Changes in Estrogen Receptor, Progesterone Receptor and Her-2/neu Status with Time: Discordance Rates Between Primary and Metastatic Breast Cancer

REUBEN J. BROOM[1], PATRICIA A. TANG[1], CHRISTINE SIMMONS[1], LOUISE BORDELEAU[2], ANNA MARIE MULLIGAN[2], FRANCES P. O'MALLEY[2], NAOMI MILLER1, IRENE L. ANDRULIS[2,3], DARREN M. BRENNER[3] and MARK J. CLEMONS[1]

[1]*Princess Margaret Hospital, Toronto;*
[2]*Mount Sinai Hospital, Toronto;*
[3]*Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada*

Abstract. *Background: Changes in the receptor profile between primary and metastatic breast cancer tissue have been suggested. The degree of hormone receptor discordance in archival paired pathological samples was evaluated. Materials and Methods: Archival data were collected on 100 patients for whom tissue from primary and metastatic sites was available. Estrogen receptor (ER), progesterone receptor (PR) and Her-2/neu status in the primary and metastasis were compared. Results: The discordance rate for ER was 17.7% (2-sided p=0.0039) with 9.7% of tumours changing from ER-positive to ER-negative and 8.0% changing from ER-negative to ER-positive. The discordance rate for PR was 37.3% (2-sided p<0.0001), with all of these tumours changing from PR-positive to PR-negative. No significant discordance for Her-2/neu was found. Conclusions: This series suggests that significant discordance exists for hormone receptor status between primary and metastatic breast cancer samples. Loss of PR was particularly frequent.*

Hormone receptor discordance between primary and metastatic breast cancer specimens from the same patient has been recognized for both estrogen receptor (ER) (1-4) and progesterone receptor (PR) (2-5). Her-2/neu receptor discordance is also described but with less consistent results (6-9). There is however, a paucity of data on hormone receptor discordance in the context of Her-2/neu receptor status and this warrants further investigation. This is especially important given the postulated interactions between the hormone and Her-2/neu receptors. Following the pivotal trial by Slamon *et al*. that demonstrated a survival advantage in the addition of trastuzumab to chemotherapy in the metastatic setting (10), many cancer centres began performing Her-2/neu testing of primary tumour specimens in patients who had subsequently developed metastatic disease. It is only much more recently, following the results of the adjuvant trastuzumab trials (11, 12) that routine Her-2/neu testing on primary breast cancer specimens at the time of diagnosis has been performed. Even though some centres were testing for Her2/neu prior to these timelines for prognostication, there is less data available for Her-2/neu receptor discordance rates then there is for hormone receptors.

Considerable controversy surrounds the issue of hormone receptor discordance, and while a number of possible mechanisms have been proposed, none are widely accepted. Furthermore, it is suspected that technical issues related to specimen analysis and variation in staining methodology may contribute to "pseudo-discordance". True receptor discordance, however, may have important clinical implications with respect to systemic therapy decisions. Therefore, the existence of true discordance would support an argument for obtaining metastatic tissue in patients for whom there is clinical or radiological suspicion of disseminated breast cancer.

The aim of this current study was to evaluate the degree of hormone and Her-2/neu receptor discordance between paired pathology samples of primary and metastatic breast cancer specimens from the same patient.

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Correspondence to: Dr. Reuben James Broom, Medical Oncologist, Building 7, Auckland City Hospital, Park Road, Grafton, Auckland, New Zealand. Tel: +64 9 3074949 ext 23868, Fax: +64 9 3074984, e-mail: ReubenB@adhb.govt.nz

Key Words: Hormone receptors, discordance, Her-2/neu, breast cancer, metastases.

Table I. *Patient demographics and primary tumour characteristics.*

	Patients (n=100)
Gender (%)	
Female	100
Age at diagnosis (years)	
Median	50
Range	29-79
Histology (%)	
Ductal	77
Lobular	8
Mixed	3
Unknown	12
Grade (%)	
One	6
Two	30
Three	47
Unknown	17
T stage (%)	
One	48
Two	35
Three	5
Four	3
Unknown	9
N stage (%)	
Zero	62
One	15
Two	13
Three	2
Unknown	8

Table II. *Metastasis biopsy details.*

	Patients (n=100)
Time to metastasis biopsy (years)	
Median	4.0
Average	5.4
Range	1-35
Date of metastasis biopsy (%)	
Pre 2001	36
During or post 2001	64
Site of metastasis biopsy (%)	
Bone or bone marrow	33
Lymph node or skin	24
Thorax / lung	24
Abdomen / liver	15
Central nervous system	3
Other	1

Table III. *Receptor assay methods (n=100).*

	EIA	IHC	FISH	Not available
HR-primary	53	29		18
HR-metastasis	1	57		42
Her-2/neu-primary		41	7	52
Her-2/neu-metastasis		29	11	60

EIA: Enzyme-linked immunoassay, IHC: immunohistochemistry, FISH: fluorescent *in situ* hybridization.

Materials and Methods

Pathology databases at two Toronto hospitals (Princess Margaret Hospital and Mount Sinai Hospital) were searched to identify 100 patients for whom both primary and metastatic breast cancer specimens were available. The main search terms used were "metastatic" and "breast" but excluded "axillary" lymph node samples. Such ipsilateral lymph node samples are often termed "metastatic breast cancer" in pathology reports of node positive patients and these specimens were not included in the current analysis. Patients were only included in this analysis if they had both an accessible clinical record for review and pathology information available (*i.e.* a report) on both the primary and the metastatic specimens including at least one of ER, PR or Her-2/neu status. The patient's chart and pathology reports were reviewed and demographic, tumour and treatment characteristics recorded. Logistic regression analysis was performed to see if any of the following variables influenced hormone receptor discordance; adjuvant hormone therapy, Her-2/neu status, adjuvant chemotherapy use, time from primary diagnosis to metastatic biopsy, site of metastatic biopsy and primary tumour grade. Research Ethics Board approval was obtained from both the institutions involved.

Results

Patient and primary tumour characteristics. One hundred patients were identified that met all inclusion criteria. Patient demographic information as well as the primary tumour characteristics are shown (Table I).

Metastatic biopsy details. The metastatic biopsy details are shown in Table II. Over one third of samples were collected prior to 2001 when Her-2/neu testing was rarely performed. The most common site from which a metastatic biopsy was taken was the bone or bone marrow. Many of these samples were collected for investigation of cytopenia or were taken at the time of orthopedic intervention for treatment of an impending or actual pathological fracture.

The receptor assay methods used are depicted in Table III. The type of assay used for the hormone receptor analysis in the primary [predominantly enzyme-linked immunoassay (EIA)] differed greatly from that used for the metastasis [predominantly immunohistochemistry (IHC)]. This mirrors a

Table IV. *Hormone receptor results (n=100).*

	ER		PR	
	Primary	Metastasis	Primary	Metastasis
Positive	73	52	60	26
Negative	24	12	35	36
Not available	3	36	5	38

Table V. *Her-2/neu receptor results (n=100).*

	Primary	Metastasis	Primary or metastasis
Positive	7 (14.6%)	6 (15.0%)	13 (18.6%)
Negative	41	34	57
Not available	52	60	30

shift in practice over time towards using IHC hormone receptor assays. In assessing methods of Her-2/neu testing, there was proportionally more fluorescent *in situ* hybridization (FISH) testing compared to IHC performed on the metastasis compared with the primary specimen. This may have been because metastases specimens were more likely to be in the form of core biopsies where FISH testing is favoured by many pathologists as it is thought to be more technically reliable.

Hormone receptor results. The hormone receptor status of all the primary or metastases samples is outlined in Table IV. Unfortunately, over a third of patients did not have hormone receptor status testing completed on the metastatic lesion. Out of the 100 patients included in this analysis, there were paired estrogen receptor samples available in 62 patients. Discordance for ER was found in 17.7% of these cases, with 6 tumours (9.7%) switching from being positive in the primary to negative in the metastasis and 5 tumours (8.0%) switching from negative to positive. This discordance rate for ER was statistically significant with a two-sided *p*-value of 0.0039. Paired progesterone assay results were available in 59 patients. Among these patients, there was a highly significant PR discordance rate of 37.3% ($p<0.0001$) and all of the 22 tumours that changed status switched from being PR-positive to PR-negative.

Her-2/neu receptor results. The Her-2/neu status in patients' primary or metastatic specimens is shown in Table V. Although most patients were not tested for Her-2/neu on both their primary and their metastatic specimens, 70% of patients were tested for Her-2/neu on either one of their samples. Paired Her-2/neu samples were available in 18 patients and one (5.5%) of these patients exhibited discordance ($p=0.114$). This patient switched from being positive in the primary to negative in the metastasis.

Logistic regression analysis. Logistic regression analysis was performed to evaluate whether adjuvant hormone therapy, Her-2/neu status, adjuvant chemotherapy use, time from primary diagnosis to metastatic biopsy, site of metastatic biopsy or primary tumour grade influenced hormone receptor discordance. None of these variables were significantly associated with the occurrence of hormone receptor discordance.

Discussion

In this retrospective analysis, significant discordances were found for the hormone receptor status between primary and metastatic breast pathology samples. A change in ER status over time occurred in 17.7% of patients (switching occurred both from ER-positive to -negative and *vice versa*) and for PR it occurred in 37.3% of patients (all of these tumors lost PR). This meant that a total of 45.1% of patients had some sort of hormone receptor change (*i.e.* a change in either ER or PR). These results are not dissimilar to what has been previously reported in the literature. In an analysis of 232 patients, Hull *et al*. found that 19% of patients in their series lost ER and 13% gained ER over time (1). Lower *et al*. found a discordance rate for ER of 30 % in a chart review of 200 patients with 19.5% of tumours losing ER and 10.5% gaining ER (2). For PR, this group found a discordance rate of 39.3%. Mobbs *et al*. performed a retrospective pathology specimen review of 129 cases and found discordance rates of 24 and 30% for ER and PR respectively (3). Gross *et al*., in their series of 161 cases, found that 44% of patients lost PR, however 8% of patients gained PR (5). And finally a meta-analysis of 8 observational studies was performed by Franco *et al*. totaling 658 paired ER samples and 418 paired PR samples (4). They found a discordance rate of 29 and 27% for ER and PR respectively.

There are less data available on changes in Her-2/neu status with time, however all the series published to date suggest that Her-2/neu status is more stable (6-9). The highest rate of Her-2/neu discordance was found by Zidan *et al*. in a series of 58 patients whose tumours had been tested by either FISH or IHC (6). They found a discordance rate of 14%, with 12% of patients losing Her-2/neu positivity and 2% gaining it over time. Other published series have found lower rates of Her-2/neu discordance including one series which found that none of the 56 cases analysed had changed status (7). In the presented series of 100 patients with paired primary and metastatic pathology samples, who had had 'some' receptor analysis performed on both specimens, a significant discordance rate for Her-2/neu was not found, with only one patient losing Her-2/neu positivity. However, because Her-2/neu testing had not been performed in both specimens from most of these patients, few paired Her-2/neu samples were available to analyse for discordance.

There are three possible reasons for the lack of Her-2/neu testing on the patients included in this series. Firstly, there may have been an assumption by clinicians and pathologists that Her-2/neu status in the metastasis is the same as in the primary, therefore the test was not requested. Secondly, over a third of metastatic samples were collected before 2001, when Her-2/neu testing was not routinely performed. Thirdly, the most common site of metastasis biopsy was bone and there may be technical issues related to Her-2/neu testing in decalcified bone making it more difficult to perform.

Incidentally it was noted that there was a large proportion of patients with T1 and N0 tumours in this series of patients who had all relapsed with metastatic disease. One possible reason for this is that these patients would have been thought to have been at a lower risk of relapse and therefore when they presented with clinical or radiological evidence of metastatic disease, clinicians may have been more eager to obtain a biopsy to confirm or refute the diagnosis of metastatic breast cancer. This may have meant that this series of patients was skewed to be different from the typical population of patients with metastatic breast cancer with respect to their primary tumour characteristics. This is one limitation of this study. There are several other important limitations of this study. Firstly, a central pathology review was not performed. This was a chart review with the receptor status being taken typically from the pathology report and some patients had their primary tumour tested at a different pathology laboratory (commonly another Toronto community hospital) to that where their metastatic sample was analysed (Princess Margaret or Mt. Sinai Hospitals). This analysis is therefore subject to interlaboratory variation. Secondly, for both hormone receptor and Her-2/neu receptor analysis there were often different assay methods used on the primary and metastatic specimens and this could have confounded the results. EIA was the main method utilized for hormone receptor analysis on the primary specimen whereas IHC was almost invariably used when the metastasis was analysed. This represents a change in practice over recent years. FISH (as the definitive test for Her-2/neu) was used proportionally more often in the analysis of the metastasis compared to the primary and this is likely because it is thought to be a more reliable method when a core biopsy is done. Thirdly, a label of either positive or negative was ascribed for each receptor as interpreted from the pathology report. It is possible that a tumour which changes its PR status from being weakly positive to negative may have less clinical meaning than a change of greater magnitude and the analysis did not capture this information. The definition of hormone receptor "positive" varied depending on which laboratory and by what method the analysis was done. As was already mentioned, there was a considerable amount of missing data, especially for Her-2/neu status, and this negatively impacted on the power of this study.

Despite the limitations of this study, this review does add weight to the literature that significant hormone receptor discordance exists for both ER and PR. This has a number of potential clinical implications for the management of patients. Firstly, a proportion of patients with metastatic disease may be sub-optimally treated in the absence of a biopsy. This may be especially so if a patient's receptor status has become positive over time and, if this is not known, they may be deprived of potentially life-prolonging targeted treatment such as endocrine therapy. Secondly, patients may be inappropriately enrolled in clinical trials of systemic therapy. Many such trials are powered to detect small differences in efficacy between therapies. It is therefore possible that because of this discordance phenomenon, unknown imbalances in receptor status between the arms of trials (as eligibility is often based on the status of the primary tumour) may influence the results.

One strong theme that is perpetuated in both this article and elsewhere is the high rate of "loss of PR" over time. Out of all three receptor discordance phenomena discussed here, loss of PR is the one that has generated the most biological research. PR is an ER-regulated gene which is expressed in two isoforms, PR-α and -β. PR mediates the effects of progesterone on the development of mammary glands in healthy individuals and is implicated in the development of breast cancer. There is an increased breast cancer incidence in patients taking combined estrogen and progesterone hormone replacement therapy compared to estrogen therapy alone (13). ER-positive/PR-negative metastatic tumours tend to have a more aggressive course and are associated with a reduced overall survival compared to those retaining PR (5, 14). Therefore it is not surprising that there are more PR-negative tumours amongst the metastatic samples compared to the primaries. There is an increased association between Her-2/neu overexpression and ER-positive/PR-negative tumours (25%) compared to ER-positive/PR-positive (10%) tumours (15). There is also an association between the ER-positive/PR-negative phenotype and high tumour grade (14). Furthermore ER-positive/PR-negative tumours have higher levels of EGFR expression compared to ER-positive/PR-positive tumours (16). These associations with the ER-positive/PR-negative phenotype implicate high growth factor activity with a decrease or loss of PR. Therefore one of the predominating theories explaining loss of PR is growth factor-mediated downregulation of PR, independent of ER (17).

There are several other postulated mechanisms for loss of PR. One is genetic loss of the *PR* gene locus or loss of heterozygosity. Another is hypermethylation of the *PR* promoter, which is found in 21-40% of ER-positive/PR-negative tumours compared with none of the tumours which

exhibit the ER-positive/PR-positive phenotype (18). One other theory attempting to explain the ER-positive/PR-negative phenotype is that in these tumours ER is non-functional and therefore unable to stimulate PR production (19). However, some ER-positive/PR-negative tumours may simply result from low levels of circulating endogenous estrogens in post-menopausal patients that are insufficient to induce PR expression even though the ER pathway is intact (20).

In conclusion, this study has demonstrated significant discordance for hormone receptor status between primary and metastatic specimens and this may have implications for the systemic treatment of patients' metastatic disease. In particular, loss of PR was found in 37.3% of paired samples, and this may reflect (or even cause) a shift to a more aggressive tumour biology. Her-2/neu status seems more stable than either ER or PR although this analysis was not sufficiently powered, because of missing data, to directly assess this. This was not a prospective study and therefore this analysis carries the burden of all the pitfalls inherent to a retrospective review. The Authors' group has recently completed what is understood to be the first prospective study assessing the rate and practical implications of hormone and Her-2/neu receptor discordance (*i.e.* asking the question "Does a confirmatory metastasis biopsy alter clinical management?"). The preliminary results available on twenty-nine individuals have recently been presented and demonstrate a discordance rate for hormone receptor status of 40% and for Her-2/neu of 8% (two patients becoming positive for Her-2/neu in the metastatic sample) (21). The results of a confirmatory biopsy altered management in 20% of patients. These preliminary results further support the phenomenon of receptor discordance and reinforce the importance of obtaining a confirmatory biopsy when patients present with suspicion of metastatic disease.

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