Review
HER2 therapy

HER-2/neu diagnostics in breast cancer

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Abstract

HER-2/neu status of the primary breast cancer (PBC) is determined by immunohistochemistry and fluorescent *in situ* hybridization. Because of a variety of technical factors, however, the PBC may not accurately reflect the metastatic tumor in terms of HER-2/neu status. Recently published guidelines recommend that tumors be defined as HER-2/neu positive if 30% or more of the cells are 3+. Circulating levels of the HER-2 extracellular domain can be measured in serum using a test cleared by the US Food and Drug Administration, and increased serum HER-2/neu levels to above 15 ng/ml can reflect tumor progression. Studies comparing tissue HER-2/neu status of the PBC and HER-2/neu levels above 15 ng/ml in metastatic breast cancer patients are also reviewed.

Introduction

For many years estrogen receptor (ER) status has guided the administration of hormone therapy to patients with breast cancer. Positive ER status narrows the pool of patients eligible for hormone therapy and increases the likelihood of favorable response. However, ER status indicates that approximately 50% of patients will respond, but it does not predict which patients will respond to hormone therapy. Similarly, the HER-2/neu status of breast carcinoma narrows the pool of candidates eligible for HER-2/neu directed therapies, but it does not definitively select those who will or will not respond.

The HER-2/neu oncoprotein continues to be an important target in the development of a variety of new cancer therapies, which include mAb-based therapy, small-molecule drugs directed at the internal tyrosine kinase portion of the HER-2/neu oncoprotein, and vaccines. The most widely known HER-2/neu-directed therapy is trastuzumab (Herceptin; Genentech, South San Francisco, CA, USA). Trastuzumab is a humanized recombinant mAb that specifically targets the HER-2/neu extracellular domain (ECD). The effectiveness of trastuzumab therapy has been proved not only in metastatic breast cancer (MBC) patients [1] but also in early-stage breast cancer patients receiving adjuvant trastuzumab therapy [2,3]. Recently, the US Food and Drug Administration (FDA) approved lapatinib [4] (Tykerb, GSK, Philadelphia, PA, USA) for clinical use. In addition, several tyrosine kinase inhibitors are currently in clinical development, including HKI-272, (Wyeth, Cambridge, MA, USA) [5] and AEE 788 (Novartis, Hanover, NJ, USA) [6]. Therefore, accurate determination of the HER-2/neu status is extremely important in guiding therapy, and the reliability of the diagnostic method used to determine HER-2/neu status is critical in selecting the most appropriate patients for HER-2/neu directed therapies.

The present review is focused on HER-2/neu testing, and so we address reports concerning the tissue tests used to determine HER-2/neu status as well as tests to quantitate circulating levels of the HER-2/neu ECD. Although there is approximately 80% to 85% accuracy in determining HER-2/neu status by tissue analysis, studies collectively show that the HER-2/neu status of the primary breast cancer (PBC) does not always accurately reflect the HER-2/neu status of the MBC. We highlight some of the factors that contribute to inaccurate assessment of HER-2/neu status by tissue testing, in the hope that this will improve assessment of HER-2/neu status. We also review reports on assays that have been used to quantitate circulating levels of the HER-2/neu ECD, and we present evidence that not all HER-2/neu assays have been adequately validated, which has resulted in inaccurate

ASCO = American Society of Clinical Oncology; BCIRG = Breast Cancer International Research Group; CAP = College of American Pathologists; CISH = chromogenic *in situ* hybridization; ECD = extracellular domain; ER = estrogen receptor; FDA = Food and Drug Administration; FISH = fluorescent *in situ* hybridization; IHC = immunohistochemistry; mAb = monoclonal antibody; MBC = metastatic breast cancer; NSCLC = non-small-cell lung carcinoma; PBC = primary breat cancer; TCH = docetaxel, platinum salt (cisplatin or carboplatin), and trastuzumab; TH = docetaxel and trastuzumab.

data and uncertainty in the value of measuring the ECD of the HER-2/neu oncoprotein. We describe studies that measure the HER-2/neu ECD in the serum of MBC patients and discuss evidence that increasing serum levels reflect tumor progression whereas decreasing levels reflect response to therapy or stable disease. We also review current thinking on the potential clinical utility of measuring circulating ECD in MBC and speculate on future applications.

Tissue methods used to determine HER-2/neu status

Reports published during the past several years have demonstrated that HER-2/neu tissue status can routinely be assessed using two methods: immunohistochemistry (IHC), which measures over-expression of the HER-2/neu full-length oncoprotein (p185); and fluorescent *in situ* hybridization (FISH), which measures the number of HER-2/neu gene copies [7]. A third tissue method (not yet approved by the FDA) that is growing in popularity is known as chromogenic *in situ* hybridization (CISH) [8]. CISH also measures gene amplification and has several technical advantages over FISH. All three tissue tests are performed on breast cancer tissue that has been formalin fixed and embedded in paraffin, and findings are reviewed by trained pathologists to determine HER-2/neu status.

There are two FDA-approved IHC tests for determining HER-2/neu status: Herceptest (DAKO, Carpeteria, CA, USA), which is a polyclonal antibody; and CB11 (Pathway, Ventana Medical Systems, Tucson, AZ, USA), which is a mAb. Most laboratories employing IHC generally use either Herceptest or CB11 to measure the level of p185 expression on breast cancer cells. To assess HER-2/neu status, a pathologist will determine the percentage of tumor cells that stain with a 3+ intensity. In National Comprehensive Cancer Network guidelines reported in 2006 by Carlson and coworkers [7], at least 10% of tumor cells must stain 3+ by IHC for a patient to be designated HER-2/neu positive. However, in a recent 2007 joint report from the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP), it was recommended that patients be considered HER-2/neu positive only if more than 30% of the tumor cells stain 3+ by IHC [9]. It is interesting to note, however, that HER-2/neu positivity can depend on the antibody used to assess HER-2/neu status. For instance, Fornier and coworkers [10] reported that 95% of patients evaluated using mAb CB11 were found to be 2+/3+, but in the same report they found that 84% of patients evaluated using the polyclonal Herceptest antibody were 2+/3+. Such variation in tissue results has important implications for patients with tumors that are potentially treatable with trastuzumab and chemotherapy.

The FISH method directly measures the number of HER-2/neu genes, and when there is an increase in the number of genes compared with normal it is referred to as 'gene amplification'. FISH testing results are semiquantitative and are based on the average ratio of HER-2/neu signals to CEP 17 signals in nonoverlapping interphase nuclei of the lesion. Three FISH tests are FDA-approved for selecting patients for treatment with trastuzumab. The Path Vysion (Vysis Inc., Downers Grove, IL, USA) test requires a ratio (HER-2/neu to CEP 17) of 2.0 or greater for the sample to be considered amplified. The INFORM test (Ventana Medical Systems) requires that at least 5.0 gene copies of HER-2/neu be present if a sample is to be considered amplified. The third HER-2/neu FISH test (DAKO) requires a ratio (HER-2/neu to CEN 17) of at least 2.0 for a sample to be considered amplified.

Although IHC and FISH methods are strongly correlated to histologic tissue, evaluation of tumor morphology by FISH is difficult. However, the limitations of FISH were recently improved upon by CISH. The latter technology visualizes the amplification product along with morphologic features using a peroxidase reaction, results can be viewed using a standard light microscope, and CISH is cheaper and can be stored as a permanent record. A majority of studies report concordance between FISH and CISH of 83% to 100%, which is leading to CISH gaining popularity in the research setting, but it is not yet FDA approved for selecting patients for trastuzumab therapy [7-9].

Variation in tests used to establish HER-2/neu status

Despite considerable efforts devoted to standardizing methods to determine HER-2/neu status by IHC or FISH, there are still several conditions that result in false-positive or false-negative results. Over the past few years numerous reports have been published identifying discrepancies between Herceptest and CB11, between IHC and FISH, and between FISH and CISH, as well as discrepancies between the various IHC and FISH methods from different manufacturers. Because all three tests use fixed tumor tissue, there are several sources of potential error. For instance, the HER-2/neu epitope can be destroyed by formalin fixation and many years of storage. In many cases the primary tumor is removed, formalin fixed, embedded in paraffin, and stored until the time of recurrence. Therefore, antigen loss may occur in up to 20% of HER-2/neu positive samples. Several other conditions can contribute to false-positive or false-negative IHC results, including tissue processing, reagent variability, antigen retrieval methods, scoring interpretation, tumor heterogeneity, and the semiquantitative nature of the test [7-12].

Discrepancies in HER-2/neu status can be laboratory dependent

In a 2006 report by Perez and coworkers [11], HER-2/neu status determined by IHC and FISH were compared between local pathology laboratories and central laboratories. A high degree of discordance was found to exist between HER-2/neu testing in the local laboratory and that in the central laboratory. However, in cases of discordance between local and central laboratories, there was a high degree of

agreement between the central laboratory and reference laboratories. These results support the importance of using high-volume experienced laboratories to determine HER-2/neu status. Similarly, Reddy and coworkers [12] concluded that use of high-volume HER-2/neu testing reference laboratories will improve the process of selecting patients who are likely to benefit from trastuzumab by accurate determination of HER-2/neu status.

Comparison of HER-2/neu status: primary tumor versus metastatic tumor

Currently, treatment of MBC patients with HER-2/neu positive tumors is based on HER-2/neu status derived from the primary tumor, which was generally removed many years previously and stored as paraffin-embedded blocks. In a 2005 report by Zidan and coworkers [13] it was pointed out that HER-2/neu status of the primary tumor may not accurately reflect the HER-2/neu status of the metastatic tumor, and that this should be taken into account when making treatment decisions. Those investigators demonstrated 14% discordance between primary and metastatic tumors by IHC. Twelve per cent (7/58) of the patients were HER-2/neu positive in the metastatic tumor yet negative in the corresponding primary tumor. Interestingly, three of the seven patients who were HER-2/neu negative in the primary tumor but positive by IHC in the metastatic tumor responded to trastuzumab-based therapy. The references list of that report directed us to several other papers comparing HER-2/neu status, as determined using IHC and FISH, between primary breast tumor and metastatic tumor from the same patient. Evidence supporting the observation that a primary breast tumor can be HER-2/neu negative while the metastatic tumor can positive is illustrated below with a few examples.

Edgerton and coworkers [14], employing IHC and FISH, reported 20% discordance between the primary and metastatic tumor, which was due to normal HER-2/neu expression in the primary tumor and HER-2/neu overexpression in the metastatic tumor. Gancberg and colleagues [15] compared HER-2/neu status of the primary breast tumor with that of at least one distant metastatic tumor in 107 patients using both IHC and FISH. There was a 6% (6/100) rate of discordance with IHC between the primary and metastatic tumor. In the six cases of discordance, there was greater HER-2/neu staining in the metastatic tumor tissue than in the primary tumor tissue. By FISH analysis, 7% (5/68) of the cases were discordant. Three of the five discordant patient specimens exhibited amplification in the metastatic tumor but not in the primary tumor. It was also reported that if all metastatic tumor sites were included in the analysis, then the HER-2/neu positive metastatic tumors with a corresponding negative primary tumor were more frequent than the converse, suggesting that HER-2/neu expression in primary tumors might represent an underestimation of reality. In another study [16], 80 paired primary tumors and metastatic tumors from the same patients were evaluated for HER-2/neu expression using tissue tests. A change from HER-2/neu negative status in the primary tumor to HER-2/neu positive status in the metastatic tumor occurred in 17% of cases. In a study conducted by Regitnig and coworkers [17] there was a 48% rate of discordance between primary and metastatic tumor when 31 paired samples were evaluated by both IHC and FISH. However, the 48% discordance rate included changes from 0 to 1+ or 0 to 2+ by IHC; in four cases was conversion to 3+ observed, yielding a discordance rate of 13.5%. In the 17 samples assessed by FISH, the discordance rate was 25%.

Collectively, these reports strongly support the contention that HER-2/neu status can be different between the primary and metastatic tumor from the same patient when they are evaluated using the standard tissue methods of IHC and FISH. They provide evidence that there exists a population of patients who are HER-2/neu negative in the primary tumor yet HER-2/neu positive in the metastatic tumor sample. Although this may be a small population of patients (in the 10% to 30% range), it is clearly of concern that these patients may not be eligible for trastuzumab therapy based on the initial tissue test. The overall message from Zidan and coworkers [13] is the recommendation that the discordance in tissue results be taken into consideration when making treatment decisions.

Guidelines to define HER-2/neu positive status

A recent report by Carlson and coworkers [7] presents the recommendations of a multidisciplinary panel of 24 experts on how to determine HER-2/neu status accurately. According to those recommendations, an IHC score of 0 or 1+, an average HER-2/neu gene/chromosome 17 ratio of less than 1.8, or an average number of HER-2/neu gene copies/cell of four or fewer (as determined by FISH) is considered to represent HER-2/neu negative status. An IHC score of 3+, an average HER-2/neu gene/chromosome 17 ratio of greater than 2.2 by FISH, or an average number of HER-2/neu gene copies/cell of six or more is considered to represent HER-2/neu positive status. A tumor with an IHC score of 2+ should be tested by FISH. Tumor samples with an average HER-2/neu gene/chromosome ratio of 1.8 to 2.2, or an average number of HER-2/neu gene copies/cell in the range from more than four to fewer than six are considered to be borderline.

However, a 2007 report published in the *Journal of Clinical Oncology* [9] provided guidelines from members of both ASCO and CAP on improving the accuracy of HER-2/neu testing in invasive breast cancer; the report also summarized the utility of HER-2/neu as a predictive marker. This publication also reported that approximately 20% of current HER-2/neu tissue testing results may be inaccurate. The authors concluded that when carefully validated tissue testing is performed, the available data do not clearly demonstrate the superiority of either IHC or *in situ* hybridization as a predictor of benefit from anti-HER-2/neu therapy. Overall, the panel recommended that HER-2/neu status be determined for all

invasive breast cancer patients and that a testing algorithm that relies on accurate, reproducible test performance, including newly available tests such as CISH, be considered.

In these more recent recommendations, a positive HER-2/neu result is defined as IHC staining of 3+ (uniform, membrane staining) in more than 30% of invasive tumor cells. A FISH result of more than six HER-2/neu gene copies per nucleus or a FISH ratio of HER-2/neu gene signals to chromosome 17 signals of more than 2.2 also indicate HER-2/neu positivity. A negative result is an IHC staining of 0 or 1+, or a FISH result of fewer than four HER-2/neu gene copies per nucleus, or FISH ratio of less than 1.8. Equivocal results require additional action to arrive at a final determination. It was recommended that, to perform HER-2/neu testing, laboratories should demonstrate 95% concordance with another validated test for positive and negative assay values. The panel strongly recommended validation of laboratory assay or modifications, use of standardized operating procedures, and compliance with new testing criteria, to be monitored with the use of stringent laboratory accreditation standards, proficiency testing, and competency assessment. The panel recommended that HER-2/neu testing be done in a laboratory accredited by the CAP or in a laboratory that meets the accreditation and proficiency testing requirements set out by the document [9].

Circulating levels of HER-2/neu extracellular domain in metastatic breast cancer patients

Many studies have established that the ECD of the p185 HER-2/neu is a p97-115kDa fragment that can be released from normal or cancer cells and can be reproducibly and accurately measured in serum using a test (for review, see Carney and coworkers [18]) referred to as serum HER-2/neu. The test was first cleared by the FDA in September 2000 on an automated platform (Immuno-1) and in the microtiter plate format (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). Studies have demonstrated a strong correlation between the automated and the manual HER-2/neu tests [19]. In 2003, the serum HER-2/neu test was also cleared for use on another automated platform, namely the ADVIA Centaur (Siemens Medical Solutions Diagnostics). The serum HER-2/neu test is currently available in both a manual and an automated platform (Siemens Medical Solutions Diagnostics) [20]. In addition to the test receiving FDA clearance, a set of high, medium, and low HER-2/neu controls to quality control the assay and the results were also cleared by the FDA. It should be noted that clearance by the FDA not only requires a considerable amount of validation conducted in clinical samples but it also requires documentation to prove reproducibility of samples as well as the reproducibility in the manufacture of multiple lots of reagents. This is often what is missing in homebrew assays and those for research use only.

The HER-2/neu test cleared by the FDA is intended to measure HER-2/neu ECD quantitatively in serum of women with MBC. The use of the HER-2/neu test is indicated for follow up and monitoring of patients with MBC whose initial serum HER-2/neu value is above 15 ng/ml. HER-2/neu values should be used in conjunction with information available from clinical and other diagnostic procedures in the management of MBC. The clinical utility of the serum measurement of HER-2/neu as a prognostic indicator for early recurrence and in the management of patients on immunotherapy regimens has not been fully established. A patient has an elevated serum HER-2/neu level if it is above 15 ng/ml. The upper limit of normal, defined as the lowest concentration that exceeds 95% of the values for normal healthy premenopausal and postmenopausal women, was determined as part of the data submitted to the FDA. The patient population included 121 serum samples from healthy premenopausal women and a total of 120 serum samples drawn from healthy postmenopausal women. In the population of 241 healthy women, 95% of the serum HER-2/neu values were found to be below 13.37 ng/ml. There was no significant difference in the HER-2/neu values between 121 premenopausal and 120 postmenopausal women. Based on this same population, the upper limit of normal was defined as the mean plus two standard deviations, which was 14.78 ng/ml, and 15 ng/ml was defined as the normal cutoff value. Any HER-2/neu values equal to or above 15 ng/ml are considered elevated.

Since the introduction of this test as an *in vitro* diagnostic in 2000, many studies have monitored serum HER-2/neu levels in serial samples of MBC patients; these show that increases and decreases in serum HER-2/neu levels correlate with the clinical course of disease [21-24]. For example, Cook and coworkers [21] monitored 103 MBC patients who received various regimens of hormonal or chemotherapy, and demonstrated that changes in serum HER-2/neu levels reflected changes in the clinical course of disease. There was an 88.6% concordance between increasing levels of serum HER-2/neu reflecting disease progression and decreasing levels reflecting therapy response or stable disease. Schippinger and colleagues [22] monitored serum HER-2/neu levels in approximately 3,000 serum samples from 286 MBC patients receiving either hormone therapy or chemotherapy, or both. They showed that changes in serum HER-2/neu levels paralleled the clinical course of disease. In addition, they found that those patients in whom serum HER-2/neu level was continuously elevated to above 15 ng/ml for the entire course of disease had significantly worse overall survival than did patients whose serum HER-2/neu levels were continuously below 15 ng/ml. As part of the FDA clearance procedure, it was found that a 20% difference from sample to sample was necessary for the manual microtiter format, whereas a 15% difference from point to point was necessary to obtain statistically significant data [19,21].

An earlier review of the literature [18], which included the vast majority of publications measuring HER-2/neu, showed

that 23% to 80% (mean 43%) of MBC patients had HER-2/neu levels above the control population included in each study. In contrast, 0% to 38% (mean 18.5%) of PBC patients had HER-2/neu levels above those of the corresponding control individuals. The wide range of serum HER-2/neu values reported may be due to the lack of enzyme-linked immunosorbent assay standardization that occurred in the early days of HER-2/neu testing and before the FDA test cleared 15 ng/ml as the cutpoint. Most of the early studies measuring HER-2/neu levels in control individuals and patients used research or homebrew assays without standardization or diagnostically approved methods of validation. More recent reports using the FDA cleared test for serum samples found a higher percentage of patients with elevated HER-2/neu levels. Potential reasons for this are described below.

Comparison of tests that measure the circulating HER-2/neu extracellular domain levels

It is important when measuring levels of any circulating marker with clinical importance that strict assay development guidelines be implemented and that the assay be well validated and characterized from a quality point of view. Before the decision by the FDA to clear the serum HER-2/neu test with a standardized cutpoint of 15 ng/ml, many reports used different research assays with various, uncharacterized antibody specificities, calibrators, and cutoff values to measure circulating HER-2/neu level. For instance, in a previous review [18] it was shown that there were 21 references to the Triton-Ciba-Chiron HER-2/neu assay but 11 different cutoffs, ranging from 5 to 30 units/ml or from 120 or 450 fmol/ml. We found five references to the Nicherei assay, three references to the Calbiochem or the Oncogene Research Products assay, three references to the Dianova assay, and two references to the Bender assay. We could not find any references that described antibody specificities or standardization of these assays, and neither were the findings compared with standardized results. All of the assays mentioned above were available for research use only, which means that the performance characteristics of the assay had not been determined. Unlike FDA-cleared *in vitro* diagnostic products, there are no manufacturing guidelines for research use only products, which often results in inconsistent findings.

The Bender assay was claimed to measure circulating soluble p185, but there has never been been a scientific report of a circulating full-length p185 and neither does the manufacturer present data to support the claim. It has clearly been shown that the circulating fragment is a p97-115kDa, and so it is unclear what the manufacturer meant by 'circulating soluble p185'.

In a study published in 1998 [25], using a homebrew HER-2/neu assay and mAb 4D5 as the capture reagent, it was reported that 259 out of 443 (58%) patient sera did not have measurable circulating HER-2/neu levels. In contrast, reports using the FDA-cleared serum HER-2/neu test reported that all individuals tested, both normal (male and female) and cancer patients, had some level of circulating serum HER-2/neu. In a 1999 report [26], using the same enzyme-linked immunosorbent assay based on mAb 4D5, baseline serum concentrations of the circulating HER-2/neu were below the detectable concentration in 73 out of 191 patients (38%). The article concluded that no significant correlation could be demonstrated between shed HER-2/neu concentrations and patient response status. However, the conclusions were based on data from a nonvalidated immunoassay with no standardization, and no references demonstrating the specificity of the research assay were presented. However, an explanation for these results can be derived from a report by Wong and Mass [27] presented at the ASCO 2000 annual meeting. In the report they compared the homebrew mAb 4D5 assay with the FDA-cleared test. The poster compared serum samples from the same MBC patients using both the mAb 4D5-based assay and the FDA-cleared assay. The authors concluded that the mAb 4D5 assay was not as sensitive as the FDA-cleared assay, which helps explain the results reported by the above-cited studies as well as other studies using the homebrew mAb 4D5 assay [25,26].

In a similar abstract published in 2004 at the annual ASCO meeting [28], Leyland-Jones and coworkers reported on a study in which they measured HER-2/neu levels in 366 cancer patients, again using the homebrew mAb 4D5 assay. Included was a combination of stage 2 and stage 3 breast cancer patients and patients with non-small-cell lung carcinoma (NSCLC). In the study it was concluded that no obvious relationship existed between baseline HER-2/neu levels and patient response, and in all cases the levels dropped with antiproliferative therapy. Of the 366 patients included in the 2004 poster, 103 patients were from the NSCLC study. Combining breast cancer and NSCLC with stage 2 and 3 cancer studies does not seem appropriate in light of the FDA cleared indication for patients with MBC. Despite the abstract and poster presented in 2000 by Wong and coworkers [27] showing that the 4D5 assay had less analytical sensitivity than the FDA-cleared assay, the authors of the 2004 ASCO poster used the homebrew 4D5 assay.

In addition, the FDA assay is cleared for monitoring patients with values above 15 ng/ml. In the 2004 poster by Leyland-Jones and coworkers [28], conclusions were also based on plotting values below 15 ng/ml. To date, these data have not been published in a peer-reviewed journal.

In summary the homebrew, mAb 4D5 assay is not indicated for use in NSCLC patients or for measuring serial values below 15 ng/ml. Given the nature of the mAb 4D5, being a homebrew assay, and therefore the potential for high coefficients of variation, it is questionable whether differences in values below 15 ng/ml are accurate or statistically

significant. Therefore, the findings and conclusions reported in the articles cited [25-27] that used a nonstandardized and unvalidated assay, which has been shown to be less sensitive than the FDA-cleared test, should be viewed with caution, especially in light of the numerous reports employing the FDA-cleared serum HER-2/neu assay and the reproducible finding of a 15 ng/ml cutoff.

Trastuzumab-treated metastatic breast cancer patients and serum HER-2/neu levels

Schondorf and coworkers [23] and Esteva and colleagues [24] evaluated patients with MBC who were treated with trastuzumab-based therapies (trastuzumab plus various combinations of chemotherapy); these patients were found to exhibit serial changes in serum HER-2/neu levels that correlated with clinical course of disease. In addition, reports by Köstler and coworkers [29] and Esteva and colleagues [30] illustrated that changes in serum HER-2/neu level paralleled the clinical course of disease, and in some cases changes in serum HER-2/neu levels preceded changes in clinical course of disease. Collectively, studies in which patients were treated with hormone therapy, chemotherapy, or trastuzumab/chemotherapy indicate that serial changes in serum HER-2/neu levels paralleled changes in the clinical course of disease [20-24].

The report by Köstler and coworkers [29] also demonstrated that a significant decrease in serum HER-2/neu level from the pretreatment baseline to 30 days after treatment was an early predictor of outcome with trastuzumab therapy. Subsequent reports from Esteva [30], Fornier [10], and Tse [31] and their colleagues supported the observation that a significant decrease in serum HER-2/neu from pretreatment level to 30 days after treatment was a predictor of outcome to trastuzumab therapy.

Because these earlier reports found different cutoff levels, Ali and coworkers [32] coordinated a multicenter/multinational study of 307 MBC patients that monitored changes in serum HER-2/neu levels during trastuzumab-based therapies. The data were presented at the 2006 San Antonio Breast Cancer Conference in abstract form. Serum HER-2/neu levels at baseline were compared with serum HER-2/neu levels from blood drawn at a median of 30 days after trastuzumab initiation. Patients who did not achieve a greater than 20% decline in serum HER-2/neu levels had a lower response rate, shorter duration of response, shorter time to progression, and decreased overall survival compared with those patients who did achieve a decline of more than 20%. A report by Kashiwaha and coworkers [33] presented as a poster at the 2006 San Antonio Breast Cancer Conference also supported that a decline in serum HER-2/neu level by 20% predicts outcome with trastuzumab-based therapies. However, the number of patients included in the study was not defined. These observations may be clinically useful in stratifying patients with a lower probability of optimal response to trastuzumab and chemotherapy, and therefore these findings may be exploited to identify candidates for additional HER-2/neu-directed therapies such as the tyrosine kinase inhibitors [4-6].

Monitoring metastatic breast cancer patients receiving lapatinib

Lapatinib (Tykerb, GSK, Philadelphia, PA, USA) is an oral dual tyrosine kinase inhibitor that targets HER-2/neu and epidermal growth factor receptor positive tumors in MBC. It was recently approved by the FDA for use in combination with capecitabine for the treatment of patients with advanced breast cancer or MBC whose tumors over-express HER-2 and who have received prior therapy with an anthracycline, a taxane, and trastuzumab.

At the 2004 ASCO meeting, Blackwell and coworkers [34] reported that a decrease in serum HER-2/neu levels at 4 and 8 weeks after initiation of lapatinib correlated with response to therapy and was an early indicator of clinical response. Additional studies are currently being evaluated to confirm these findings.

Serum HER-2/neu levels and clinical outcome

Some studies have suggested that clinical outcomes can be improved by maintaining serum HER-2/neu levels below 15 ng/ml. For instance, Schippinger and coworkers [22] reported results in 286 MBC patients; they found that those individuals with continuously elevated (>15 ng/ml) serum HER-2/neu levels had a significantly poorer survival after disease recurrence than did patients with HER-2/neu levels continuously or temporarily below 15 ng/ml. A decrease in elevated serum HER-2/neu levels to under 15 ng/ml or levels continuously below 15 ng/ml during the course of disease correlated with significantly longer survival. In a 2005 report by Lipton and coworkers [35], it was shown that an increase in serum HER-2/neu level to above 15 ng/ml at the time of disease progression on first-line hormone therapy correlated with significantly shortened survival. The median survival from initiation of therapy was 47.8 months for patients whose serum HER-2/neu level was continuously below 15 ng/ml. In those patients who exhibited an increase in serum HER-2/neu at the time of disease progression, the median survival was 26.5 months. The survival of patients in whom serum HER-2/neu level changed from under 15 ng/ml to greater than 15 ng/ml did not differ significantly from the survival of patients whose serum HER-2/neu level was above 15 ng/ml from the start of therapy (median 20.8 months). Survival from the time of disease progression was longest (28.1 months) for those patients whose serum HER-2/neu level remained under 15 ng/ml, and it was shortest (12.5 months) for those patients whose serum HER-2/neu level remained elevated to above 15 ng/ml. However, patients whose serum HER-2/neu level changed from below 15 ng/ml to above 15 ng/ml had a median survival of 15.2 months.

In the multicenter study conducted by Ali and coworkers [32], it was found that patients who had a serum HER-2/neu level continuously below 15 ng/ml had an overall survival of more than 1,000 days, whereas those patients whose serum HER-2/neu level was continuously above 15 ng/ml had an overall survival of only 618 days. In patients who started therapy with a serum HER-2/neu level above 15 ng/ml but which declined to below 15 ng/ml during therapy, the overall survival was in excess of 1,000 days and similar to that in the patients whose serum HER-2/neu level remained below 15 ng/ml.

Pretreatment serum HER-2/neu levels associated with poor response to therapies

In 1989 Wright and coworkers [36] reported that HER-2/neu over-expression, based on IHC, was associated with a reduced rate of response to first-line hormone therapy in MBC patients. Since then, several studies [37-39] have demonstrated that elevated pretreatment serum HER-2/neu levels are associated with a significantly lower rate of response to endocrine therapy. In a first-line hormone therapy trial reported by Lipton and coworkers [39], MBC patients with elevated pretreatment serum HER-2/neu levels had a shorter duration of response, shorter time to progression, and shorter overall survival than did patients with serum HER-2/neu levels below 15 ng/ml. Sandri and coworkers [40] reported that increased pretreatment serum HER-2/neu identified those patients with more aggressive tumors and lesser response to chemotherapy. In addition, Colomer and colleagues [41] found that pretreatment serum HER-2/neu levels correlated significantly with rate of response to chemotherapy. Patients with pretreatment levels higher than normal were less responsive than were patients with serum HER-2/neu less than normal. Several other reports (for review, see Carney and coworkers [18]) have shown that elevated pretreatment serum HER-2/neu levels are associated with poor clinical outcomes.

Serum HER-2/neu levels in metastatic breast cancer and HER-2/neu positivity by tissue tests

In several reports, HER-2/neu tissue positivity of the primary tumor was compared with serum HER-2/neu levels in the same patients with MBC. In a 2001 study reported by Harris and coworkers [42], strong concordance was identified between IHC/FISH results for the primary tumor and elevated serum levels in the same patients with MBC. Similar findings were reported by Tse and coworkers [43], Mouller and colleagues [44], and, more recently, by Kong and coworkers [45]. The latter group found a strong correlation between serum HER-2/neu levels in patients with MBC and the IHC and FISH findings for the primary tumor. They showed that the HER-2/neu tissue negative group had a median serum HER-2/neu level of 22.2 ng/ml, whereas the tissue positive group had a mean serum HER-2/neu level of 363 ng/ml. In one study [44], some patients who were HER-2/neu negative by tissue tests had very high serum HER-2/neu levels with the emergence of MBC.

In a multicenter, phase III randomized trial conducted by the Breast Cancer International Research Group (BCIRG; study 007) [46], docetaxel and trastuzumab (TH) was compared with docetaxel, platinum salt (cisplatin or carboplatin), and trastuzumab (TCH) as first-line chemotherapy in women with MBC. Women enrolled in the study were required to have primary breast tumors with HER-2/neu amplification, as determined by centralized FISH analysis. At the time of recurrence, a baseline serum HER-2/neu level was measured. The goal was to determine the percentage of patients with HER-2/neu amplification who had elevated (>15 ng/ml) baseline serum HER-2/neu before initiation of trastuzumab-based therapy. Baseline was considered to be the last available determination within 21 days before first treatment. The median baseline serum HER-2/neu level for all patients at the time of recurrence ($n = 123$) was 75.8 ng/ml (range 8 to 3,280 ng/ml), with no statistically significant difference between patients randomly assigned to receive TH ($n = 64$; median 65.9 ng/ml) and those randomized to receive TCH ($n = 59$; median 89.9 ng/ml). Overall, 89.5% of the 123 patients with HER-2/neu amplified primary tumors had serum HER-2/neu levels above 15 ng/ml at the time of metastatic disease (86% in TH versus 92% in TCH).

Many previous reports have identified an approximately 80% to 90% correlation between tissue HER-2/neu positivity and elevated serum HER-2/neu levels at the time of MBC. However, not all reports are in agreement. For instance, Hoopmann and coworkers [47] reported 40% of 20 HER-2/neu 3+ patients had elevated serum HER-2/neu. Also, Bethune-Volters and colleagues [48] found that only 55% of the 33 patients who were IHC 3+ or FISH amplified had elevated serum HER-2/neu levels. However, based on previously published data and the controlled 007 BCIRG study [46], patients with HER-2/neu tissue positivity in the primary tumor might be excellent candidates for monitoring for increasing serum HER-2/neu levels after surgery as a means of detecting early breast cancer recurrence. However, such an indication has not been cleared by the FDA.

Frequency of elevated serum HER-2/neu levels in breast cancer patients

To appreciate the frequency with which elevated serum HER-2/neu levels are identified in patients with breast cancer, we reviewed papers that reported serum HER-2/neu levels in patients that were above control levels. In the 2004 report published in *Clinical Chemistry* [18] a total of 60 studies, representing 7,639 breast cancer patients, were reviewed. In the 25 studies representing 2,622 patients with PBC, approximately 18.1% had elevated serum HER-2/neu levels. However, it should be noted that these figures were derived from all HER-2/neu reports, regardless of whether the assay was validated. However, Manouni and coworkers [49] recently demonstrated a 28.2% incidence of elevated serum HER-2 in patients with PBC receiving neoadjuvant therapy. In contrast, however, one report [50] found that only 6% of 128

PBC patients had an elevated serum HER-2/neu level. It is possible that PBC patients with elevated serum HER-2/neu levels have more extensive disease than understood.

In 50 publications representing 5,044 patients with MBC, we found (on average) that 48.6% (range 23% to 80%) had elevated serum HER-2/neu levels. In more than 40% of the reports, it was found that 50% or more of the MBC patients had elevated serum HER-2/neu levels [16]. Several recent papers [18,51-53] reported that 50% to 60% of MBC patients had an elevated serum HER-2/neu. This difference may have several explanations, such as differing patient populations or wider use of the standardized FDA cleared serum HER-2/neu test. It also may be that some reports employ hormone receptor-positive patients only whereas others include mixed populations. These data are in sharp contrast to the many publications indicating that only 20% to 30% of patients with PBC have HER-2/neu over-expression by IHC or FISH amplification. It also raises the possibility that HER-2/neu status can change, by whatever mechanism, from PBC to the MBC setting.

Comparison of HER-2/neu status in primary breast tumor and serum HER-2/neu level in metastatic breast carcinoma

In a search of the literature, we also found numerous publications that demonstrated the existence of a population of women with PBC who were designated HER-2/neu negative by tissue testing but who developed serum HER-2/neu levels above 15 ng/ml in MBC (for review, see the report by Carney and coworkers [18]). For example, Andersen and coworkers [54] found that 28 of the 82 patients (34%) who had IHC negative PBC developed elevated serum HER-2/neu levels during MBC. Fehm and colleagues [53] also found that 34% of patients designated HER-2/neu negative by tissue testing had elevated serum HER-2/neu levels at the time of MBC.

The observation that a population of patients with a HER-2/neu negative primary tumor can develop elevated serum levels at MBC is consistent with the report by Zidan and coworkers [13]. Those investigators demonstrated the presence of a population of patients in whom HER-2/neu status was negative in the primary tumor but with HER-2/neu tissue positivity in the metastatic tumor. This also raises the question of whether circulating serum HER-2/neu can be used as a surrogate marker for the presence of a HER-2/neu positive tumor.

HER-2/neu is more than just another tumor marker

Several reports suggest that serum HER-2/neu levels are associated with tumor burden, HER-2/neu over-expression, and receptor activation. Molina and coworkers [55] showed that cleavage of the ECD leads to increased phosphorylation of the intracellular tyrosine kinase. This observation suggests that circulating HER-2/neu level not only is a marker of tumor over-expression but also is indicative of the degree of receptor activation.

Ali and coworkers [56] and Fehm and colleagues [53] examined the question of whether serum HER-2/neu reflects tumor aggressiveness or is simply a surrogate marker of disease bulk. In the report by Ali and coworkers [56] pre-treatment samples from 566 MBC patients were tested for serum HER-2/neu levels as well as for levels of CA15-3, a surrogate marker of tumor burden. They concluded that serum HER-2/neu is a significant independent predictive and prognostic factor in hormone receptor positive MBC patients, even when adjusted for tumor burden using CA15-3. In addition, Fehm and colleagues [53], using a multivariate analysis, also concluded that when serum HER-2/neu results were adjusted for tumor load with CA15-3, serum HER-2/neu remained an independent marker of tumor aggressiveness and reflected the biologic behavior of the tumor. Several other studies have also concluded that the combination of serum HER-2/neu and CA15-3 identifies a subset of patients with a worse prognosis when compared with one marker alone. However, it has also been reported on several occasions that there is a weak correlation between serum HER-2/neu and CA 15-3, but each provides important information about the biology of the tumor.

Monitoring serum HER-2/neu for detection of early recurrence

Several reports have shown that monitoring serum HER-2/neu levels after surgery in patients with HER-2/neu positive tissue may be beneficial in that it may permit earlier detection of breast cancer recurrence. In a report by Pichon and coworkers [57], serum HER-2/neu levels were measured before first metastasis in 128 out of 701 breast cancer patients, and 45% of these women had elevated serum HER-2/neu. In recurrent breast cancer, elevated serum HER-2/neu levels may permit early detection of occult metastasis and identification of patients with a high probability of shortened survival. This study also showed that elevated serum HER-2/neu levels before first metastasis were indicative of aggressive disease.

Fehm and colleagues [53] found that 27% of breast cancer patients with recurrence had elevated serum HER-2/neu levels 6 months before clinical signs or clinical diagnosis. This percentage increased to 50% of the patients who had elevated serum HER-2/neu levels at 3 months before clinical diagnosis. Isola and coworkers [58] reported that 37% of patients could be predicted to have recurrent breast cancer on the basis of increasing serum HER-2/neu levels 6 months before the actual clinical diagnosis.

Overall, a search of the literature found approximately 12 publications that reported an increase in serum HER-2/neu levels that could be measured 2 to 9 months before the actual clinical diagnosis of recurrent breast cancer [59].

Monitoring serum HER-2/neu levels in all HER-2/neu tissue positive patients might be a way to detect breast cancer recurrence earlier.

Conclusion

HER-2/neu status is important information that is needed to guide therapy in patients with HER-2/neu positive breast cancer; therefore, accurate determination of HER-2/neu status by IHC or FISH is essential to providing appropriate treatment. Many studies now demonstrate that the HER-2/neu status of the primary tumor may not accurately reflect the HER-2/neu status of patients receiving trastuzumab therapy in either adjuvant or metastatic setting. Therefore, one potential clinical use for the serum HER-2/neu test is to identify patients with an elevated serum HER-2/neu test but a negative HER-2/neu tissue test. This could be a reason to re-evaluate the original tumor or a metastatic tumor by IHC and FISH for HER-2/neu positivity. Serum HER-2/neu is a test that should complement tissue testing, but it should not be used in place of IHC or FISH. However, use of the serum HER-2/neu test is not approved for selecting patients for trastuzumab therapy. No clinical studies have been performed to determine how patients with elevated serum levels will respond to trastuzumab.

Because most IHC and FISH studies report 20% to 30% HER-2/neu positivity in PBC, and several studies now show a 20% false-negative rate in primary breast tumors, a 50% proportion of MBC patients with elevated serum HER-2/neu is certainly a possibility. It is also possible, in light of the BCIRG 007 data [46], that recent reports in which the FDA-cleared assay indicated that 50% to 60% of MBC patients can have elevated serum levels are real. Variations in this percentage observed over the years has been confused by the use of nonstandardized assays as well as studies that included patients with stage 2 or 3 breast cancer, such as the study conducted by Leyland-Jones and coworkers [28].

The most probable reason for a patient to be found to be HER-2/neu negative, however, is that the patient's tumor has less than the 10% 3+ positive cells required to be designated HER-2/neu positive. However, it is also likely that there are enough HER-2/neu positive cells in the primary tumor (regardless of cutoff) to generate a HER-2/neu positive metastatic breast cancer. Whatever the percentage of HER-2/neu positive cells is in the primary breast tumor, they could be the tumor cells with the biologic growth advantage and those most likely to migrate to distant sites and create metastatic tumors. However, based on recent recommendations by ASCO and CAP that more than 30% of cells be HER-2/neu 3+ by IHC for a patient to be considered HER-2/neu positive, this should decrease of the rate of false-negative tissue results.

Several studies conducted during the past few years also support that as many as 50% to 60% of unselected MBC patients can have elevated serum HER-2/neu levels. However, the frequency of serum HER-2/neu elevation is higher in MBC patients selected for primary tumor HER-2/neu positivity, as recently demonstrated by the BCIRG 007 study [46]. That study showed that 89.5% of MBC patients identified as HER-2/neu positive in the primary tumor by FISH amplification had elevated serum HER-2/neu levels (>15 ng/ml).

It is also possible that serum HER-2/neu testing could have clinical value in managing patients who specifically have HER-2/neu positive tumors. Studies are starting to emerge that show that the patients with the better clinical outcomes have HER-2/neu levels that are continuously below 15 ng/ml [22,32,34]. In trastuzumab-treated patients, studies also show that a drop of less than 20% in serum HER-2/neu level from pretreatment to post-treatment is associated with poor clinical outcome, as compared with those patients with a drop greater than 20%. Those patients who do not achieve a 20% decline might benefit from additional HER-2/neu inhibitors that are currently in clinical development. Clearly, these observations are exciting, and additional studies are needed to determine whether serum HER-2/neu can have clinical value in HER-2/neu positive patients and in patients receiving HER-2/neu directed therapies such as Tykerb.

With the emergence of many targeted therapies, it will be increasingly important that new mechanism-based circulating biomarkers, such as HER-2/neu, epidermal growth factor receptor, and vascular endothelial growth factor, be used in the metastatic cancer setting to monitor the efficacy of the targeted therapies and to select patients for targeted therapies. The goal of new diagnostic tests should be to use mechanism-based biomarkers, such as serum HER-2/neu, to routinely monitor patients from the time of first diagnosis and tumor removal in order to detect early breast cancer recurrence, with the intention being to institute therapeutic intervention earlier and hopefully to increase overall survival.

Competing interests

WC and RN are employees of Siemens Medical Solutions DX. AL and KL are employees of Penn State/Hershey Medical Center, Hershey Pa. SA is an employee of Lebanon Veterans Association Medical Center and Hershey Medical Center. SA and KL have received speking honoraria from Siemens and reimbursement for travel to scientific conferences. WC, RN, SA and AL do not own stock in Siemens.

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Other articles in the series can be found online at http://breast-cancer-research.com/articles/review-series.asp?series=BCR_HER2

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