Evaluation of the Quantitative Analytical Methods Real-Time PCR for HER-2 Gene Quantification and ELISA of Serum HER-2 Protein and Comparison with Fluorescence in Situ Hybridization and Immunohistochemistry for Determining HER-2 Status in Breast Cancer Patients

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Background: HER-2 status is generally determined by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH). Both methods are only semiquantitative, require a tumor sample, and can be difficult to reproduce. We compared these methods with 2 quantitative approaches, one measuring HER-2 gene copy number in tissue by real-time quantitative PCR (qPCR), and the other measuring shed HER-2 protein in serum by ELISA in patients with metastatic disease.

Methods: We analyzed 52 cases of metastatic breast cancer for which both serum collected at the diagnosis of metastasis and stored primary breast tumor specimens were available. The within- and between-run imprecision of real-time qPCR and ELISA were evaluated according to Clinical and Laboratory Standards Institute (formerly known as NCCLS) recommendations. Concordance among the 4 methods was assessed by calculating the κ statistic and its 95% confidence interval (95% CI).

Results: The CVs for within- and between-run imprecision were both <10% with qPCR and ELISA. There was good agreement of results between qPCR and IHC (κ = 0.81; 95% CI, 0.64–0.99), qPCR and FISH (κ = 0.77; 95% CI, 0.58–0.96), ELISA and IHC (κ = 0.65; 95% CI, 0.41–0.89); and ELISA and FISH (κ = 0.69; 95% CI, 0.46–0.92).

Conclusions: Measurements of HER-2 gene expression by qPCR and of serum HER-2 protein by ELISA are highly reproducible approaches for determining HER-2 status in metastatic breast cancer. In addition, ELISA eliminates the need for biopsy.

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The HER-2 gene encodes p185, a 185-kDa transmembrane tyrosine kinase growth factor receptor belonging to the epidermal growth factor receptor family. The specific ligand for p185 is unknown. HER-2 is expressed in epithelial cells and is involved in cell differentiation and growth (1). The extracellular domain (ECD)5 of the receptor protein (also called p105) can be cleaved from the cell surface by matrix metalloproteases and then released into blood (2). HER-2 gene amplification and overexpression both have important biological implications, as they rep-
resent prognostic markers and predict breast tumor responsiveness to conventional therapies (3).

Immunotherapy based on a humanized anti-HER-2 monoclonal antibody, trastuzumab (Herceptin®), represents a new treatment option for women with metastatic breast cancer overexpressing HER-2. Patients with strong overproduction of HER-2 protein or HER-2 gene amplification show a survival benefit when treated with a combination of chemotherapy and trastuzumab (4). Accurate assessment of HER-2 status is thus crucial for management of breast cancer patients, and several methods have been proposed. Methods such as immunohistochemistry (IHC), Western blotting, and ELISA can be used to measure HER-2 protein concentrations in the tumor (IHC, Western blotting, and ELISA) or in serum (ELISA). Southern blotting, fluorescence in situ hybridization (FISH), chromogenic in situ hybridization, and quantitative PCR (qPCR) can detect gene amplification in the tumor. No single assay has been universally accepted as the “gold standard” for HER-2 status. Approximately 25% of primary invasive breast carcinomas are associated with HER-2 overexpression and/or amplification. Currently, patient selection for immunotherapy with trastuzumab is based on HER-2 concentrations. Thus, IHC is regarded as the optimal first-line testing method that detects most positive tumors and, at the same time, is cost-effective.

Recently, assessment of HER-2 status by FISH was found to be more predictive of clinical response to trastuzumab than IHC and was therefore proposed as the best method to select patients eligible for this therapy (5). Although HER-2 gene amplification is a surrogate for overproduction of HER-2 protein, except in a small percentage of cases, and does not represent the target of trastuzumab, it does represent, in experienced hands, a more accurate method to assess HER-2 status and a reliable comparison method for calibrating the IHC method (6, 7). Therefore, IHC and FISH are currently the most commonly used methods because they are suitable for routine analysis and analysis of archival paraffin-embedded tissues and because their methods of detection involve direct visualization of tumor cells (8). Recent data suggest that real-time qPCR on paraffin-embedded tissue may represent an alternative standardized approach to HER-2 status (9, 10). Moreover, the tissue-based methods give information on the HER-2 status of the primary tumor, and it is assumed that this is indicative of metastatic potential. Measurement of circulating HER-2 ECD in serum (s-HER-2) has been shown to be useful for assessing the prognosis and for predicting the response to trastuzumab (11, 12).

In this study we evaluated real-time qPCR and ELISA assessment of HER-2 status. We first determined the within- and between-run imprecision of the 2 quantitative methods; we then compared the 3 tissue-based techniques, i.e., IHC, FISH, and real-time qPCR, in a selected series of metastatic breast cancer cases. Finally, we examined the concordance of ELISA results with primary tumor status as determined by IHC and FISH.

**Materials and Methods**

**CASE SELECTION**

The study material consisted of specimens from 52 cases of metastatic breast cancer in which stored serum and primary tumor samples were available. The majority of the patients were enrolled in a phase IV multicenter study (the HERMES trial) designed to evaluate the economic impact of weekly trastuzumab and paclitaxel therapy in women with metastatic breast cancer (13).

A representative block of paraffin-embedded tumor tissue from each patient was selected and used to prepare four 5-μm sections for IHC and FISH. Two additional 10-μm sections were taken from the same block for DNA extraction and real-time qPCR. To minimize dilution of the PCR signal by nontumoral and nonamplified cells, sections containing >50% tumor cells were selected when possible. At the time of diagnosis of metastasis (before treatment initiation), blood was drawn into standard test tubes (BD Biosciences) and allowed to clot; serum was then separated by centrifugation, divided into aliquots, and stored at −20 °C.

**IHC AND FISH**

Immunohistochemical studies were performed with the polyclonal antibody A0485 (Dako Corporation). Our in-house IHC technique was calibrated to FISH. All steps of our test (antigen retrieval with a microwave, antibody dilution optimized at 1:800, use of a citrate buffer at pH 7.3) were standardized as recommended by the French multicenter GEFPICS study (14). The Ventana Enhanced DAB detection reagents and a Nexes R staining system (Ventana Medical Systems) were used for chromogenic visualization. In each run, a composite slide bearing 3 formalin-fixed human breast cell lines representing 3 concentrations of HER-2 protein (MDA-231 for normal concentrations, MDA-175 for mildly increased concentrations, and SKBR-3 for highly increased concentrations) was used as control. In addition, 1 slide from each case was incubated with normal rabbit serum instead of the polyclonal antibody (negative control). Interpretation of IHC results was performed by the same pathologist, who scored HER-2 staining on invasive carcinoma cells as positive only if the immunostaining intensity on the tumor cells was markedly higher than the staining on normal epithelium. The intensity of membrane staining was evaluated with the Dako scoring system (15). Scores of 0 and 1+ were considered negative for HER-2 overproduction, and scores of 2+ and 3+ were considered positive.

FISH was performed with the Inform test (Ventana Medical Systems) according to the manufacturer’s instructions and with reagents, probes, and positive controls purchased from the manufacturer. This system uses a biotin-labeled oligonucleotide probe detected with an
PCR was performed with the LightCycler® (Roche Diagnostics) according to the manufacturer’s instructions. Master Pure DNA purification reagents (Epicentre Technologies) were independently extracted with avidin-biotin phenylisothiocyanate system for signal amplification. Fluorescence signals were counted by use of a Zeiss fluorescence microscope equipped with an appropriate filter set. For each specimen, the gene copy number was assessed in 2 areas containing at least 20 nonoverlapping tumor nuclei. Cases were scored as amplified when the mean number of fluorescent signals per nucleus was >2 (16). HER-2 gene-amplified tumors were further subdivided into 3 categories: low amplification if no polysomy of chromosome 17 (mean of 2–5 signals per nucleus), moderate amplification (5–20 signals per nucleus), and high amplification (>20 signals per nucleus).

**Quantification of HER-2 Gene Copy Number by Real-time qPCR**

Two 10-μm tissue sections from the same block of paraffin-embedded tissue were independently extracted with Master Pure DNA purification reagents (Epicentre Technologies) according to the manufacturer’s instructions. PCR was performed with the LightCycler® HER-2/neu DNA quantification assay (Roche Molecular Biochemicals), which is based on relative quantification, normalization against a DNA calibrator, and efficiency correction (17). The assay simultaneously amplifies 2 fragments located on chromosome 17: a 112-bp fragment of the HER-2 gene and a 133-bp fragment of a single-copy reference gene; the assay uses 2 pairs of hybridization probes to detect both amplicons. The reference gene, located on the same chromosome with HER-2, provides a control for DNA quality and loading and also serves as an internal control gene for polysomy. PCR was run in duplicate according to the manufacturer’s instructions. Two independent DNA extractions, 2 PCR amplifications per DNA extract, and 4 measurements were performed for each sample. Results were expressed as the ratio of crossing threshold values of the HER-2 and reference genes in the sample compared with the same ratio in a calibrator DNA sample, which is a linearized plasmid DNA containing 1 copy each of the HER-2 and reference genes. Normalization against calibrator DNA helps to adjust for inter-PCR run variations and sets a value for comparison and standardization. The relative amounts of gene products for HER-2 compared with the reference gene were calculated by use of the model of efficiency-adjusted relative quantification (RelQuant® software; Roche Molecular Biochemicals). This model incorporates all important factors that potentially affect quantification by including the reference gene, the calibrator, and the individual PCR-defined reaction efficiencies for HER-2 and the reference gene in each reaction. Final results were recorded as a mean ratio calculated from the 4 measurements performed for each sample.

In each experiment, DNAs extracted from the T47-D and SKBR-3 breast cancer cell lines (ATCC) were included as controls for the nonamplified and amplified HER-2 gene copy, respectively.

**ELISA Measurement of Serum HER-2 Protein**

HER-2 ECD in serum was measured with a US Food and Drug Administration-cleared 2-site sandwich immunoassay (Oncogene Science/Bayer Diagnostics). This test uses 2 monoclonal antibodies: one to capture human HER-2 ECD and the second, biotinylated, to detect it. The supplied calibrators were prepared from a recombinant HER-2 ECD fragment and were calibrated in micrograms per liter. Samples were diluted 1:50 in sample diluent, and the s-HER-2 concentration was measured according to the manufacturer’s instructions.

Three controls patented by Oncogene Science and containing known concentrations of recombinant shed HER-2 protein (low, medium, and high) were included in each series.

**Imprecision Study**

The 2 breast cancer cell lines, T47-D and SKBR-3, and the 3 ELISA controls were used to estimate the imprecision of the 2 methods. We assessed within-run imprecision by analyzing several control samples on the same day. Between-run imprecision studies were conducted over a 30-day period by measuring HER-2/reference gene ratios and s-HER-2 concentrations in the control samples. The within- and between-run CVs were determined. For both within- and between-run imprecision, a CV of 10% was considered beforehand to represent acceptable performance. Total imprecision was then calculated according to Clinical and Laboratory Standards Institute (CLSI; formerly known as NCCLS) recommendations.

**Statistical Analysis**

Dichotomous variables are reported as numbers (%), and continuous data are reported as medians and ranges. Data were compared with the Fisher exact test and nonparametric tests as appropriate.

The ROC curve was drawn to determine the optimal cutoff value in terms of sensitivity and specificity for

<table>
<thead>
<tr>
<th>Her-2 Status</th>
<th>Positive</th>
<th>Negative</th>
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<tbody>
<tr>
<td>No. of Patients (%)</td>
<td>30 (60)</td>
<td>20 (40)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Metastatic Sites</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>&gt;3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Patients (%)</td>
<td>24 (46.2)</td>
<td>17 (32.7)</td>
<td>6 (11.5)</td>
<td>5 (9.6)</td>
</tr>
</tbody>
</table>

*PBC, primary breast cancer.

**Table 1. Characteristics of the 52 patients.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Age at PBC* diagnosis, years</th>
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<tr>
<td></td>
<td>Median</td>
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<tr>
<td>Months with no recurrent disease</td>
<td>40</td>
</tr>
<tr>
<td>Nodal status, b no. of patients (%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>20 (40)</td>
</tr>
<tr>
<td>Positive</td>
<td>30 (60)</td>
</tr>
<tr>
<td>Number of metastatic sites, no. of patients (%)</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>24 (46.2)</td>
</tr>
</tbody>
</table>

* PBC, primary breast cancer.

b Data not available for 2 patients.
detecting HER-2-positive tumors by real-time qPCR and ELISA. Because no “true” standard method for HER-2 status assessment has been established, we used IHC as the comparison method, but we also compared the qPCR assays and ELISAs with FISH.

To measure the degree of concordance of the results of the different HER-2 assays, the $\kappa$ statistic and its 95% confidence interval (95% CI) were calculated. $\kappa$ values were interpreted as follows: $\kappa = 0.00–0.20$, poor agreement; $\kappa = 0.21–0.40$, fair agreement; $\kappa = 0.41–0.60$, moderate agreement; $\kappa = 0.61–0.80$, good agreement; $\kappa = 0.81–1.00$, near-perfect agreement (18). The McNemar test was used to test for the null hypothesis, according to which there was no concordance between 2 methods.

All probabilities were 2-tailed, and $P$ values $<0.05$ were considered statistically significant. All statistical calculations were performed with the Statview and SAS software packages.

### Results

The characteristics of the 52 patients with metastatic breast cancer are given in Table 1.

### Analytical Assessment of Imprecision

The mean (SD), ranges, and within- and between-run CVs for the qPCR and ELISA tests, and the total imprecision for each control sample, were determined (see Table 1 of the Data Supplement that accompanies the

<table>
<thead>
<tr>
<th>Table 2. Comparison of HER-2 assay results: IHC vs FISH (n = 52).</th>
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<tr>
<td>IHC score</td>
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<tr>
<td>0 and 1+</td>
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<tr>
<td>2+ and 3+</td>
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</table>

<table>
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<tr>
<th>Table 3. HER-2/reference gene ratio in groups negative and positive by IHC and FISH (n = 51).*</th>
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<tbody>
<tr>
<td>Group</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>IHC score</td>
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<tr>
<td>0 or 1+</td>
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<tr>
<td>2+ or 3+</td>
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<tr>
<td>FISH</td>
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<tr>
<td>Negative</td>
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<tr>
<td>Moderate</td>
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<td>High</td>
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* All comparisons used Kruskal–Wallis test.

Fig. 1. Real-time qPCR vs IHC.

(A), ROC curve for real-time qPCR assay based on 51 patients with metastatic breast cancer, with IHC as the comparison method. The area under the curve (SE) is 0.94 (0.03); 95% CI, 0.84–0.99. (B), scatter plot of HER-2/reference gene ratios (expressed in relative copy numbers) determined by real-time qPCR for patients with positive (scores 2+ and 3+) and negative (scores 0 and 1+) IHC. The horizontal line indicates the optimal cutoff for the HER-2/reference gene ratio (2.2), which gave a sensitivity of 87.5% and specificity of 100%.
COMPARISON OF IHC AND FISH
The results of both IHC and FISH analyses were assessable in every case. Because of the design of the HERMES trial, most of the 52 women had positive HER-2 status: 13 cases (25%) scored negative (11 cases with a score of 0+ and 2 with a score of 1+) and 39 cases (75%) scored positive (3 cases with a score of 2+ and 36 with a score of 3+; Table 2). FISH showed HER-2 gene amplification in 38 cases (13 with moderate and 25 with high amplification; Table 3). None of the cases negative by IHC was positive by FISH, whereas 38 of the 39 cases with increased HER-2 protein concentrations showed HER-2 gene amplification by FISH (Table 2). The results of IHC and FISH were therefore concordant in 51 of the 52 cases. The concordance coefficient (κ) was 0.95 (95% CI, 0.85–1.00; \( P = 0.32 \), McNemar test).

HER-2 status by real-time qPCR
Real-time qPCR results were assessable in 51 cases. The calculated HER-2/reference gene ratios were higher in IHC-positive and FISH-positive cases than in the corresponding negative cases (Table 3). Cases with IHC scores of 3+ (n = 36) or rated as highly amplified by FISH (n = 25) had significantly higher HER-2/reference ratios than those with IHC scores of 2+ [n = 3; median (range) ratios, 11.5 (1.8–58.9) vs 3.6 (1.8–21.4); \( P < 0.0001 \)] or rated as moderately amplified by FISH [n = 13; median ratios, 16.6 (2.3–58.9) vs 4.3 (1.8–24.4); \( P < 0.0001 \)].

The ROC curve obtained by comparing patients with and without HER-2 protein overproduction, as indicated by IHC, is shown in Fig. 1A, and the curve comparing patients with and without HER-2 gene amplification, as assessed by FISH, are shown in Fig. 2A. An optimized HER-2/reference gene ratio of 2.2 yielded 87.5% sensitivity and 100% specificity with reference to IHC and 89.5% sensitivity and 92% specificity with reference to FISH.

Considering a ratio >2.2 as being positive for HER-2 gene amplification, 69% (35 of 51) of cases were classified as positive by real-time qPCR. Ninety percent (46 of 51) of IHC and FISH results matched real-time qPCR results (Figs. 1B and 2B). Among the 39 cases showing overproduction by IHC and the 38 cases amplified by FISH, 5 cases with an IHC score of 3+ and 4 cases moderately positive by FISH had a mean ratio near the cutoff and were thus classified as nonamplified. When we analyzed each of the 4 values obtained before calculating the mean ratio, we found that they ranged between 1.8 and 2.5, giving a mean ratio near 2. The 5 tumor samples with discordant results showed a strong stromal component. Of 13 cases not amplified by FISH, only 1 had a ratio >2.2 and scored 3+ by IHC.
The best concordance was between qPCR and IHC (κ = 0.81; 95% CI, 0.64–0.99; P = 0.06, McNemar test). The value was 0.77 (95% CI, 0.58–0.96; P = 0.18, McNemar test) for the concordance between qPCR and FISH.

**HER-2 Status by ELISA**

ELISA results were assessable in every case. The median s-HER-2 concentration was significantly higher in IHC-positive and FISH-positive cases than in IHC-negative and FISH-negative cases (Table 4).

We determined an optimal s-HER-2 cutoff of 16 µg/L by comparing patients with and without increased HER-2 protein concentrations by IHC (Fig. 3A) and those with and without HER-2 gene amplification by FISH (Fig. 4A). At an s-HER-2 concentration of 16 µg/L, the sensitivity and specificity were 90% and 83%, respectively, with respect to IHC and 90% and 77% with respect to FISH.

Using this cutoff value, 73% (38 of 52) of the cases had increased s-HER-2 concentrations. Most of the patients with HER-2-positive tumors by IHC (36 of 38) and FISH (35 of 38) had an s-HER-2 concentration above the cutoff at diagnosis of metastatic disease (Figs. 3B and 4B). Of the 40 cases positive by IHC and 39 cases positive by FISH, 4 cases showed s-HER-2 concentrations within the reference interval (11–16 µg/L) but a HER-2/reference gene ratio > 2.2 by qPCR (range, 2.3–24.4). Of the 12 cases showing no overproduction of HER-2 and no HER-2 gene amplification in the primary tumor, 2 had a slightly increased s-HER-2 concentration (17 and 40 µg/L). The case with an s-HER-2 concentration of 40 µg/L scored 1+ by IHC.

The degree of concordance between ELISA and IHC and between ELISA and FISH was 0.65 (95% CI, 0.41–0.89; P = 0.71, McNemar test) and 0.69 (95% CI, 0.46–0.92; P = 1.00, McNemar test), respectively.

**Discussion**

Trastuzumab is one of the few drugs whose indication depends on the results of a diagnostic test, and accurate and precise determination of HER-2 status is therefore essential (19). At present, it is recommended that more than 1 method be used for this purpose (8).
Our report is the first comparing 4 different methods to assess HER-2 status in 52 patients with metastatic breast cancer, 75% of whom were eligible for trastuzumab therapy. We found the expected high concordance between centralized IHC and FISH, in keeping with the reported concordance values of 85%–95% when immunostaining is interpreted as either negative (scores 0 and 1) or strongly positive (score 3) (20, 21).

Real-time qPCR and ELISA are true quantitative methods, whereas IHC and FISH are morphologic in situ and semiquantitative tests. We obtained good concordance between the 3 tissue-based methods: real-time qPCR, IHC, and FISH. The optimal cutoff ratio for qPCR was 2.2, in good agreement with the arbitrary cutoff of 2 generally accepted for gene quantification methods. Most discrepancies between qPCR and IHC/FISH were qPCR false negatives, probably resulting from dilution of cells carrying amplified genes among nontumor cells (22–25). Tumors with qPCR ratios showing great variability of results between 1.8 and 2.5 should therefore be interpreted with care. For tumors with a strong stromal component, manual or laser-assisted microdissection is recommended to allow reliable detection of low-level gene amplification by real-time qPCR (10, 25). Only 1 case was positive by qPCR and negative by FISH in our series, and it is noteworthy that the relevant tissue sections were old and of poor quality. Recent FISH guidelines recommend that cut-tissue sections should not be stored for more than 6–12 months (8).

An immunoassay for serum HER-2 ECD that was recently cleared by the US Food and Drug Administration has a cutoff of 15 µg/L, a value very similar to the optimal value determined here (16 µg/L). Very similar proportions of our patients had HER-2 overproduction by IHC (75%), HER-2 gene amplification by FISH (73%), and increased s-HER-2 concentrations (73%). These rates are consistent with the good concordance found between the primary tissue tests of IHC and FISH and the ELISA that measures the concentration of circulating HER-2 ECD. We observed marked variations in s-HER-2 values (from slightly under the cutoff to >1000 µg/L) in patients with strong HER-2 overproduction or amplification, suggesting that the HER-2 ECD concentration correlates positively with tumor burden (11). The clinical or biological significance of HER-2 ECD shedding is unknown, but it is probably attributable to up-regulation of metalloproteases, which are involved in proteolytic cleavage of HER-2 and have been linked to tumor invasion and metastasis (26).

s-HER-2 concentrations were not increased in 4 patients whose tumors were HER-2-positive by IHC or FISH. Conversely, s-HER-2 was increased in 2 patients with HER-2-negative tumors. There are several possible explanations for such discrepancies between the HER-2

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**Fig. 4. ELISA vs FISH.**

(A), ROC curve for ELISA based on 52 patients with metastatic breast cancer with FISH used as the comparison method. Area under the curve (SE), 0.94 (0.03); 95% CI, 0.84–0.99. (B), scatter plot of s-HER-2 (p105) concentrations determined by ELISA for patients with positive and negative FISH results. The horizontal line indicates the optimal cutoff for s-HER-2 concentrations (16 µg/L), which gave a sensitivity of 90% and a specificity of 77%.
status of primary breast tumors and serum HER-2 ECD concentrations measured at diagnosis of metastatic disease. Clonal changes between the primary breast tumor and distant metastases may also affect HER-2 status (27). Patients with negative tumor HER-2 status at the time of biopsy may become HER-2-positive as they progress to metastatic disease (28). This could explain discrepancies between negative tissue testing and increased serum HER-2 concentrations in metastatic breast cancer (29). Moreover, reduced metalloprotease activity could lead to decreased s-HER-2 concentrations.

HER-2 status assessment based on real-time PCR and immunoassay appears promising (9–11, 30). IHC is currently used to estimate HER-2 protein production in tissue samples, in which staining is subjectively scored from 0 to 3+. Accuracy can be improved by use of the FISH method, which detects HER-2 gene amplification in tissue but requires a high degree of technical expertise. Furthermore, there is increasing evidence that variability in fixation and staining techniques and scoring methods can lead to significant discrepancies in HER-2 test results.

Our preliminary data suggest that real-time qPCR and ELISA correlate well with IHC and FISH. In addition, the analytical performance, such as precision and trueness (data for trueness are not shown here), is assessable according to CLSI recommendations, contrary to IHC and FISH. Real-time qPCR is highly sensitive (as few as 10 copies of the HER-2 gene can be detected) and specific (primers and hybridization probes are sequence specific for human HER-2 and control genes). Moreover, HER-2 gene quantification is objective and offers good precision, particularly when the robust mathematical model of efficiency-adjusted relative quantification is used. The ELISA offers precise quantification of HER-2 ECD in serum. It shows a weaker correlation with IHC and FISH than does real-time qPCR and probably reflects metastatic status rather than primary tumor status. This could have important therapeutic implications for patients not eligible for the trastuzumab-based therapy at the time of primary breast cancer. Moreover, the ELISA for s-HER-2 is a dynamic test that can be performed at any time. Various studies have demonstrated that increasing s-HER-2 concentrations are associated with progressive disease and that decreasing concentrations indicate a response to therapy (11, 13).

In summary, real-time qPCR is an alternative method for determining HER-2 status in paraffin-embedded tissue sections from breast cancer patients. When performed on tissue sections selected by the pathologist and containing >50% tumor cells, this method provides rapid and accurate HER-2 gene quantification. Nevertheless, when tissue sections contain few tumor cells, microdissection of tumor cells is greatly recommended to improve the accuracy of the test. The ELISA can be used when primary tumor samples are unavailable, and eliminates with the need for biopsy. ELISA could also be helpful when tissue results are discordant. Both techniques are rapid, cost-effective, and fully automated, thereby offering a high degree of standardization and reproducibility.

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References


