Lack of HER-2/neu Overexpression in Non-Hodgkin’s Lymphoma

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Abstract. Objectives: The HER-2/neu oncogene is overexpressed in many types of cancer and especially in 25% to 30% of breast cancers. Single reports mention HER-2/neu positivity in hematological malignancies like Hodgkin’s disease and even diffuse large-cell lymphoma. Objective. To test for HER-2/neu overexpression in patients with non-Hodgkin’s lymphoma and the possible role of the recombinant monoclonal anti-HER-2/neu antibody trastuzumab (Herceptin®) in the treatment of non-Hodgkin’s lymphoma. Materials and Methods: Serum samples from 87 consecutive unselected patients with non-Hodgkin’s lymphoma were retrospectively retrieved from a serum bank and tested for the shed antigen of HER-2/neu using the Oncogene Science® ELISA assay (Cambridge, MA, USA). From those lymphoma patients, the paraffin-embedded lymph-node specimens of 25 cases with diffuse large-cell lymphoma were stained with the HER-2/neu DAKO HercepTest®. Results: In 87 lymphoma patients, the serum level of HER-2/neu ranged from 3.6 to 244.1 ng/ml (median 8.0 ng/ml). Only 2 patients showed a marginal or increased HER-2/neu level with 15 ng/ml (which is the upper limit of normal) and 244.1 ng/ml, respectively. No patient with diffuse large-cell lymphoma showed HER-2/neu overexpression by immunhistochemistry of the lymph node. The paraffin block of the one patient with a very high HER-2/neu serum level was also stained for HER-2/neu overexpression. In this patient, suffering from a high-grade T-cell non-Hodgkin’s lymphoma, no staining could be found. Conclusion: HER-2/neu is not overexpressed in non-Hodgkin’s lymphoma and especially not in diffuse large-cell lymphoma, using a standardized immunochemistry technique with complementary serum testing. Thus, specific anti-HER-2/neu-targeted therapy should play no role in the treatment of non-Hodgkin’s lymphoma.

The activation of cellular oncogenes plays an important role in the development of human cancer. One important oncogene for the development and therapy of solid tumors encodes the growth factor receptor known as HER-2/neu (human epidermal growth factor receptor) or c-erbB-2 (1, 2). This transmembrane protein is found on cells of epithelial origin and exerts its effect on cell growth through autophosphorylation. The full-length protein has a molecular weight of 185 kDa (p185) and is composed of three domains: the internal tyrosine kinase portion, a short transmembrane section and the extracellular ligand-binding domain that is heavily glycosylated (referred to as the extracellular domain or ECD). The C-terminal, cytoplasmic end of the molecule is responsible for the tyrosine kinase activity and initiation of signal transduction. A hydrophobic transmembrane portion connects the internal tyrosine kinase region and the ECD.

The HER-2/neu protein is a normal epithelial protein that is present on many organs such as lungs, colon, prostate and breast and therefore is not specific to any tissue (3). Overexpression of HER-2/neu has been found in a variety of human cancers, especially in breast cancer where it has been identified in 25% to 30% of patients (4, 5). In breast cancers and many other neoplasms, overexpression of HER-2/neu is correlated with aggressive tumor behavior, advanced stages of disease and a worse outcome (6-8).

The method most frequently employed for measuring HER-2/neu expression is immunohistochemical detection of the HER-2/neu receptor in paraffin sections of tumor material (9). As HER-2/neu is a transmembranous protein, the ECD portion, after proteolytic processes mediated by metalloproteases, is shed into the circulation and can be

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measured in serum (10). This shed ECD has been shown to be present in the sera of healthy subjects and to be elevated above the normal range in patients with malignant tumors. In breast cancer patients, the shed antigen of HER-2/neu has been shown to correlate with prognosis (11).

The determination of HER-2/neu expression has become more important, not only as prognostic marker, but also to identify patients for whom the recombinant monoclonal antibody trastuzumab (Herceptin®) could be used for treatment (12). Interestingly, in several studies, HER-2/neu overexpression is postulated not only in solid tumors but also in malignant lymphoma (13-16). In this study, we systematically measured the level of HER-2/neu in sera of 87 patients with non-Hodgkin’s lymphoma (NHL) of any histology. From these 87 patients, the paraffin-embedded lymph node specimens of 25 individuals, who presented with diffuse large-cell lymphoma (DLCL), were stained with the HER-2/neu DAKO HercepTest® since in one case report, HER-2/neu overexpression by immunohistochemistry was found in this lymphoma entity (14).

Materials and Methods

Patients. Serum samples from 87 consecutive unselected patients with NHL of our haematological unit were collected and, after centrifugation, were stored at -30°C until batch analysis. All serum samples were retrospectively tested for the shed antigen of HER-2/neu. Among those 87 patients with NHL were 48 cases with diffuse large-cell lymphoma (DLCL). The paraffin-embedded primary lymph-node specimens of 25 out of these 48 patients were stained with the HER-2/neu DAKO HercepTest. Clinical staging of the patients at initial diagnosis was performed following the Ann Arbor staging system and histology revised according to the R.E.A.L. classification. For the whole patient cohort, the remission status of the lymphoma at the time of the blood sample was documented.

Measurement of serum HER-2/neu. Serum levels of the shed antigen of HER-2/neu were tested using the Oncogene Science® ELISA method (Cambridge, MA, USA). The Oncogene Science assay is a sandwich enzyme immunoassay. Monoclonal anti-HER-2/neu antibodies, immobilized on the interior surface of microplate wells, specifically bind to the ECD of HER-2/neu. The serum sample (20 µl) is mixed with 980 µl of sample diluent. One hundred µl of this serum dilution is then added to the microplate wells. After an incubation time of 3 hours at 37°C and 3 washes, 100 µl of mouse monoclonal antibody is added to each well. This step is followed by another hour of incubation at 37°C and 3 washes. During this incubation, the detector reagents react with the immobilized ECD HER-2/neu antigen. The amount of detector antibody-antigen-complex is measured after reaction with a streptavidin-horseradish peroxidase conjugate (100 µl) which is added to each well. The streptavidin-horseradish peroxidase conjugate catalyzes the conversion of the chromogenic substrate o-phenylenediamine into a colored product. This chromogenic substrate (100 µl) is added to the microplate wells after 30 minutes of incubation at room temperature and a final 3-cycle wash. The plate is then incubated for 45 minutes at room temperature, followed by stopping the reaction with 100 µl of stop solution (2.5 N H2SO4). The colored reaction product is quantified by spectrophotometry at 490 nm. To establish the calibration curve, 6 different standards of recombinant HER-2/neu protein fragment p105 are run with each assay. The standard curve is constructed by plotting the average absorbance value for the HER-2/neu standards versus the known HER-2/neu standard concentration (ng/ml). The assay has a lower detection limit of 3.4 ng/ml and an upper linear dilution limit of 36 ng/ml. The cut-off level for HER-2/neu positivity in serum was previously established as 15 ng/ml (17).

Immunohistochemical study of HER-2/neu expression in lymph node specimens. Immunohistochemical staining for HER-2/neu protein expression was performed according to the instructions provided by the manufacturer. Four 4-µm sections of formalin-fixed, paraffin-embedded breast cancer tissue were mounted onto silanized slides. Tissue sections were dewaxed at 60°C in xylene (2x 5 min) and rehydrated in decreasing concentrations of ethanol to distilled water. The tissue sections were exposed to the epitope retrieval solution (1:10) in a water bath at 95°C for 40 minutes in a 10 mmol/l citrate buffer (pH 0.06), followed by a 20-minute cool-down period at room temperature (RT). After washing, 100 µl of hydrogen peroxide solution (5 minutes at RT) was used to block endogenous peroxidase activity, followed again by several washing courses. The slides were incubated with the prediluted primary rabbit polyclonal antibody for 30 minutes and then washed three times. The immunoreaction was visualized by incubating the slides with the DAKO Visualization
Reagent (dextran polymer conjugated with horseradish peroxidase and goat-anti-rabbit immunoglobulin) for 30 minutes at RT. This step was then followed by additional washing steps. 3,3′-Diaminobenzidine tetrahydrochloride (DAB; 30 µl) was used as the chromogen. The sections were counterstained with hematoxylin and washed several times with distilled water. Corresponding sections, included to serve as negative controls, were processed similarly with the exception that the primary antibody was omitted. Staining results were validated by the simultaneous processing of control slides that were provided as part of the kit and which were composed of three cell lines with different HER-2/neu expression levels. Semi-quantitative scoring of the HercepTest results was performed as per the instructions provided by the manufacturer and discussed in detail elsewhere (18). Specifically, scoring was as follows: no staining at all or membrane staining in <10% of the cells → score 0; faint perceptible membrane staining in ≥ 10% of the cells → score +1; weak to moderate staining in ≥ 10% of the cells → score +2; strong staining of the entire membrane in ≥ 10% of the cells → score +3. Only scores of +2 and +3 staining were defined as overexpression.

Results

Patient characteristics. The clinicopathologic features of the 87 patients with NHL are summarized in Table I. The median age of the group was 60 years (range: 17 to 80 years). There was a greater proportion of men (55 men and 32 women). Sixty-seven patients (77%) had intermediate- or high-grade lymphomas and 60 patients (69%) had stage III/IV disease at initial diagnosis. For 44 patients (50.5%), blood samples were obtained at initial diagnosis of the lymphoma or at the beginning of chemotherapy. For the other patients, remission status of the disease at the time of the blood samples was noted (Table I). Only 3 patients (3.4%) were in complete remission of the lymphoma at the time of the blood draw.

Serum levels of HER-2/neu. Among the 87 NHL patients, the serum levels of HER-2/neu ranged from 3.6 to 244.1 ng/ml (median 8.0 ng/ml). Only 2 patients showed a marginal or increased HER-2/neu serum level with 15 ng/ml and 244.1 ng/ml, respectively. The first patient was a 61-year-old female with a progressive mantle cell lymphoma at an advanced stage (IVA). The second patient, with the highest HER-2/neu serum level, was diagnosed as having a high-grade T-cell lymphoma with lymphadenopathy, bone marrow and skin involvement, which later showed to be unresponsive to all systemic chemotherapy.

HER-2/neu expression in lymph nodes – Correlation with clinicopathological features. None of the analysed lymph nodes of 25 patients with DLCL showed HER-2/neu overexpression by immunohistochemistry. The paraffin block of the one patient with a very high HER-2/neu serum level and high-grade T-cell lymphoma was stained for HER-2/neu overexpression according to the immunohistochemistry method as described by the DAKO package insert, too. No staining could be found and the slide was scored as HER-2/neu-negative (DAKO score 0) (Figure 1).

Discussion

Although HER-2/neu overexpression has been primarily detected in a variety of solid tumors, it has also been reported in two cases of aggressive DLCL (13, 14) and in
58.5\% of patients with Hodgkin’s disease (16). In a recent study on serum levels of HER-2/neu among 93 patients with ovarian masses, two patients presenting with ovarian NHL had elevated serum concentrations of HER-2/neu (15). However, details on the lymphoma histology of these two cases were not provided. On the other hand, in the biggest study published so far, HER-2/neu expression was examined by an immunohistochemical technique using the HercepTest. None of the 50 lymphoma specimens demonstrated overexpression or even any expression of HER-2/neu (19). Our results differ from the analysis of Bairey et al. (19), as we measured the serum level of HER-2/neu in 87 NHL patients and retrospectively studied lymph node specimens from 25 patients with DLCL by immunohistochemical staining. An elevated serum level of HER-2/neu was found in only 2 of our 87 patients. However, the lymph node specimens of both the patients with the highest elevated serum level and the 25 patients with DLCL were negative for HER-2/neu expression. Our findings indicate the lack of HER-2/neu overexpression in patients with NHL. The highly increased HER-2/neu serum level must be considered as unspecific or at least unrelated to lymphoma.

Although the ideal method for determining the HER-2/neu status is not yet known, immunohistochemical staining is the most common method as it can be used for paraffin-embedded as well as frozen tissue sections and is less expensive and technically easier than fluorescence-in situ-hybridization, the second most common technique (20). Evaluation of HER-2/neu overexpression by immunohistochemical staining may be subject to several problems, including variation in tissue fixation and processing, types of primary antibody used and difference in scoring criteria (21, 22). To avoid the considerable variability among the results obtained with the different immunohistochemical testing protocols, we used a commercially available, standardized HER-2/neu testing kit, the HercepTest (DAKO) that has been approved by the US Food and Drug administration.

The lack of HER-2/neu expression in this series of lymphoma patients can not be attributed to problematic antigen retrieval since, on the contrary, it has been reported that antigen retrieval, achieved with protease digestion or, as we practiced it, with heat treatment in citrate buffer, may increase the incidence of HER-2/neu positivity (23). Of course, we can never exclude that archival tissue blocks have reduced, or complete loss of, immunostaining intensity for some antigens/antibodies after prolonged storage (24).

HER-2/neu overexpression in solid tumors has been reported to be associated with other poor prognostic factors, including advanced stage, poor histological differentiation and resistance to drug therapy. In the current study, the only patient with high elevated serum level of HER-2/neu had a high-grade T-cell lymphoma with bone marrow and skin involvement and progression despite systemic chemotherapy. However, because the majority of our patients presented with active lymphoma at advanced stage, and since the correlation of increased serum HER-2/neu levels revealed the best sensitivity for advanced stage in solid tumors, above all breast cancer (16), we can affirm that serum HER-2/neu testing has no clinical utility among patients with NHL.

Conclusion

In conclusion, our data suggest a lack of HER-2/neu overexpression in patients with NHL. Specific anti-HER-2/neu-targeted treatment modalities like the monoclonal antibody trastuzumab (Herceptin®) do not seem to be an appropriate treatment of this malignancy.

References


