assays from different manufacturers can give significantly different results for the same serum [86–88]. The method used should therefore be stated on the laboratory report. When it is necessary to change the method used to monitor a patient during follow-up, this should be undertaken with considerable care, e.g., by assaying a specimen using both methods for a period of time, so as to minimize the risk of misinterpretation of trends in marker levels.

EGTM Recommendation

Well-documented and relevant IQC and EQA procedures must be in place and should be followed whenever tumor markers are measured. If it is necessary to change the method during serial monitoring, this must be undertaken with considerable care.

Tissue Markers

While serum markers in breast cancer are mostly used for monitoring patients with diagnosed disease, tissue-based markers are primarily measured in order to determine prognosis and predict response to therapy. Clinically, the most useful tissue-based markers in breast cancer are estrogen receptor (ER), progesterone receptor (PR) and HER-2 (also known as c-erbB-2 or neu). Although not yet in widespread clinical use, urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) are potential markers for determining prognosis in lymph node-negative breast cancer patients.

ER and PR

ER and PR are transcriptional factors which mediate the actions of estrogens and progesterone, respectively [89, 90]. Both receptors are now known to exist in two different forms. For ER, these forms are known as ER-alpha and ER-beta [91], and for PR, the two forms are known as PRA and PRB [92]. To date, for ER, a clinical role has only been established for the alpha form. Existing assays for PR do not discriminate between the two forms.

The main clinical application of ER (i.e. ER-alpha) and PR in breast cancer is selecting patients likely to respond to hormone therapy. In both early and advanced disease, hormone receptor-positive patients have a significantly greater probability of responding to hormone therapy than patients lacking receptors [93–95]. Therefore, the EGTM panel recommends that ER (i.e. ER-alpha) and PR assays be performed on all patients with newly diagnosed breast cancer. Similar recommendations have been previously made by the EGTM [26, 96], as well as by other expert panels such as the American Society of Clinical Oncology (ASCO) [17, 97], the National Academy of Clinical Biochemistry [96], the European Society of Medical Oncology [98], the European Society of Mastology [99] and a National Institute of Health developmental panel [100]. At this stage, the assay of ER-beta cannot be recommended for predicting response to endocrine therapy.

While the primary purpose for performing hormone receptor assays lies in selecting a likely response to endocrine therapy, information on receptor status or concentration may also be of prognostic value. Generally, for the first 4–5 years after diagnosis, ER-positive patients have a better outcome than ER-negative patients [101, 102]. However, after this period, the favorable prognostic impact of ER is lost. A further limitation of ER as a prognostic factor is that it is of little value in lymph node-negative patients [101, 102]. Although less work has been carried out on the prognostic impact of PR, patients with tumors expressing PR also tend to have a better prognosis than those lacking this receptor [95, 103].

Measurement of ER and PR

Three well-established assays exist for measuring hormone receptors, namely ligand binding, ELISA and immunohistochemistry. It is important to state that all the early studies showing a clinical utility for hormone receptors were performed with ligand-binding assays. However, more recently, immunohistochemistry has largely replaced the older biochemical assays for determining receptor levels. Immunohistochemistry is simpler to perform than either ligand-binding assays or ELISA and has the distinct advantage of being the only assay that can be used for small tumors. Furthermore, at least for ER, immunohistochemistry assays have been shown to perform as well as the ligand-binding assays for predicting response to hormone therapy [104–110]. Therefore, for the present, the EGTM recommends that immunohistochemistry should be used to determine hormone receptors in breast cancers.

For immunohistochemistry, some of the most widely validated antibodies include 6F11 MAb or antibody ID5 for ER [104, 105, 111, 112] and antibodies PR88 KD68 MAb or MAb 1294 for PR [105, 111–113]. Scoring of immunohistochemical stain may be based on either percentage of cells stained or a combination of percentage of cells stained plus intensity of stain [111, 112]. The hormone receptor report should indicate this semi-quantitative score, rather than merely stating a negative or positive finding [111, 112].

A new approach, using a combination of DNA flow cytometry and immunophenotyping on single cell suspensions from formalin-fixed paraffin-embedded tissue, has the advantage of both objective quantitation and application to small amounts of tissue. Furthermore, the quality of the DNA histograms serves as an internal control for the preservation of receptor proteins. Although this is a novel method for the quantitative determination

of markers in tissue and has been subjected to several studies [114, 115], it has not yet found wide acceptance, since a possible improved correlation with clinical outcome has not yet been documented.

EGTM Recommendations

An assay of hormone receptors (i.e. ER and PR) is mandatory in the selection of patients with both early and advanced breast cancer for treatment with hormone therapy. Patients with hormone receptor-positive tumors should be treated with some form of endocrine therapy, while receptor-negative patients should receive an alternate form of therapy.

Immunohistochemistry with validated antibodies should be used to determine hormone receptors in breast cancer.

Since both ER and PR are relatively weak prognostic factors in breast cancer, these factors should not be used alone for differentiating between patients with indolent and aggressive breast cancers. However, hormone receptors may be combined with established prognostic factors in determining outcome.

HER-2

HER-2, which is also known as c-erbB-2 or neu, encodes a transmembrane protein belonging to the epidermal growth factor receptor family [for a review, see ref. 116]. The HER-2 gene is either amplified or overexpressed in 15–30% of invasive breast cancers. Amplification of the HER-2 gene means that instead of having only 2 copies of the gene per cell, there may be 50–100 gene copies per cell. The number of HER-2 proteins per cell can then increase from 20,000–50,000 to as high as 2 million [117].

HER-2 has a number of potential uses in breast cancer, including determining prognosis, predicting relative resistance to both hormone therapy and adjuvant cyclophosphamide, methotrexate, 5-fluorouracil (CMF) therapy, selecting for enhanced response to adjuvant anthracycline-based therapy and identifying patients for treatment with Herceptin (trastuzumab). The role of HER-2 in these specific areas is now briefly discussed.

Predicting Response to Herceptin

Currently, the primary and only mandatory reason for determining HER-2 levels in breast cancer lies in selecting patients with advanced breast cancer for treatment with Herceptin (trastuzumab). Herceptin is a humanized MAb that binds with high affinity to the extracellular domain of HER-2, thereby blocking its role in signal transduction [118]. Herceptin, alone or in combination with chemotherapy, is now widely used for the treatment of HER-2-positive patients with advanced breast cancer. Furthermore, Herceptin is currently being evaluated in clinical trials for the adjuvant treatment of HER-2-positive breast cancer [119].

Based on cell culture and animal model experiments [117, 118], it is generally believed and highly likely that overexpression of HER-2 is necessary for Herceptin to induce tumor regression. Consequently, at this stage, Herceptin should only be administered to breast cancer patients showing either gene amplification or overexpression of HER-2. In agreement with the ASCO expert panel [97], the EGTM panel recommends an assay of HER-2 for selecting patients with advanced breast cancer for treatment with Herceptin.

Predicting Resistance to Endocrine Therapy

Most but not all published reports conclude that overexpression of HER-2 is associated with relative resistance to hormone therapy in patients with breast cancer [for reviews, see ref. 120, 121]. Most of these studies have methodological limitations including a retrospective assay of HER-2, the use of different HER-2 assays and inclusion of relatively small numbers of patients. Furthermore, different forms of hormone therapy were used and different subgroups of patients were studied in the different trials. Based on present information, the EGTM panel is unable to recommend an assay of HER-2 for selecting endocrine resistance in patients with breast cancer. The ASCO [97] and National Institute of Health panel [100] have made similar recommendations.

Predicting Response to Chemotherapy

Similar to the situation with hormone therapy, controversy also exists on the relationship between HER-2 and response to adjuvant CMF. Although most studies conclude that patients with HER-2-positive tumors derive less benefit from adjuvant CMF than patients with HER-2-negative cancers, there are again conflicting reports [for reviews, see ref. 120, 121]. In general, studies on the relationship between HER-2 and response to CMF suffer from the same limitations as discussed above for endocrine therapy. Based on current findings, the EGTM panel, in agreement with the ASCO panel [97], is unable to recommended assay of HER-2 for predicting response to adjuvant CMF.

Although overexpression of HER-2 may be associated with relative resistance to adjuvant CMF, a number of retrospective studies suggest that increased levels of this oncoprotein predicts an enhanced response from anthra-

cycline-based adjuvant therapy [for reviews, see ref. 120, 121]. Indeed, the ASCO panel stated 'that high levels of HER-2 may identify patients who particularly benefit from anthracycline-based adjuvant therapy but that levels of HER-2 should not be used to exclude patients from anthracycline treatment' [97]. The EGTM panel supports this statement.

Compared with the response to CMF or anthracycline-based therapy, relatively few studies have investigated the interaction between HER-2 and the benefit of taxanes. While some reports concluded that patients with HER-2-positive tumors exhibited higher response rates to taxanes in advanced breast cancer than those with HER-2-negative cancers [122], others were unable to confirm these findings [123, 124]. In a recent retrospective study, Konecny et al. [125] reported that patients with HER-2-positive tumors had a greater objective response than patients with HER-2-negative tumors to treatment with epirubicin/paclitaxel when used as first-line chemotherapy for metastatic breast cancer. However, HER-2 was not associated with response to epirubicin/cyclophosphamide. Despite these promising findings, HER-2 cannot, at present, be recommended in selecting for response or resistance to taxanes [97].

Determining Prognosis

Most published reports on axillary node-positive breast cancer patients conclude that either HER-2 gene amplification or overexpression correlates with an adverse outcome in patients with breast cancer [for a review, see ref. 126]. However, the prognostic impact of HER-2 in node-negative breast cancer patients is less clear [126]. It is important to point out that most of the studies relating HER-2 to patient outcome were retrospective in design, contained relatively low numbers of patients and used a variety of HER-2 assays. Furthermore, in many of the studies, patients received different types of adjuvant therapy, making it difficult to differentiate a prognostic from a predictive impact. Based on the available information, the EGTM panel recommends that HER-2 should not be used alone for determining outcome in patients with breast cancer. However, as HER-2 is being measured on an increasing number of patients with breast cancer, information on HER-2 status may be combined with standard prognostic factors for determining outcome in patients with breast cancer.

Measurement of HER-2

Although multiple methods have been used to measure HER-2 gene amplification/protein overexpression in breast tumors, the two most widely employed methods are immunohistochemistry and fluorescent in situ hybridization (FISH). The advantages of immunohistochemistry include its wide availability, simplicity and relatively low costs. Its disadvantages include subjectivity in evaluating the staining score, possible loss of HER-2 protein as a result of tissue storage and fixation and variable results depending on both the antibody and staining procedure used.

In contrast to immunohistochemistry, FISH can theoretically provide a more objective scoring system. It also has the advantage of a built-in internal control consisting of two HER-2 gene copies in the non-malignant cells within the specimen. The disadvantages of FISH include its high costs, the requirement for a fluorescent microscope and the inability to preserve the slide for storage and review. In addition, reading the fluorescent signal in a sufficiently large number of cells for reliable individual scoring is difficult.

To avoid fading of the fluorescent signal, chromogenic in situ hybridization may be used. However, this approach is also unable to solve the problem relating to the limited number of cells that can be evaluated. Chromogenic in situ hybridization remains to be validated in prospective studies. As regards monitoring the quality of the preservation of HER-2 protein during tissue fixation and embedding, see above for ER. Combining the DNA histogram as a quality control aspect of tissue preservation with the specific quantification of HER-2 in the epithelial compartment by multiparametric analysis can be an alternative [127].

EGTM Recommendation

HER-2 should be assayed in order to select patients with advanced breast cancer for treatment with Herceptin. However, at present, HER-2 is not recommended for predicting response to adjuvant CMF, taxanes or endocrine therapy in patients with breast cancer.

HER-2 should not be used alone for determining outcome in patients with breast cancer. However, HER-2 may be combined with other prognostic factors for predicting patient outcome.

For determining the HER-2 status in breast cancer, immunohistochemistry (calibrated against FISH) should be employed. For samples with an immunohistochemistry score of 2+, confirmation by FISH should be carried out.

uPA and PAI-1

uPA is a serine protease implicated in cancer growth, invasion and metastasis [for a review, see ref. 128]. PAI-1 is an endogenous inhibitor of uPA, but paradoxically is also involved in tumor progression [128]. Multiple single institutional studies have shown that both uPA and PAI-1 are potent and independent prognostic factors in breast cancer [129, 130]. This prognostic impact of uPA and PAI-1 has been shown in both lymph node-negative and lymph node-positive breast cancer patients. Recently, the prognostic impact of uPA and PAI-1 in lymph node-negative breast cancer patients was validated in both a randomized prospective trial and a pooled analysis ($n > 8{,}000$ patients) of single institution studies [131, 132].

At present, the main use of these factors lies in selecting lymph node-negative patients who do not need or are unlikely to benefit from adjuvant chemotherapy. Patients with low levels of both these proteins are at a relatively low risk of developing recurrent or metastatic disease and, consequently, may be able to avoid the toxic side effects and costs of adjuvant chemotherapy.

uPA and PAI-1 should be determined by ELISAs that have undergone both technical [133, 134] and clinical validation [131, 132]. For the determination of uPA and PAI-1 by ELISA, a small piece of breast tumor must be rapidly frozen in liquid nitrogen following histopathological examination. Recently, a microassay (i.e. a micro ELISA) was described for the measurement of uPA and PAI-1 [135, 136]. Although not yet clinically validated, this assay showed that uPA and PAI-1 levels in core biopsies correlated well with corresponding levels in surgically removed tissue. Although multiple studies have described the use of immunohistochemistry to detect uPA and PAI-1 in breast cancer, none have shown a significant correlation with either ELISA values or patient outcome. Thus, at present, immunohistochemistry cannot be recommended for the clinical determination of uPA and PAI-1 in breast cancer.

EGTM Recommendation

According to the EGTM panel, uPA and PAI-1 may be used for determining prognosis in breast cancer, especially in the group of patients with lymph node-negative disease. ELISAs validated for both analytical and clinical performance should be used for determining these proteins.

Gene Expression Profiling

Instead of measuring individual markers, the use of DNA microarray or gene expression profiling is a popular current research approach for determining prognosis. With a microarray, the expression of tens of thousands of genes (e.g., in a sample) can be determined simultaneously. In recent years, a number of investigators have shown that microarray techniques can predict outcome in patients with breast cancer [137–141].

In one of these studies, using this technique on lymph node-negative breast cancer patients, van't Veer et al. [137] identified a 70-gene signature that correctly predicted the later appearance or absence of clinical metastasis in 65/78 lymph node-negative patients. The application of this prognostic classifier to an independent set of 19 breast cancers resulted in only two incorrect classifications.

The 70-gene expression profile was more recently validated in a series of 295 consecutive breast cancer patients [138]. In this larger study, the probability of remaining free of distant metastasis at 10 years after surgery was 85.2% in the patients with the good prognostic signature and 50.6% in those with the poor signature. The estimated hazard ratio for distant metastasis in the patients with the poor prognostic signature as compared with the group with the good prognostic signature was 5.1 (95% confidence intervals 2.9–9.0; $p < 0.001$). A similar significant difference in outcome between patients with the good and poor signature was seen in patients with either lymph node-negative or lymph node-positive patients. Multivariate analysis showed that the gene signature was an independent factor in predicting disease outcome. Although these results with DNA microarray are promising, this technology is technically demanding, time-consuming and expensive. Before clinical use, this technology must be simplified, standardized and evaluated in external quality assessment schemes. Furthermore, these preliminary results must be confirmed in a large prospective study or a meta-analysis/pooled analysis of individual studies.

Rather than using large numbers of genes, Paik et al. [142] recently identified a 21-gene panel (16 test genes and 5 reference genes) that predicted breast cancer recurrence in 668 lymph node-negative ER-positive patients that received adjuvant tamoxifen therapy. Using multivariate analysis, the gene panel predicted the outcome independently of patient age and tumor size. Importantly, the authors were able to carry out RT-PCR on RNA extracted from formalin-fixed and paraffin-embedded tissue. However, as with the gene microarray results discussed above, these findings require validation in independent laboratories.

EGTM Recommendation

Although initial results are promising, DNA microarray should not be used at present for predicting outcome in patients with breast cancer.

Other Tissue-Based Markers

Based on available evidence, a routine assay of other breast cancer tissue markers, such as p53, c-myc, cathepsin B, cathepsin D, S phase or ploidy [98, 134], cannot be recommended at present.

Conclusions

MUC-1 antigen and CEA are the most useful serum markers in patients with breast cancer. Serial determination of these markers may be useful in routine therapy monitoring and for early detection of recurrence and progression during follow-up. However, interpretation of sequential measurements is a task for specialists who are able to integrate information at a multidisciplinary level in collaboration with the end users, general practitioners, surgeons and oncologists. Steroid receptors and HER-2 are the tissue-based markers accepted in clinical practice, having an established role in predicting hormone sensitivity or in Herceptin treatment, respectively. uPA and PAI-1 are recently validated as prognostic factors for lymph node-negative breast cancer patients and thus may be of use in selecting those node-negative patients who may not need to receive adjuvant chemotherapy. Other potential markers for breast cancer such as p53, cathepsin B, cathepsin D, S phase and ploidy look promising, but further research is necessary before their clinical utility is established.

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