Correlation of Human Epidermal Growth Factor Receptor 2 (HER2), Estrogen Receptor (ER), and Progesterone Receptor (PR) Expression as Predicted by Core Biopsy with the Immunohistochemical Results of Surgical Breast Cancer Specimens

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Key Words

Quality assurance · Core biopsy · Immunohistochemistry · Steroid hormone receptors · HER2 overexpression · Breast cancer

Summary

Background: Representative breast cancer specimens can be obtained by core biopsy. Immunohistochemistry (estrogen receptor: ER, progesterone receptor: PR, and human epidermal growth factor receptor 2: HER2) of core specimens and excised tumors should correlate. Antigen retrieval and tumor heterogeneity may impair immunohistochemical procedures and lead to unreliable results. **Patients and Methods:** Data was collected from 110 patients with operable invasive breast cancer who were diagnosed by core biopsy between 2001 and 2006, subsequently underwent primary surgery, and had complete immunohistochemical evaluation of biopsy and surgical specimens. ER, PR, and HER2 status were semiquantitatively evaluated. **Results:** Categorical ER and PR expression correlated in 54% and 55%, respectively. Clinically relevant ER status (positive versus negative) correlated in 97% and the PR status in 88%. HER2 overexpression was observed in 18% of core biopsies and 28% of surgical specimens with a concordance in 69%. Correlation of categorical HER2 expression (0, 1+, 2+, 3+) was present in only 46%. **Conclusion:** In our patient population, steroid hormone receptor status could be predicted by core biopsy. However, the rather weak concordance of the HER2 status must be further examined. To evaluate whether core biopsies or surgical specimens are better suited to predict the more accurate HER2 status, gene amplification of both specimens should be correlated. Until then, diagnosis of HER2 expression in core biopsy specimens should be interpreted with care.

Schlüsselwörter

Qualitätssicherung · Stanzbiopsie · Immunhistochemie · Steroidhormonrezeptoren · HER2-Überexpression · Brustkrebs

Zusammenfassung

Hintergrund: Mittels perkutaner Stanzbiopsie kann man eine repräsentative Gewebeprobe aus Mammakarzinomen gewinnen. Für den Nachweis der Expression des humanen epidermalen Wachstumsfaktorrezeptors-2 (HER2) und des Hormonrezeptorstatus (Östrogen-Rezeptor: ER, Progesteron-Rezeptor: PR) wird eine hohe Korrelation zwischen Stanzbiopsie und Operationspräparat erwartet. Antigenerkennung und Tumorheterogenität können diese Korrelation beeinflussen. **Patientinnen und Methoden:** Retrospektiv wurden 110 Patientinnen erfasst, die ein stanzbiopsisch gesichertes invasives Mammakarzinom mit nachfolgender operativer Therapie und vollständiger immunhistochemischer Auswertung beider Gewebeproben aufwiesen. **Ergebnisse:** Der Grad der ER-Expression stimmte in 54% und der der PR-Expression in 55% überein. Der ER-Status (positiv versus negativ) korrelierte in 97%, der PR-Status in 88%. Eine HER2-Überexpression wiesen 18% der Stanzbiopsiepräparate und 28% der Operationspräparate auf. Hinsichtlich der klinisch relevanten Befunde zur HER2-Überexpression fand sich in 69% eine Übereinstimmung. Der HER2-Expressionsgrad (0, 1+, 2+, 3+) stimmte in 46% überein. **Schlussfolgerung:** In unserer Patientenpopulation war die Korrelation des Hormonrezeptorstatus hochsignifikant. Für die HER2-Expression fanden wir eine signifikante, aber geringere Übereinstimmung. Um zu klären, welches Material den HER2-Status in der Immunhistochemie besser widerspiegelt, muss eine Korrelation mit der Genamplifikation hergestellt werden. Bis dahin sind immunhistochemische Ergebnisse aus der Stanzbiopsie mit Vorsicht zu interpretieren.

Introduction

In the event of continuous quality-assured medicine, especially in the diagnosis and treatment of breast cancer, even established standard procedures must sometimes be re-evaluated. As the number of breast cancer patients treated at the Center for Breast Disease at the Martin Luther University Hospital in Halle/Germany continues to increase, with approximately 10–15% of patients clinically eligible for primary systemic therapy, the accurate prediction of cellular tumor characteristics by analysis of diagnostic core biopsy specimens is mandatory.

The correct analysis of human epidermal growth factor receptor 2 (HER2) in core biopsy specimens can optimize primary systemic therapy by allowing the addition of trastuzumab treatment where indicated [1] and avoid unnecessary and costly therapy in other cases. In patients experiencing complete pathological remission, the core biopsy specimen remains the only tissue available for tumor profiling. Correct prediction of steroid hormone receptor status is equally important where neoadjuvant endocrine therapy is an option.

Representative breast cancer specimens may be obtained through quality-assured percutaneous core biopsy. Since major changes in the expression of hormone receptors or HER2 in the time interval between core biopsy and definitive local therapy in patients not receiving primary systemic therapy is highly unlikely, immunohistochemical properties (estrogen receptor: ER, progesterone receptor: PR, and HER2) of core biopsy specimens and surgically excised tumors should correlate significantly. On the other hand, the antigen retrieval of tissue samples may be altered by fixation and embedding processes. Slamon et al. [2] have reported that a proportion of breast cancers known to have gene amplification and overexpression of HER2 may lose membrane staining after paraffin embedding. The American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer [3] suggest that fixation time alters protein antigen expression.

We report here on the correlation between immunohistochemical results of ER, PR, and HER2 in core needle biopsy specimens and material obtained from tumor excision.

Patients and Methods

In this retrospective analysis, data was collected from patients with operable breast cancer who were treated at the Martin Luther University Hospital in Halle/Germany between 2002 and 2006. Patients were only eligible for inclusion if complete immunohistochemical data (ER, PR, and HER2 status) of biopsy specimens as well as excised tumors were available for evaluation. A total of 110 patients between the ages of 32 and 86 years (median 62 years) met these criteria for HER2. Hormone receptor status was complete in 103 cases, ranging in age from 32 to 86 years with a median of 63 years.

ER and PR status was determined by immunohistochemical analysis of formaldehyde-fixed (neutral buffered 4.5% formaldehyde) and paraffin-embedded tissue using the SP1 monoclonal anti-ER and SP2 monoclonal anti-PR antibodies [4, 5] (both LabVision Corp., Fremont CA, USA). The immunoreactive score (IRS) according to Remmele and Stegner [6] defines the intensity of the immunohistochemical reaction of the tumor cells and thereby the content of immunohistochemically detectable receptor proteins. The IRS is the product of the subjective measure of staining intensity (SI): 0 (no staining), 1 (weak staining), 2 (moderate staining), 3 (strong staining) and the objective percentage of positive cells (PP): 0 (no breast cancer cell stained), 1 (<10% of breast cancer cells stained), 2 (10–50% of breast cancer cells stained), 3 (51–80% of breast cancer cells stained), 4 (>81% of breast cancer cells stained). Thus, IRS can measure 0 (no expression) to 12 (maximal expression).

Patients with a positive ER and/or PR expression are considered steroid hormone receptor positive and may benefit from the addition of a selective estrogen receptor modulator (tamoxifen) or an aromatase inhibitor to adjuvant systemic therapy. The IRS considered to define a positive expression of ER and PR may vary from institution to institution. In this case, an IRS of 3 and above denoted a positive expression. HER2 status was determined by using the HercepTest® (DAKO Corp., Carpinteria, CA, USA) [7] immunohistochemical assay of formaldehyde-fixed (neutral buffered 4.5% formaldehyde) and paraffin-embedded tissue. The categorical score is determined by semiquantitative evaluation of cell membrane staining. Possible results of HER2 expression range from 0 to +3, whereby 0 and 1 are defined as negative, +2 as equivocal, and +3 as HER2 overexpression [8]. Breast cancer patients with HER2 overexpressing tumors are considered eligible for and may benefit from the addition of trastuzumab (Herceptin®) to adjuvant or neoadjuvant systemic therapy.

Statistical Methods

Expression of ER, PR, and HER2 was categorized as described above. Based on these categorical variables, we calculated Spearman rank correlation coefficients (r) to quantify the association between expressions based on core biopsies and excised tumors. Values of r vary between +1 and -1 with a positive sign indicating a direct relationship and a negative sign indicating an inverse relationship between the variables. Data were analyzed with SAS for windows, version 9.1., 2002 (Cary, NC, USA) as well as SPSS 12.0 (SPSS Software GmbH, Munich, Germany). Statistically significant correlations were calculated for $p < 0.05$.

Results

Steroid Hormone Receptor Status

A comparison of steroid hormone receptor status in biopsy and tumor tissue was possible in 103 cases. Of 103 patients, 76 (74%) had ER-positive biopsy specimens versus 75 (73%) ER-positive tumors upon excision, whereas 62 (60%) showed PR expression in biopsy tissue in comparison with 58 (56%) in excised tumors. A total of 58 (56%) patients showed an expression of both hormone receptors in biopsy as well as tumor tissue (tables 1, 2).

Categorical expression of the ER in biopsy and tumor tissue correlated in 56 (54%) cases ($r = 0.671$; $p < 0.0001$). The correlation of the PR was given in 57 (55%) cases ($r = 0.744$; $p < 0.0001$). If these values are correlated according to clinically relevant ER and/or PR status, i.e. negative (IRS 0, 1, 2) or positive (IRS 3, 4, 6, 8, 9, 12), a correlation is seen in 97% (100) of all cases for ER ($r = 0.882$; $p < 0.0001$) and 88% (91) of all cases for PR ($r = 0.858$; $p < 0.0001$).

Table 1. Correlation of ER expression in tumor tissue obtained from core biopsies and excised tumors ($r = 0.671$; $p < 0.0001$)

ER expression in biopsy specimen	ER expression in excised tumor				Total
	negative	weak	moderate	strong	
Negative	26	1	0	0	27
Weak	2	6	6	5	19
Moderate	0	12	13	8	33
Strong	0	5	8	11	24
Total	28	24	27	24	103 (missing: 7)

Table 2. Correlation of PR expression in tumor tissue obtained from core biopsies and excised tumors ($r = 0.744$; $p < 0.0001$)

PR expression in biopsy specimen	PR expression in excised tumor				Total
	negative	weak	moderate	strong	
Negative	37	4	1	0	41
Weak	6	9	5	9	29
Moderate	0	6	5	6	17
Strong	2	3	4	6	15
Total	45	22	15	21	103 (missing: 7)

Table 3. Correlation of HER2 expression in tumor tissue obtained from core biopsies and excised tumors, listed according to scoring results (DAKO HercepTest®); ($r = 0.587$; $p < 0.0001$)

HER2 expression in biopsy specimen	HER2 overexpression in excised tumor				Total
	0	1+	2+	3+	
0	14	6	0	2	22
1+	20	13	10	7	50
2+	3	4	6	5	18
3+	0	1	2	17	20
Total	37	24	18	31	110

Table 4. Correlation of HER2 expression in tumor tissue obtained from core biopsies and excised tumors, categorized according to overexpression (DAKO HercepTest® 3+) vs. equivocal expression (DAKO HercepTest® 2+) vs. no overexpression (DAKO HercepTest® 1+, 0), ($r = 0.580$, $p < 0.0001$) as well as overexpression (DAKO HercepTest® 3+) vs. no overexpression (DAKO HercepTest® 1+, 0) excluding equivocal expression (DAKO HercepTest® 2+), ($r = 0.713$, $p < 0.0001$)

HER2 overexpression in biopsy specimen	HER2 expression in excised tumor			Total
	negative	equivocal	positive+	
Negative	53	10	9	72
Equivocal	7	6	5	18
Positive	1	2	17	20
Total	61	18	31	110

HER2

Of 110 patients included in this analysis, 20 (18%) showed HER2 overexpression in tumor biopsy specimens upon immunohistochemical analysis. In material from surgical specimens 31 (28%) of the tumors overexpressed HER2. An equivocal expression of HER2 was seen in 18 (16%) cases both of biopsy specimens and excised tumors. Weak HER2 staining

was seen in 50 (46%) biopsy specimens, but only in half as many (24; 22%) excised tumors. The remaining 22 (20%) biopsy specimens showed no HER2 expression whatsoever, compared to 37 (34%) excised tumors (table 3).

An agreement between categorical HER2 expression results of both types of tumor specimens can be seen in only 50 (46%) of all cases ($r = 0.587$; $p < 0.0001$).

In general, biopsy specimens tended to show a lower overall HER2 expression. A total of 66% of all biopsy specimens showed negative or weak HER2 expression, compared to 56% of excised tumors. On the other end of the scale, almost half of the excised tumors (44%) had a DAKO HercepTest® score of 2+ or 3+, while only 34% of biopsy specimens showed an equivocal or definite HER2 overexpression.

If HER2 expression is compared with regard to clinical significance (overexpression present vs. absent), a much lower concordance between biopsy specimens and excised tumor tissue is seen in the case of HER2 overexpression (55%) as opposed to lack of overexpression (87%) of HER2 (equivocal results excluded) (table 4).

Overall, a clinically significant correlation of HER2 status in biopsy specimens compared to excised tumor tissue was observed in 69% of all investigated cases ($r = 0.713$; $p < 0.0001$).

Discussion

Patients with ER and/or PR expression equal to or greater than IRS 3 are considered steroid hormone receptor positive and may benefit from adjuvant endocrine therapy. ER expression was accurately predicted by immunohistochemical profiling of core biopsy tissue in all but three tumors. All but one ER expressing tumor was accurately identified by immunohistochemical profiling of core biopsy tissue. A total of 2 PR-positive tumors were not identified by this method. In the above cases, a negative expression in core biopsy was followed by a weak expression in excised tumor tissue. Nonetheless, not a single patient with a hormone receptor positive tumor > IRS 4 (for either ER or PR) upon excision would have had endocrine therapy withheld, since these patients showed either ER or PR (but not both) expression in core biopsy tissue. Although clinically significant correlation (ER: 99% and PR: 88%) is statistically highly significant, it is interesting to note that categorical expression of ER (54%) as well as PR (55%) is, although also statistically significant, relatively unsatisfactory. Since there is neither a consistent up or down scoring in ER or PR expression of biopsy and tumor tissue, an alteration of antigenicity caused by fixation or embedding processes is unlikely. The most likely explanation for the observed inconsistency in categorical immunohistochemical results is the inhomogeneity of the tumor itself.

Mann et al. [9] comment that tumor heterogeneity and sampling errors in core biopsy could explain some variations in results, however, not those cases with strong staining in the core biopsy but none in the surgical specimen. We did not observe this discrepancy in ER or PR staining results.

The correlation of HER2 expression levels (46%) was disappointing. Although the immunohistochemical analysis of core biopsy tissue identified 87% of the HER2 negative tumors, HER2 overexpressing tumors (3+) were identified in only 55%. When considering the therapeutic consequences of these

results, 13% of patients with HER2/neu negative tumors upon excision showed moderate to strong HER2/neu expression in core biopsy tissue, while 45% of patients with HER2/neu overexpressing tumors did not show overexpression in core biopsy tissue. The significant benefits of trastuzumab therapy [10, 11] combined with potential cardiotoxicity in approximately 3% and the high cost of therapy mandate the highest possible accuracy in HER2 profiling. Ideally, sensitivity and specificity should approach 100%.

In practice, immunohistochemical analysis of core biopsy specimens is a standard in providing the basis for planning primary systemic therapy. Coupled with the immunohistochemical profile of a tumor upon excision in the case of primary operative breast cancer therapy, these data also provide the basis for definitive therapy planning. As far as hormone receptor expression is concerned, the preceding results have shown this practice to be suitable. The overall correlation of clinically significant HER2 expression in this retrospective analysis, while statistically significant at 69%, definitely seems worthy of improvement. In a standard situation, further testing is deemed mandatory only where HER2 expression is equivocal (2+). In these cases, the determination of HER2 gene amplification by fluorescent in situ hybridization (FISH) is recommended. Cahill et al. [12] report that diagnostic core biopsy (DCB) correctly ascribed ER, PR, and HER2 receptor status in most cases (68%, 71%, and 60% respectively) and accurately identified all tumors with clinically important ER and HER2 expression. Hence, they come to the conclusion that DCB reliably categorizes patients with regard to (neo)adjuvant therapy. Taucher et al. [13] came to similar conclusions. On the other hand, although Mann et al. [9] report on concordance for clinically significant staining of HER2 in 80%, when raw scores were compared, there was concordance in only 42%. Without applying FISH in all of (their) cases, they did not find it possible to determine whether core biopsy or surgical specimen HER2 immunohistochemical assessment is more accurate. Mann et al. [9] report that they do not rely on core biopsy immunohistochemistry for HER2 when making treatment decisions. After analysis of our results, we must come to a similar conclusion. We currently consider only patients with immunohistochemical overexpression of HER2 in both biopsy and tumor tissue to be eligible for trastuzumab therapy without further investigation of gene amplification. Any discrepant immunohistochemical findings which include the results 2+ or 3+ in one of both tissue specimens are verified by gene amplification. In order to determine whether the core biopsy specimen or the excised tumor provides the more relevant information pertaining to HER2 status, it is necessary to correlate immunohistochemical and gene amplification results. Only then can we approach the level of accuracy necessary to plan the most suitable primary systemic as well as adjuvant therapy regarding the implementation of trastuzumab in patients with breast cancer.

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