Review

Serum HER-2/neu in the management of breast cancer patients

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Abstract

The clinical role of HER-2/neu, a 185 kD epithelial transmembranous protein, has evolved after the approval of the anti-HER-2/neu targeted monoclonal antibody trastuzumab (Herceptin) for the therapy of metastatic breast cancer. The extracellular domain of HER-2/neu undergoes proteolytic cleavage from the full-length protein by metalloproteases, and is shed into the blood as a circulating antigen. While HER-2/neu gene amplification and/or protein overexpression are detected in approximately 25% of primary breast cancers, serum HER-2/neu levels are elevated beyond the upper limit of normal in 50 to 60% of stage IV breast cancer patients. HER-2/neu in serum can be detected by enzyme immunoassays (manual and automated versions). It has been shown to have prognostic and predictive information in breast cancer patients. Monitoring for recurrence by serum HER-2/neu reaches a high sensitivity for HER-2/neu positive tumors. Longitudinal follow-up of patients during any kind of systemic therapy allows for monitoring of the therapeutic success. When utilized in these applications, serum HER-2/neu testing is complementary to HER-2/neu tissue results and to the determination of classical tumor markers such as CA 15–3, CA 27.29 and CEA, which are not targeted by specific forms of systemic therapy. © 2003 The Canadian Society of Clinical Chemists. All rights reserved.

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1. Introduction

The clinical role of HER-2/neu (c-erbB-2) has evolved in recent years, especially after the approval of the anti-HER-2/neu targeted monoclonal antibody trastuzumab (Herceptin®, Genentech® Inc., South San Francisco, CA) for the treatment of advanced breast cancer. Since the determination of the HER-2/neu status is the basis of patient selection for Herceptin therapy, its laboratory evaluation must be highly reliable, valid and reproducible. Serum HER-2/neu is a circulating oncoprotein, which is cleaved from the full-length, membrane-bound HER-2/neu protein. It can be detected in the serum of healthy individuals as well as in patients with various solid tumors. This review will summarize the role of HER-2/neu in the biology of breast cancer, the determination of the HER-2/neu status and the clinical value of testing for serum HER-2/neu. Furthermore, we suggest an algorithm for the use of serum HER-2/neu over the disease course of breast cancer from locally confined disease to metastatic spread, with patient stratification based on the HER-2/neu status of the primary tumor.

2. Structure and biology of HER-2/neu

HER-2/neu is a 185 kD glycoprotein normally expressed in the epithelia of numerous organs such as lungs, bladder, pancreas, breast, and prostate [1–3]. Overexpression and/or overamplification of HER-2/neu in epithelial tumors leads to a strong increase in the density of HER-2/neu in the cell membranes [4]. HER-2/neu is a member of the epidermal growth factor receptor (EGFR) family, which is comprised of 4 family members, designated HER-1 (synonym: EGFR) through HER-4. The HER-2/neu receptor protein has three domains: an intracellular tyrosine kinase portion, a hydrophobic transmembrane domain, and an extracellular domain (ECD), which is the ligand-binding portion of the receptor [5–7]. The ECD undergoes proteolytic cleavage from the full-length protein by metalloproteases, and is shed into the blood as a circulating antigen [8]. The HER-2/neu ECD, a
glycoprotein of 97 to 115 kD, is sometimes referred to as p105, but is generally known as ‘serum HER-2/neu’.

HER-2/neu gene amplification and/or protein overexpression are detected in approximately 25 to 30% of primary breast cancers [9]. HER-2/neu positive tumors tend to be steroid hormone receptor negative, have a positive lymph node status, a high grading score and S-phase fraction. These tendencies provide for a higher probability of relapse and treatment-resistant disease. Increased aggressiveness, metastatic potential and therapeutic resistance of HER-2/neu positive tumors may also depend on the shedding process [10,11]. When the HER-2/neu ECD is cleaved, the truncated intracellular tyrosine kinase retains its signaling ability [12,13]. Thus, serum HER-2/neu levels may reflect the activation-state associated with the shedding process. In addition, elevated serum HER-2/neu levels reflect the HER-2/neu expression status of the tumor as well as tumor burden. Molina et al. hypothesize that the inhibitory effect on HER-2/neu shedding induced by Herceptin may be one mechanism of action and that it is probably a result of steric hindrance [11].

3. Tissue testing for HER-2/neu

3.1. Immunohistochemistry

In the Herceptin pivotal trials, immunohistochemical analysis of tumor material was used to assess the HER-2/neu status. Since then, immunohistochemistry (IHC) has been considered as the reference method, or gold standard, in HER-2/neu testing [14]. IHC detects the degree of HER-2/neu protein overexpression in paraffin-embedded tissue samples by using mono- or polyclonal antibodies, which bind to the HER-2/neu expressed on the cell membranes. The resultant antigen-antibody complexes are visualized due to conjugation of detector antibodies to peroxidase or some other chromogenic substance. IHC offers semiquantitative, graded scores, ranging from 0 to 3+. Scores of 2+ or 3+ are considered to be HER-2/neu positive, i.e., more than 10% of the malignant cells demonstrate at least moderate staining of the complete cell membrane. However, IHC results may be compromised due to the loss of antigenic determinants during the tissue fixation process. IHC results are also subject to variance in antibody reactivity and to inter-observer interpretation as demonstrated in one recent account where variances ranging from 26% to 42% were observed [15].

3.2. Fluorescence-in situ-hybridization

Fluorescence-in situ-hybridization (FISH) measures the number of HER-2/neu gene copies per nucleus, i.e., DNA amplification. To employ FISH, a specific DNA probe consisting of a fluorescein-labeled oligonucleotide is allowed to anneal to complementary HER-2/neu nucleic acid sequences. Since FISH detects the exact number of hybridization signals per nucleus, and since DNA is less susceptible to preparation procedures than epithelial proteins, FISH is often considered more objective than IHC [15]. However, FISH is costly, more time-consuming and more technically complex than IHC. Furthermore, it is often too labor-intensive for many hospital pathology departments.

While the IHC and FISH methods can be done on paraffin-embedded tissue using standardized and FDA-approved techniques and kits, the mRNA determination has only been used in research settings using single-center based, nonstandardized assays. A major reason for this development must be seen in the relatively low stability of mRNA and the preferential use of fresh frozen tissue which is generally not available in the standard clinical setting.

4. Serum Testing for HER-2/neu

Both HER-2/neu gene amplification and protein overexpression can be evaluated in tumor tissue. However, circulating levels of serum HER-2/neu can also be used to evaluate the HER-2/neu status (Figure 1). Since HER-2/neu bearing epithelial cells shed the extracellular domain (ECD) into the serum, serum HER-2/neu levels can be detected by enzyme-linked immunosorbent assays (ELISA). Currently, two FDA-approved ELISA assays are available that measure the concentration of circulating HER-2/neu: a manual microtiter plate assay (Oncogene® Science/Bayer Corporation, Cambridge, MA, USA) or an automated version on the Bayer Immuno1® platform (Bayer Diagnostics, Tarrytown, USA). Both assays utilize the same two monoclonal antibodies that are directed against different epitopes of the HER-2/neu ECD.
4.1. Manual serum HER-2/neu testing

In the manual microtiter plate HER-2/neu ELISA assay, two monoclonal anti-HER-2/neu antibodies, designated NB-3 and TA-1, which specifically bind to independent binding sites on the HER-2/neu ECD, are utilized. The NB-3 mouse monoclonal antibody is immobilized on the interior surface of the microtiter plate wells and TA-1 biotinylated mouse monoclonal antibody is used for detection of HER-2/neu ECD [16]. The upper limit of normal for serum HER-2/neu has been independently established at 15 ng/mL for the microtiter plate ELISA kit as well as for the automated assay described below [17]. The microtiter plate ELISA has a lower detection limit of 3.4 ng/mL and an upper linear dilution limit of 36 ng/mL.

4.2. Automated serum HER-2/neu testing

The automated Bayer Immuno1®HER-2/neu assay is a magnetic particle separation immunoassay that uses the same monoclonal antibodies as the manual assay described above. The assay is linear from 0.1 to 250 ng/mL and there is no hook effect up to 10,000 ng/mL. Maximum within-run imprecision is less than 2.0% [18].

Serum HER-2/neu testing does not show any significant cross-reactivity with other members of the EGF receptor family (< 0.6%). There is no interference from human antismouse antibodies, heterophilic antibodies or rheumatoid factor. Most importantly, there is no interference between circulating levels of HER-2/neu and the therapeutic monoclonal antibody, Herceptin. This may be explained by the finding that the monoclonal antibodies utilized in the assay and the therapeutic monoclonal antibody recognize different and nonoverlapping binding sites of the HER-2/neu ECD molecule. Serum HER-2/neu levels are not correlated with race (white vs. nonwhite), but do slightly increase in postmenopausal women [19,20]. However, this increase does not compromise the use of the upper limit of normal of 15 ng/mL for both pre- and postmenopausal women. High serum HER-2/neu levels correlate with advanced or late stage disease. In breast cancer, the sensitivity of the serum HER-2/neu assay increases with stage of disease, from 0% for stage I up to 40 to 50% for stage IV disease [17]. The specificity for healthy women and women with benign breast disease is between 97% and 98%. This is an expected finding since HER-2/neu is an oncogene, and would not be expected to be nonspecifically elevated in the manner of other mass tumor markers.

Serum HER-2/neu testing provides some important benefits. The manual and automated ELISA testing methods are standardized, routine methodologies and are not subject to variable interpretation of a reviewer. The ELISA results are quantitative allowing for the formation of biologic subgroups within the subsets of patients who were identified as being HER-2/neu positive by semiquantitative tissue testing. The method is noninvasive and allows for repeat testing and monitoring of breast cancer patients undergoing all forms of systemic therapy. The most important advantage, however, is the ability to conduct ‘real-time’ testing. This means that the patient’s HER-2/neu status can be evaluated precisely at the time of therapeutic decision-making. This is especially relevant for those patients who are being considered for anti-HER-2/neu-targeted treatment options and for whom decisions are currently made based solely on the status of tumor tissue obtained historically.

5. Clinical role of serum HER-2/neu testing

The specific clinical benefits of serum HER-2/neu testing are being clarified by numerous investigations ongoing worldwide. Several studies indicate a role for forecasting of disease-free and overall survival (i.e., prognostication). Other trials, specifically in metastatic breast cancer populations, compare patient subgroups with elevated baseline serum HER-2/neu levels vs. the subgroup with a normal serum HER-2/neu baseline concentration for predicting response to treatment (i.e., a predictive role).

5.1. Prognosis

In a number of retrospective studies that included significant numbers of patients, serum HER-2/neu levels have demonstrated prognostic significance with respect to disease-free survival (DFS) and overall survival (OS). Mehta et al. reported that elevated prechemotherapeutic serum HER-2/neu levels were prognostic of a shorter disease-free survival in a population (n = 79) of stage II and III patients undergoing standard regimens of chemotherapy [21]. Nodal-positive patients having serum HER-2/neu levels greater than 100 fmol/mL (the defined upper limit of normal for a noncommercial ‘research use only’ serum HER-2/neu test) had a significantly shorter disease-free survival (24 months) than those patients with 100 fmol/mL or less (78 months). In addition, the postchemotherapy HER-2/neu level was the second most significant prognostic indicator for disease-free survival with only the patient’s nodal status being more significant. Moreover, serum HER-2/neu levels increased and were positively correlated with the number of positive lymph nodes.

In a separate retrospective study involving patients with localized primary breast cancer (n = 700), elevated serum HER-2/neu levels (> 13 ng/mL) were also prognostic of a significantly shorter DFS with a p-value of 0.003 [22]. Consistent with the results as demonstrated by Mehta et al., elevated pretreatment serum HER-2/neu levels were associated with larger tumor size, positive lymph nodes and high grading score. It should be noted that in this study only a small percentage (6.7%) of primary breast cancer patients showed elevated serum HER-2/neu levels before initiation of adjuvant therapy, reflecting the correlation of HER-2/neu levels and overall tumor load.
In a third study, elevated pretreatment serum HER-2/neu levels (≥ 10.5 ng/mL) were shown to be significantly associated with a shorter overall survival in 242 patients with metastatic breast cancer [23]. In a univariate subset analysis, elevated pretreatment serum HER-2/neu levels were associated with a worse overall survival when compared with patients with normal levels of serum HER-2/neu (non-elevated levels) among those patients who were treated with endocrine therapy (megestrol acetate; n = 103), underlining the prognostic and predictive information which can be taken from the serum HER-2/neu status. This analysis did not hold up for those patients treated with chemotherapy (mainly anthracycline-containing regimens; n = 139) or when analyzed by multivariate analysis. Furthermore, in contrast to other studies, the rates of response to either endocrine or chemotherapy were similar regardless of serum HER-2/neu levels in patients with metastatic disease.

In summary, elevated serum HER-2/neu levels in breast cancer patients following primary surgery as well as immediately after the completion of adjuvant chemotherapy provide prognostic information. These results suggest that those patients exhibiting elevated levels of serum HER-2/neu might benefit from specific anti-HER-2/neu-target treatment options early in the course of their disease, i.e., in the adjuvant setting. The studies that have been described in this review are primarily retrospective, and, thus, will require confirmation through prospective trials which are, in fact, currently being performed by large American and European cooperative groups.

5.2. Monitoring for recurrence

The determination of serum HER-2/neu levels for monitoring for recurrence in patients with breast cancer was suggested by several authors using various types of enzyme immunometric assays [24,25]. Isola et al. demonstrated that elevated serum HER-2/neu levels predicted the appearance of metastases at least 6 months before clinical observation in 37% of all observed breast cancer patients [26]. However, there are relatively few data available regarding the sensitivity for the detection of recurrence in relation to the HER-2/neu tissue status of the primary tumor. Molina et al. evaluated the use of serum HER-2/neu as a circulating oncoprotein along with the classical tumor markers, CA15-3 and CEA, for the diagnosis of recurrence of disease in primary breast cancer patients [27,28]. Thirty-eight percent of the patients (95/250) included in this study developed metastases during the period of 1 to 4 yr of follow-up. An elevated level of at least one of these biologic markers was the first sign of recurrence in 69.5% of patients. The combined sensitivity, as well as the individual sensitivity for serum HER-2/neu, was greater for metastases than for local–regional relapse. Exclusion of the local–regional recurrences increased the sensitivity to 76.4%. An elevation of serum HER-2/neu levels was the first sign of recurrence in 28.3% of patients with recurrence. However, when taking into consideration the HER-2/neu tissue status of the primary tumor (i.e., when evaluating only those patients who were considered to be HER-2/neu positive by the tissue test), the sensitivity of the serum HER-2/neu test for the detection of recurrence increased to 83.3%. Considering these findings and the current recommendations for the prospective determination of the HER-2/neu status in all newly diagnosed breast cancer patients, serum HER-2/neu testing in HER-2/neu positive tumors could change diagnostic standards for monitoring for recurrence of breast cancer patients. This may be especially relevant as anti-HER-2/neu targeted treatment options might become available for adjuvant therapy in the near future.

5.3. Prediction

In several phase II- and phase III-studies, cohorts of metastatic breast cancer patients were subdivided into groups to compare patients with increased levels vs. normal levels of serum HER-2/neu before treatment initiation (baseline determination). In a multicenter, phase II trial of first-line paclitaxel and doxorubicin combination therapy for metastatic breast cancer (n = 58), Colomer et al. concluded that serum HER-2/neu was a significant predictor of treatment efficacy independent of other clinical variables [29]. Elevated serum HER-2/neu levels (>450 fmol/mL) correlated with a significantly lower probability of complete response (0% vs. 26%; p < 0.021) and a significantly shorter duration of response (7.5 months vs. 11 months; p = 0.035).

Our own group found similar results. In a study on metastatic breast cancer patients receiving weekly fractionated paclitaxel as 2nd- or 3rd-line therapy, no significant difference in response rate was found when comparing patients with elevated (>15 ng/mL) and nonelevated levels of serum HER-2/neu. This lack of difference in response rate between patients with elevated and nonelevated serum HER-2/neu levels might reflect some sensitivity of HER-2/neu positive breast tumors to paclitaxel therapy. As previously demonstrated by Colomer et al., we found that elevated serum HER-2/neu values were correlated with a significantly shorter (25.7 weeks vs. 65.2 weeks; p = 0.042) duration of response with a trend toward shorter progression-free survival (31.2 weeks vs. 53.2 weeks; p = 0.098). This trend prompted further statistical evaluation. When using an arbitrary cut-off of 22 ng/mL rather than 15 ng/mL, a statistically significant shorter progression-free survival for patients with serum HER-2/neu values ≥ 22 ng/mL was identified. The higher the hypothetical, biologic cut-off was chosen, the better the level of significance became (p = 0.0089 for a serum HER-2/neu discrimination level of 25 ng/mL or p = 0.0039 for a serum HER-2/neu discrimination level of 30 ng/mL).

A significant correlation between elevated serum HER-2/neu levels and shorter overall survival was also reported by Harris et al., in the evaluation of metastatic breast cancer.
patients \((n = 242)\) undergoing induction with doxorubicin-based therapy followed by high-dose alkylating agents with autologous stem cell support \([30]\). The authors found a significant correlation between both preinduction and postinduction serum \(\text{HER}-2/\text{neu}\) levels and shorter overall survival \((p = 0.045\) and 0.096, respectively\). Interestingly, neither FISH nor IHC tissue results correlated with overall survival. These results support the idea that different methods of \(\text{HER}-2/\text{neu}\) testing provide complementary biologic information and, thus, one method cannot necessarily replace another. As previously stated, serum \(\text{HER}-2/\text{neu}\) levels seem to provide a real-time assessment of \(\text{HER}-2/\text{neu}\) status while providing predictive value.

Lipton et al. evaluated the serum \(\text{HER}-2/\text{neu}\) status among metastatic breast cancer patients \((n = 719)\) receiving either megestrol acetate or an aromatase inhibitor \((\text{fadrozole or letrozole})\) \([31]\). Thirty percent \((30\%; 219/711)\) of patients had elevated levels of serum \(\text{HER}-2/\text{neu}\) \(>(15 \text{ ng/mL})\). Patients that were both estrogen receptor positive \((\text{ER}+)\) and serum \(\text{HER}-2/\text{neu}\) positive and who underwent any of these hormonal therapies had a significantly worse clinical benefit rate \((\text{complete response plus partial response plus stable disease: 23\% vs. 45\%})\). These patients demonstrated a shorter duration of response \((11.7 \text{ months vs. 17.4 months})\), a shorter time to progression \((90 \text{ days vs. 180 days})\) and a shorter time to treatment failure \((93 \text{ days vs. 175 days})\) than patients with nonelevated levels of serum \(\text{HER}-2/\text{neu}\). Most importantly, the improved outcome for patients with nonelevated levels of serum \(\text{HER}-2/\text{neu}\) was reflected in a longer duration of survival with 29.6 months vs. 17.2 months for patients with elevated serum \(\text{HER}-2/\text{neu}\) concentrations. Additionally, this trial showed a trend toward improved outcome for serum \(\text{HER}-2/\text{neu}\) positive patients with aromatase inhibitors as compared to megestrol acetate. This result is consistent with a recently published trial which demonstrated pronounced differences in response rates between neoadjuvant letrozole therapy vs. tamoxifen for patients with tumors that were classified as both \(\text{HER}-1\) \((\text{EGFR}-)\) and \(\text{HER}-2\) positive \((88\% \text{ vs. } 21\%, p = 0.0004)\) \([32]\). In contrast, patients with tumors which were negative for both \(\text{HER}-1\) and \(\text{HER}-2\) protein overexpression did not show significantly different response rates for letrozole as compared to tamoxifen \((54\% \text{ and } 42\%, \text{ respectively}, p = 0.1078)\). When considered together, these analyses suggest that \((\text{serum})\) \(\text{HER}-2/\text{neu}\) positive patients could profit especially from aromatase inhibitors for hormonal therapy of metastatic breast cancer.

### 5.4. Monitoring of advanced disease

Using the automated Bayer Immuno 1 immunoassay, Cook et al. demonstrated a very good correlation \((86.8\%)\) between longitudinal measurements of serum \(\text{HER}-2/\text{neu}\) in metastatic breast cancer patients \((n = 104)\) as compared to the clinical course of disease \([17]\). This excellent correlation has significant implications in terms of therapeutic monitoring and patient management. Most importantly, it has the potential to protect the patient from adverse events associated with the prolonged administration of ineffective therapies. This could be of extreme importance, especially in the case where patients are given a combination of chemotherapy plus Herceptin and where the discontinuation of chemotherapy must be considered after induction of a good response. Thus, Herceptin may be continued as a means of maintenance therapy, and recurrent progression can be monitored by the longitudinal measurements of serum \(\text{HER}-2/\text{neu}\) leading to re-combination with cytotoxic agents when and as needed. This means of closely following a patient could also help to minimize bone marrow insufficiency and initiate successive lines of chemotherapy without any compromise of dose and density of therapy.

Several authors have reported that serum \(\text{HER}-2/\text{neu}\) levels correlate with the course of disease under various systemic therapies including Herceptin-based regimens. Esheva et al. found that changes in serum \(\text{HER}-2/\text{neu}\) correlated very well with clinical response among metastatic breast cancer patients \((n = 30)\) receiving weekly docetaxel and Herceptin therapy \([33]\). Twenty-one patients had elevated \(\text{HER}-2/\text{neu}\) levels at baseline \(>(14.9 \text{ ng/mL})\). In terms of monitoring, serum \(\text{HER}-2/\text{neu}\) levels decreased in 87\% of patients who responded to therapy with 75\% of responding patients exhibiting a decrease in serum \(\text{HER}-2/\text{neu}\) levels to levels below the upper limit of normal. The overall response rate of the subgroup with elevated serum \(\text{HER}-2/\text{neu}\) concentrations was 76\%, compared with 33\% in those patients with normal serum \(\text{HER}-2/\text{neu}\) concentrations \((p = 0.04)\). This higher response rate in patients with elevated levels of serum \(\text{HER}-2/\text{neu}\) is intriguing and stands in contrast to all previously published studies with chemotherapy alone which showed worse outcome for patients with high serum \(\text{HER}-2/\text{neu}\) levels. While the results need confirmation, this must be interpreted as an indicator that Herceptin changes the biology of \(\text{HER}-2/\text{neu}\) positive breast cancer.

A separate, preliminary analysis of stage IV breast cancer patients \((n = 17; 369 \text{ serum samples})\) undergoing different cytotoxic therapies combined with Herceptin, had consistent findings \([34]\). In this study, the overall concordance for changes in serum \(\text{HER}-2/\text{neu}\) levels as compared with the clinical status for patients on Herceptin therapy was 78.2\% \((p < 0.005)\). Of note, the positive predictive value was very good \((81.8\%)\). This combination of high overall concordance with a high positive predictive value is very important. Aside from the information that is provided to the treating clinician, the easily obtained serum \(\text{HER}-2/\text{neu}\) test result may also provide reassurance and certainty to the patient as to the effectiveness of systemic therapy. A case report of this monitoring analysis is displayed in Figure 2.

Finally, Koestler et al. examined sera from patients undergoing Herceptin-containing therapies \((n = 75; 3122 \text{ sera})\) \([35]\). While baseline serum \(\text{HER}-2/\text{neu}\) levels failed to predict response to Herceptin-based therapy, the serum
HER-2/neu level ratio (before treatment/restaging) was significantly higher \((p \leq 0.01)\) in those patients who were responsive to treatment. The difference in serum kinetics between patients who responded vs. those with progressive disease was significant even after the first infusion of Herceptin. This type of information, provided early in the treatment regimen, might help to identify patients without benefit from therapy who could still benefit from other treatment options. In addition, taking into account the high treatment costs of Herceptin-based regimens, resources could be saved.

### 6. Algorithm for the use of serum HER-2/neu

The algorithm as shown in Figure 3 is a suggestion for the integration of serum HER-2/neu measurements into the clinical practice. It should not be interpreted being inconsistent with the recommendations for the use of tumor markers by leading oncological societies. Rather, it should be considered as a ‘work in progress’ in favor of individualized medicine (i.e., selecting optimal therapies based upon information as provided by diagnostic methods) [36].

Serum HER-2/neu testing is a complementary test to tissue testing (IHC and/or FISH) of the primary tumor. When patients are stratified according to the HER-2/neu tissue result, unnecessary serum HER-2/neu measurements can be avoided while at the same time important predictive and monitoring information can be gained. This algorithm is based on the “classical” management of diagnosed breast cancer patients who are treated first with primary surgery that is then followed by adjuvant treatment. In the case of primary, neoadjuvant chemotherapy with the intention of downstaging before secondary surgery, the determination of the serum HER-2/neu levels might provide important information for subsequent treatment strategies. This application will be dependent on the results of ongoing phase II- and III-studies on the neoadjuvant and adjuvant use of Herceptin. Of note, the serum HER-2/neu concentration before initiation of neoadjuvant treatment might influence the procedures for monitoring of relapse.

Currently, the measurement of serum HER-2/neu in the adjuvant setting is of moderate clinical importance. Irrespective of the HER-2/neu status as determined by tissue analysis, the probability of an elevated serum HER-2/neu level is between 5 and 10% for all breast cancer patients at the time of initial diagnosis. This small percentage does not necessarily justify the routine measurement of serum HER-2/neu in all breast cancer patients considering the cost aspect although the prognostic value of serum HER-2/neu has also been demonstrated in patients who had tumors that were characterized as HER-2/neu negative. Instead of “blind” testing, physicians should take into consideration the status of HER-2/neu in the tumor tissue. Currently, pathologists provide this result along with the tumor stage and the steroid hormone receptor expression. The determination of serum HER-2/neu is very unlikely to provide clinically useful information for those patients who are found to have tumor tissue that is HER-2/neu negative.
Thus, we recommend that the first measurement of serum HER-2/neu be obtained at the time of relapse in patients who had been initially diagnosed with HER-2/neu negative disease.

In those patients with primary breast cancer who are HER-2/neu tissue positive, it is suggested that the HER-2/neu serum status be determined before the initiation of adjuvant cytotoxic or hormonal therapy as well as at its conclusion. For both of these time points, increased serum HER-2/neu levels would suggest a poorer prognosis for this patient. These early measurements could become even more important in upcoming years. The preclinical and clinical data for the use of antibodies as adjuvant therapy of solid tumors suggests that Herceptin might be effective in early breast cancer.

In terms of monitoring for relapse, this individualized approach of utilizing regular measurements of serum HER-2/neu in patients who have HER-2/neu positive tumors, provides a sensitivity of more than 80%. This level of sensitivity is 2 to threefold higher than the sensitivity for the classical carbohydrate antigen (CA) tumor markers in breast cancer. After the initial diagnosis and treatment of breast cancer, we recommend that patients with HER-2/neu positive tumors be followed at 3 to 6 month intervals with serial monitoring of serum HER-2/neu levels as a means to detect relapse.

This algorithm puts more emphasis on the palliative situation than on the follow-up after first diagnosis and treatment. Again, monitoring is useful under systemic therapy every 2 to 3 months together with conventional restaging procedures in HER-2/neu tissue positive patients. Determination of serum HER-2/neu levels should be obtained in addition to tumor markers like CA15–3 or CA27.29, as the serum HER-2/neu levels show different kinetics and sometimes reflect different cell populations of the tumor mass. It is assumed that HER-2/neu expression is not consistent throughout the tumor (tumor heterogeneity) and in metastases. Serum HER-2/neu is reflective of those portions of the tumor that are overexpressing HER-2/neu while the carbohydrate antigens reflect the whole tumor mass. In patients undergoing Herceptin therapy, the serum HER-2/neu levels will fall below the upper limit of normal of 15 ng/mL with 90% probability in the case of a partial or complete remission. If the serum HER-2/neu level does not decrease by more than 35% in comparison to baseline within the first 4 weeks of treatment, benefit from therapy is unlikely and early restaging procedures should be initiated.

In 20 to 30% of breast cancer patients with tumor tissue positive for HER-2/neu, the shedding process is not very active for reasons which are not yet well understood. Thus, these patients have a normal serum HER-2/neu level despite having advanced disease. However, it is known that successful treatment with Herceptin diminishes shedding and, thus, the serum HER-2/neu level, while the autonomous, irresponsive disease is accompanied by increased shedding and elevated serum HER-2/neu levels. The significance of this is that with the loss of the extracellular binding site for Herceptin following shedding of the extracellular domain, this treatment will not be effective. However, the intracellular domain of HER-2/neu persists and can still be activated.

The complementary nature of serum HER-2/neu testing is most evident when one considers the current estimates that 15% of all breast cancers go through a change of the HER-2/neu expression over the course of the disease process [37]. Possible reasons for these discordances range from technical issues to clonal changes to therapeutic influences as seen under neoadjuvant therapy or preferential selection of HER-2/neu positive cells into metastatic spread. The higher percentage of serum HER-2/neu positivity seen upon relapse of up to 50% and up to 68% for minimal residual disease support the latter hypothesis [38]. In this complex situation, testing for serum HER-2/neu allows for a HER-2/neu assessment at the time of therapeutic decision making and takes into account the possible dynamics of HER-2/neu expression over the course of time.

7. Conclusions

Measurements of levels of serum HER-2/neu provide prognostic and predictive information to the clinician and can be used for monitoring of metastatic breast cancer patients. HER-2/neu serum testing provides complementary information to the tissue determination as the HER-2/neu status might change over time which would lead to compromised selection of patients for specifically targeted therapies. Testing for serum HER-2/neu using manual or automated ELISA assays is technically robust, does not require archival material and is independent of subjective interpretation. The clinical relevance of assessing serum HER-2/neu levels is certain to increase even moreso with the introduction of new, innovative anti-HER-2/neu targeted therapies in the form of small molecule tyrosine kinase inhibitors as well as with the utilization of anti-HER-2/neu targeted therapies in the adjuvant setting of breast cancer treatment. Furthermore, HER-2/neu overexpression is not confined to breast cancer. It is also noted in other epidemiologically important solid tumors such as bronchial-, prostate- and ovarian cancers. Thus, it is likely that the clinical role of serum HER-2/neu will evolve beyond that of breast cancer in the near future as well.

References


