

Original Article

Predictive markers in primary breast cancer compared with lymph node and bloodspread metastases

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Received November 23, 2008; accepted February 9, 2009; available online February 15, 2009

Abstract: High levels of HER2 expression identify those patients who might benefit from treatments that target HER2. Among women with metastatic breast cancer, the predictive markers may be different from the primary tumor. We compared predictive markers: Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2 of primary breast carcinomas with those of lymph node (LN) and blood spread metastases (BM). ER, PR and HER2 status were compared between the primary breast tumor and the LN metastasis and blood spread metastasis. ER, PR and HER2 were performed on primary tumor core biopsies and available FNA cell blocks and on metastatic lesions using FDA approved antibodies and HercepTest (Dako). ER and PR were positive when $\geq 10\%$. Her2 was positive (amplified/expressed) when $3+ >30\%$ by immunostain or >2.2 by FISH. Sixty four metastatic breast cancer patients were included in this analysis. Forty-eight patients had LN metastases (35 [73 %] diagnosed by FNA) and twenty seven patients had BM (16 [60 %] diagnosed by FNA). P value was determined comparing primary breast with BM and LN for ER, PR and HER2. ER p values when compared for primary breast with BM and LN were 0.45 and 0.57 respectively, and for PR were 0.31 and 0.06 and for HER2 were 0.45 and 0.07. All three predictive markers are similar in the primary and two metastatic sites (lymph node, bloodspread). Only in primary versus lymph node metastases is there a tendency for PR and HER2 (P values 0.06, 0.07) to be different. For HER2, the majority of lymph node metastases are in cell blocks (FNA), fixed in ethanol rather than formalin, which may have caused false positive HER2 expression.

Key Words: Breast carcinoma, prognostic markers, metastatic breast cancer, hormone receptors

Introduction

Annually, 182,460 American women are diagnosed with breast cancer, and 40,480 die from this disease [1]. In addition breast cancer is the most common female cancer in the United States, and the main cause of death in women ages, 45 to 55 [1]. Hormone therapy offers several advantages over cytotoxic chemotherapy for breast cancer treatment. Assay of hormone receptors such as estrogen receptor (ER) and progesterone receptor (PR) are an important component of the pathologic

evaluation of a newly diagnosed breast cancer. Women with hormone receptor-positive tumors benefit from the addition of postoperative endocrine treatments such as tamoxifen, or in postmenopausal women, the aromatase inhibitors such as anastrozole, letrozole, exemestane, or for premenopausal women, ovarian ablation or suppression. Hormone therapy is not beneficial for women with hormone receptor-negative tumors. Among women with newly diagnosed metastatic disease, approximately 30-40% will have an objective response to hormone therapy,

sometimes lasting for several years. Furthermore, a substantial number will have a clinically significant period of disease stability (i.e., neither disease progression nor regression) [2-4]. Although objective response rates are higher with first-line chemotherapy (50-60%), toxicity is worse than with hormone therapy, and responses are generally not durable.

The published clinical data also suggests that hormone receptor status is an important predictor of responsiveness to chemotherapy. The survival gains from advances in adjuvant chemotherapy over the last 20 years appear to be only marginal in patients with ER-positive breast cancer, and significantly less than those seen in patients with ER-negative tumors [5]. The latest update of the Oxford meta-analysis has also shown that in greater than 30,000 patients, the benefit of adjuvant tamoxifen is limited to patients with expression of HRs [6].

The HER2 receptor belongs to the epidermal growth factor receptor (EGFR) family of receptors, which are critical in the activation of subcellular signal transduction pathways controlling epithelial cell growth and differentiation [7, 8]. Amplification of HER2 or overexpression of its protein product is observed in 18 to 20 percent of human breast cancers [9-11]. In metastatic disease, the benefit of the anti-HER-2 monoclonal antibody trastuzumab is clearly limited to those patients with HER-2 overexpression or amplification [12]. The American Society of Clinical Oncology Tumor Marker Guidelines Panel has recommended routine testing of HER2 expression on newly diagnosed and metastatic breast cancers since 2001 [13]. A joint committee representing the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) has also published a set of guidelines that specifically address to the technical and analytical aspects of HER2 testing [14]. This committee recommended strict accreditation for laboratories providing HER2 testing.

Material and methods

The purpose of our study was to compare HER-2 amplification and HRs status in primary breast tumors versus lymph node (LN) and blood spread metastases (BM). All specimens from both primary tumors and metastatic site lesions were analyzed by a dedicated immunohistochemistry (IHC) pathologist. HRs status was evaluated by IHC and HER-2 status was evaluated by immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH).

The tumor specimens were fixed in 10% neutral-buffered formalin before routine processing and embedding. Immunohistochemical staining was performed as follows: Sections (4 microns) of formalin-fixed, paraffin-embedded tissue were tested for the presence of primary antibody using DAKO Envision®+ dual link system which is an HRP labeled polymer (DAKO, Carpinteria, CA) with heat induced antigen retrieval. The sections were deparaffinized and rehydrated to deionized water. They were then heated in citrate buffer (ph 6.0), using an electric pressure cooker for 3 minutes at 12-15 pounds per square inches (PSI) (approx 120 C), and cooled for 10 minutes prior to immunohistochemical staining. All slides were loaded on an automated system (DAKO Auto Stainer plus, DAKO, Carpinteria, CA) and exposed to 3 % hydrogen peroxide for 5 minutes, incubated with primary antibody (DAKO, Carpinteria, CA) dilution for 30 minutes, with labeled polymer for 30 minutes, 3,3'-diaminobenzidine (DAB) as a chromogen for 5 minutes, and hematoxylin as counter stain for 5 minutes. These incubations were performed at room temperature; between incubations sections were washed with Tris-buffered saline (TBS). Cover-slipping was performed using the Tissue-Tek SCA (Sakura Fintek USA, Inc, Torrance, CA) coversliper. New positive controls of known positive breast carcinoma, and negative controls with primary antibody replaced with TBS were run with the patient/study slides. ER/PR : >10% is positive;

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Table 1. Clinical data of patients included in the study

Breast cancer with lymph node and blood spread metastasis (n=64)		
SEX	Female	61 (95%)
	Male	3 (5%)
AGE	< 50	27 (42%)
	>/=50	37 (58%)
TYPE*	Infiltrating ductal CA	55 (86%)
	Infiltrating lobular CA	1 (1.5%)
	Infiltrating ductal and lobular CA	1 (1.5%)
SIZE*,#	</= 2 cm	27 (42%)
	> 2 cm	17 (27%)
GRADE*, \$\$	III	27 (42%)
	II	21 (33%)
	I	6 (9%)
DCIS*	Present	38 (59%)
	Absent	19 (29%)
LVI*	Present	21 (33%)
	Absent	36 (56%)

DCIS: ductal carcinoma in-situ; LVI: lymphovascular invasion in the breast lesion (at the time of diagnosis);

*Seven patients has no information regarding the breast primary in our record (diagnosed at outside institution); # the rest of the patients diagnosed by core biopsies so the size is not available; \$\$ two patients received neo-adjuvant chemotherapy so the grade is not applicable and one patient found to have DCIS in the breast but had multiple lymph node metastases.

<10% is negative. HER2 is considered negative when it is less than 10% and equivocal (2+) when >10% but < 30%, and it is positive when (+3) >30%. FISH: <1.8 is negative; 1.8-2.2 is equivocal and >2.2 is positive.

Overall and positive agreement with the score confidence intervals (CI) on overall agreement between the tissue types (primary breast cancer, BM and LN) was calculated. Kappa was also calculated as an alternative measure of agreement. McNemar's test for paired binary outcomes was used to compare positive percentages.

Results

This study reviewed data from sixty four patients (**Table 1**) on whom clinical follow-up was available. In comparing primary breast cancer versus BM (**Table 2**), 19 patients (30 %) had ER and 18 patients (28 %) had PR results available; 17 patients (27 %) had HER2 results available by IHC and 4 patients (6 %) by FISH. Positivity rates for ER, PR and HER2 done by IHC and FISH (P value is 0.45, 0.31, 0.45 and 0.32 respectively) shows a nonsignificant trend toward loss of hormone receptors or loss of HER2 amplification in patients with BM compared to the primary breast cancer.

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Table 2. Comparison of primary breast cancer with blood spread metastasis

Variables	N	Overall agreement% (95% CI)	Kappa	Positive, breast	Positive, blood spread metastasis	P value
<u>ER</u> : Breast vs. BM	19	68.4 (46.0, 84.6)	0.30	79%	58%	0.45
<u>PR</u> : Breast vs. BM	18	61.1 (38.6, 79.7)	0.28	61%	33%	0.31
<u>HER2</u> : Breast vs. BM	17	52.9 (31.0, 73.8)	0.28	24%	24%	0.45
<u>FISH</u> : Breast vs. BM	4	75.0 (30.1, 95.4)	0.50	50%	25%	0.32

ER Estrogen receptor, PR progesterone receptor, LN lymph node, BM blood spread metastasis

The comparison of primary breast cancer versus LN metastasis (**Table 3**), 42 patients (64%) had ER and PR available; 39 patients (61 %) had HER2 done by IHC and 10 patients (16 %) by FISH. Positivity rates for ER and FISH (P value is 0.57 and 0.39 respectively) shows a non-significant trend toward loss of hormone receptor or loss of HER2 amplification in the metastatic lymph node site comparing to the primary breast cancer. However, only in primary vs. LN metastases is there a tendency for PR and HER2 (P values 0.06, 0.07) to be different. For HER2, the majority of LN metastases are in cell blocks (FNA), fixed in ethanol rather than formalin, which may have caused false positive HER2 expression.

When comparing BM metastasis versus LN metastasis (**Table 4**), 12 patients (19 %) had ER and PR results; 11 patients (17 %) had

HER2 done by IHC. Positivity rates for ER, PR and her2 done by IHC (P value is 0.92, 0.57 and 0.57 respectively) shows a non-significant trend toward loss of hormone receptors or loss of HER2 amplification in the BM comparing to the LN metastasis.

Discussion

It is important to distinguish between a "prognostic" factor and a "predictive" factor when evaluating either traditional or newer cancer markers [15, 16]. A prognostic factor is capable of providing information on clinical outcome at the time of diagnosis, independent of therapy. Such markers are usually indicators of growth, invasion, and metastatic potential. A predictive factor is capable of providing information on the likelihood of response to a given therapeutic modality. Such

Table 3. Comparison of primary breast cancer with lymph node metastasis

Variables	N	Overall agreement% (95% CI)	Kappa	Positive, breast	Positive, lymph node	P value
<u>ER</u> : Breast vs. LN	42	76.2 (61.5, 86.5)	0.52	64%	55%	0.57
<u>PR</u> : Breast vs. LN	42	73.8 (58.9, 84.7)	0.48	48%	26%	0.06
<u>HER2</u> : Breast vs. LN	39	59.0 (43.4, 72.9)	0.28	8%	26%	0.07
<u>FISH</u> : Breast vs. LN	10	70.0 (39.7, 89.2)	0.46	30%	30%	0.39

ER Estrogen receptor, PR progesterone receptor, LN lymph node, BM blood spread metastasis

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Table 4. Comparison of blood spread metastasis with lymph node metastasis in patients with primary breast cancer

Variables	N	Overall agreement% (95% CI)	Kappa	Positive, lymph node	Positive, blood spread metastasis	P value
<u>ER</u> : BM vs. LN	12	33.3 (13.8, 60.9)	0.00	50%	33%	0.92
<u>PR</u> :BM vs. LN	12	33.3 (13.8, 60.9)	0.00	50%	17%	0.57
<u>HER2</u> : BM vs. LN	11	36.4 (15.2, 64.6)	0.00	9%	18%	0.57
<u>FISH</u> : BM vs. LN	1	-	-	-	-	-

ER Estrogen receptor, PR progesterone receptor, LN lymph node, BM blood spread metastasis

markers are either within the target of the treatment, or serve as modulators or epiphenomena related to expression and/or function of the target. Lymph node status, for example, is an important prognostic factor, but provides no information on the likelihood of response to therapy. In contrast, hormone receptor (ER and/or PR) expression is a predictive factor since it indicates the likelihood of response to endocrine therapy. According to the American Society of Clinical Oncology (ASCO), ER, PR, and HER2 overexpression should be evaluated on every primary breast cancer. Hormone receptor expression should be used to guide therapy decisions in both the adjuvant and metastatic disease settings [17]. The available evidence suggests that ER/PR-negative tumors have a worse prognosis, at least in the first five to ten years after treatment [18, 19].

Amplification or overexpression of the HER2 oncogene is present in approximately 18% to 20% of primary invasive breast cancers. The available data supports the view that HER2 overexpression in tumor tissue is associated with a poorer prognosis in early breast cancer. The International Consensus Group uses HER2 overexpression as one of the risk stratification features to select patients who are at a high enough risk to warrant chemotherapy [20].

We compared ER, PR and HER2 status between primary breast tumor and LN metastasis and BM metastasis. Most of LN metastasis and BM metastasis were diagnosed by FNA procedure with cell block preparation. Forty eight patients had LN metastasis; thirty-five of them (73 %) were diagnosed by FNA. Twenty seven patients had BM metastasis, sixteen (60%) of them were diagnosed by FNA. To the best of our knowledge there is no other study that has used the FNA-cell block material to compare between HRs and HER2 primary breast tumor and metastasis. In this study we found that the change in hormone receptors HRs (ER/PR) and the expression of HER-2, if present, is not statistically significant. However, only in primary versus LN metastases is there is a tendency for PR and HER2 (P values 0.06, 0.07) to be different. For HER2, the majority of lymph node metastases are in cell blocks (FNA), fixed in ethanol rather than formalin, which may have caused false positive HER2 expression.

In the literature, the reports are conflicting; some reports have shown a lack of concordance in the expression of these predictive factors between primary tumors and metastatic sites, as measured by IHC and/or FISH. Neubauer et al. studied 87 patients with

breast cancer who were treated with neoadjuvant chemotherapy and found that 7, 16 and 11 of these 87 patients changed the status of ER, PR and HER2 expression respectively so he recommended that HER2 status as well as ER and PR status should be re-evaluated on post-chemotherapy surgical specimens since changes can be observed [21]. Morimoto et al. reached the same conclusion when he studied the change of ER and PR status in 177 patients with metastatic breast cancer and found that 30% of the patients had a different result in the metastatic site in comparison to the primary site [22]. However other researchers like Gancberg et al. who studied HER-2 status in 107 patients with a primary breast tumor and at least one distant metastatic lesion analyzed by IHC and FISH and found that between the paired primary tumors and distant metastatic lesions, 94% and 93% of samples had concordant HER2 status when analyzed by IHC or FISH, respectively, so he concluded that these results do not support routine determination of HER-2 on metastatic sites [23]. Shimizu et al. have also studied 21 patients with breast cancer, and concluded that HER2 and p53 expression levels in breast cancer cells were almost unchanged as the disease progressed and/or in response to adjuvant therapies, regardless of the hormone receptor status [24]. Tanner et al. have also studied 46 patients with breast cancer and documented metastasis, 28% of his patients had HER2 amplification, in his study he was able to show that HER2 amplification status always remained the same between primary tumor and its metastasis, despite the fact that in some cases the metastases appeared more than 10 years after removal of the primary tumor, he concluded that amplification of HER2 measured in primary tumor reflects the status of metastasis [25].

In conclusion, our study indicates that, although there are some changes in the status of ER or HER2 over expression, these changes are not statistically significant. Although it is important to get a tissue diagnosis, if biopsies

of the metastatic sites are otherwise not needed, there is no need to subject patients to biopsies because of HER2 diagnostics or ER status. It is important to realize that our study is based -most of the times- on FNA/ cell block preparation. However more studies are needed since we did not check if the anti-hormone-treated cancer has affected the status of ER, PR or Her2, also we did not follow the Allred's procedure as many places are using them as their standard in reading the ER/PR immunostain. Also in the view of the limited number of patients in our study, more studies on the FNA-cell block-diagnosed specimens are needed.

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