Investigation of the change in the biological structure of the tumor in metastatic breast cancer

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ABSTRACT

Objective: When metastasis develops in some breast cancer patients, hormone receptors (HR) and Human Epidermal Growth Factor-2 (Her-2) status can change and the tumor alters its character. We tried to determine the rate of these changes in tumor biology in 110 patients that we followed in our clinic and performed the change of the biopsy from the metastatic site (re-bx). We aimed to determine the biological changes of tumors and, contribute to the literature by examining the relationship of these changes with the adjuvant endocrine treatments (ET) or chemotherapy type (CT).

Material and Methods: We included 110 metastatic breast cancer patients in our study. These patients had previously completed their local treatments followed by CT, and those with positive HR completed ET. After the first metastasis developed in the patients, we performed metastasectomy or biopsy from the metastatic site.

Results: The median ki-67 value was 25% at the time of primary diagnosis and 30% in re-bx. 20.9% of patients estrogen receptor (ER), 31.8% of patients progesterone receptor (PR) and 26.3% of patients Her-2 changed when metastasis developed.

Conclusions: We found that the metastatic tumor has more aggressive properties than the primary tumor. Adjuvant chemotherapy and endocrine treatments or the location of metastasis did not make a significant difference in tumor biology.

Keywords: Breast cancer, metastatic breast cancer, change of hormone receptors

INTRODUCTION

Among women, breast cancer is the most common type of cancer all over the world and second among the causes of cancer-related death (**1**). As in all other types of cancer, multidisciplinary treatment is a sine qua non for success in the treatment of breast cancer (**2**). In recent years, with the increasing number of molecular predictive biomarkers, personalized treatments have come to the fore in breast cancer. In both early-stage and advanced-stage disease, the parameters including hormone receptors (HR) such as Estrogen (ER) and Progesterone Receptor (PR), Human Epidermal Growth Factor-2 (Her-2), grade, and ki-67 proliferation index are the most important markers that determine the treatment decision (**3**). HR and Her-2 status both assess treatment options and provide information about the prognosis of the disease. Therefore, according to HR and Her-2 status, breast cancer is divided into three subgroups: Hormone receptor-positive, Her-2 positive, and triple-negative (**4**). While ER positivity is around 75%, PR positivity 60%, and Her-2 receptor positivity 20% in breast cancer, the rate of negativity of all three is about 15% (**5**). However, when metastasis develops in some patients, the tumor alters its character, and HR and Her-2 status can change. The exchange of these receptors necessitates radically changing the treatment strategy. Parameters that change with the development of metastasis are not limited to HR and Her-2. Still, we can add markers showing tumor behavior such as grade and ki-67 proliferation index to this list.

We tried to determine the rate of these changes in tumor biology in 110 patients that we followed in our clinic and performed biopsy from the first metastatic site (re-bx). Also, we aimed to contribute to the literature by examining the relationship of these changes with the endocrine treatments (ET) or chemotherapeutic type (CT) that patients received in the adjuvant period.

MATERIAL and METHODS

We included 110 metastatic breast cancer patients in our study. These patients had previously completed their local treatments and adjuvant chemotherapy for early breast cancer, and those with positive hormone receptors completed endocrine treatments or continued maintenance endocrine therapy. After the first metastasis developed in the patients, we performed metastasectomy or biopsy from the metastatic site. We excluded the patients undergoing cytological examination, those with isolated malignant pleural effusion, malignant ascites, and cerebrospinal fluid, and those younger than 18 years old. HR, Her-2, grade, ki-67 were studied immunohistochemically (IHC) in the samples taken. We compared these results with the results in the primary tumor and examined the rates of change. We discussed the relationships of these changes with the age and sex of the patients, CT in the adjuvant period, ET, local recurrence, and metastatic organ. ER and PR positivity was defined as 1% and above. Her-2 positivity was accepted as IHC: +3 or Fluorescence In Situ Hybridization (FISH) positivity.

Statistical analysis: Statistical analysis was performed using the SPSS statistical software package (Version 25.0, SPSS Inc., Chicago, IL, USA). We checked each continuous variable with Kolmogorov Smirnov and Shapiro-Wilk tests and histograms. The expression of all numerical data was median values (Minimum-Maximum) or ratio. We used the Chi-Square or Fisher Exact test to analyze the categorical variables between the groups. We compared the groups using the Student T-test or the One Way ANOVA for normally distributed data and using the Mann Whitney U test or the Kruskal Wallis test for non-distributed data. After the preliminary measurement, we performed the Mc-Nemar test or Wilcoxon test. P <0.05 value was considered statistically significant.

RESULTS

As summarized in table-1, the youngest of the patients was 25 years old and the oldest one 81. The median age was 51 years old. The average time from primary diagnosis to metastatic period was 49 months. 108 patients (98.2%) were female, and 2 (1.8%) were male. The most frequently been metastasized site was the liver (27.3%), followed by bone (20%). Only 8 patients had a local recurrence (7.3%) ER was positive in 78.2% of patients in primary diagnosis, and 73.6% in metastasis biopsy. PR positivity was 64.5% in primary diagnosis and 56.4% in metastasis biopsy. Her-2 was positive in 34.5% of patients in primary diagnosis and 35.5% in metastasis biopsy. 0.9% of the patients were grade 1, 43.6% grade 2, and 55.5% grade 3 ant initial . In the metastasis biopsy, these rates were 0%, 31.8%, and 68.2%, respectively. 28.2% of the patients received Tamoxifen, 41.8% aromatase inhibitors (AI), and 9.1% gonadotropin-releasing hormone (GnRH) analog as adjuvant ET. 20.9% of the patients had not

received adjuvant ET. 61.8% of the patients received adjuvant Taxanes, and 84.5% received adjuvant Anthracyclines. The proportion of patients who received adjuvant Trastuzumab was 38.2%. The median ki-67 value was 25% at the time of primary diagnosis, and 30% in re-bx. Among all patients, the ki-67 value of 22 patients (20%) decreased in re-bx, while the ki-67 of 12 (10.9%) did not change and that of the other 76 (69.9%) increased (p=0.0001). There was no statistically significant difference between the results of ER, PR, and Her-2 at the primary tumor and re-bx.

As summarized in table-2, ER became negative when metastasis developed in 14 (16.2%) of 86 patients who were initially ER positive. In 9 (37.5%) of 24 patients who were initially ER negative, then ER became positive. In general, in 23 (20.9%) of 110 patients, ER status in metastatic focus biopsy changed according to the primary diagnosis. 22 of the 71 patients (30.9%) who were initially positive for the PR, then the PR became negative. In 13 (33.3%) of 39 patients who were initially negative for the PR, the PR was positive in the metastatic site. In total, PR status changed in 35 of 110 patients (31.8%). Her-2 receptors became negative in 14 (36.8%) of 38 patients who were initially positive for Her-2 receptors. In 15 (20.8%) of 72 patients who were initially negative for Her-2 receptors, then Her-2 became positive. In total, Her-2 changed in 29 of 110 patients (26.3%).

As summarized in table-3, there was only one patient who was in grade 1 at the beginning, and his grade in re-bx increased to 3. In 28 (58.3%) of 48 patients who were initially grade-2, it increased to grade 3 in re-bx, and in 20 (41.6%) of them, the grade did not change. In 54 (88.5%) of 61 patients who initially graded 3, the grade was the same again in re-bx, and in 7 (11.4%), grade-2 was detected. Overall, tumor grades increased significantly after metastasis (p = 0.007). There was no statistically significant relationship between the difference in ki-67 and CT, ET or location of metastasis (p = 0.877). CT did not have a statistically significant relationship with the metastatic region. As summarized in table-4, there was no statistically significant relationship between ET and change of HR and Her-2. Also, there was no statistically significant relationship between CT and change of HR and Her-2. Her-2 results of 68 patients who did not receive trastuzumab were initially negative, while 22% were positive in re-bx (p=0.0001). Her-2 of 38 patients who received trastuzumab were initially positive, 36.8% of these patients were negative in re-bx (p=0.0001).

Table 1. Patient characteristics

		n	%
Gender	Female	108	98,2
	Male	2	1,8
Metastasis Location	Bone	22	20,0
	Lymph nodes	16	14,5
	Liver	30	27,3
	Lung	6	5,5
	Brain	8	7,3
	Soft tissue	5	4,5
	Opposite breast	8	7,3
	Other	15	13,6
Local Recurrence	Yes	8	7,3
	No	102	92,7

Table 2: Change of hormone receptors and Her-2

		Re-bx Positive	Re-bx Negative	p
Primary ER	**Positive**	72	14	0,405
	Negative	9	15	
Primary PR	**Positive**	49	22	0,175
	Negative	13	26	
Primary HER-2	**Positive**	24	14	1,000
	Negative	15	57	

Table 3: Change of the grade

		Re-bx Grade 2	Re-bx Grade 3	
Initial Grade	**1**	0	1	
	2	28	20	0,007
	3	7	54	

Table 4: Change of HR and HER-2 according adjuvant endocrine therapy

		Not received Re-bx positive	Not received Re-bx negative	Tamoxifen Re-bx positive	Tamoxifen Re-bx negative	LHRH analog Re-bx positive	LHRH analog Re-bx negative	AI Re-bx positive	AI Re-bx negative
Initial ER	Positive	1	3	23	5	9	1	39	5
	Negative	5	14	3	0	0	0	1	1
Initial PR	Positive	1	1	19	7	6	3	23	11
	Negative	3	18	4	1	1	0	5	7
Initial HER-2	Positive	9	4	6	6	1	1	8	3
	Negative	1	9	3	16	2	6	9	26

DISCUSSION

Breast cancer is the most common cancer among women, with approximately 2 million new cases annually and more than 500,000 deaths worldwide, and the second cause of cancer-related deaths in women (6-8). The main determining factor in breast cancer treatment is tumor biology. Parameters such as the status of HR, Her-2, grade, and ki-67 proliferation index are among the variables that enable us to have an idea about tumor biology. In particular, the HR and Her-2 status are predictive biomarkers that show what treatments may be more beneficial to the patient while guiding in terms of prognosis. Grade and ki-67 proliferation index, provide information about tumor aggressiveness and therefore prognosis. Generally, approximately 20-30% of patients with early breast cancer become metastatic in the future (6). HR and Her-2 may change later in some patients with metastasis. Although scientists have not fully determined the reason for this change, they have put forward some theories. The most important ones of these are tumor heterogeneity, clonal selection induced by the treatments, and the change in the genotype and phenotype of the tumor (9-11). Therefore, in an early-stage breast cancer patient, re-bx from the metastatic site when it is the first metastasis is now a standard approach worldwide. There are three rational reasons for this practice. The first is to make a malignant or benign distinction in the suspected lesion. The second reason is to distinguish a second primary tumor or breast cancer metastasis if it is malignant. The third and perhaps most important reason is to plan the treatment of breast cancer metastasis according to the new HR and Her-2 status. Published studies are showing that biopsy from metastatic focus increases survival (12-14). The discordance between the HR and Her-2 states of the primary and metastatic focus has been the subject of numerous studies before (9,15,16).

The most comprehensive of these studies is the meta-analysis performed by Aurilio G. et al. In this meta-analysis of 48 studies published between 1983-2012, the rate of ER change was 20%, PR change rate was 33%, and Her-2 change rate was 8% (15). In another more recent study, these change rates in ER, PR, and Her-2 were 18%, 34%, and 14%, respectively (17). In our study, these rates resulted in 20%, 31%, and 26%, respectively. While the hormone receptor change had similar results with the literature in our study, the rate of change in Her-2 was generally higher than in the literature. In this study, the rate of patients who were ER-positive at the beginning and then negative was 13%, and the rate of patients who were negative initially and then positive was 37%. Similarly, in our study, these values were determined as 16.2% and 37.5%, respectively. In the study mentioned above, the rate of patients who became negative when PR was initially positive was 38%, and those who became positive when they were negative at the beginning was 29%. In our study, these rates were similar, with 30.9% and 33.3%, respectively. As a result of the present study, the proportion of patients who became negative when Her-2 was positive at the beginning was 33%, and those who became positive when initially negative were only 1%, these rates were 36.8% and 20.8%, respectively in our results. As can be seen in our study, the proportion of patients who initially became negative for Her-2 and later became positive is significantly higher. The difference may arise from the change of standards of Her-2 evaluation or differences of chemotherapy agents and anti-Her-2 treatments. When the ki-67 values between the primary and metastatic disease were compared, the median Ki-67 value was 25 at the beginning, whereas in the metastatic disease this value was 30. This difference was also statistically significant. The grade of 65% of patients resulted

similarly, 6% decreased from 3 to 2, 18% increased from 2 to 3 and 1% increased from 1 to 3 in the metastatic site according to initial diagnosis. The grade was increased statistically significant in metastatic disease. However, we could not find any study in the literature where we can compare these results. The data we observed for Her-2, ki-67, and grade can be interpreted as the tumor gains more aggressive biology when metastasis develops. In our study, one of the questions was whether CT or ET affected the change in HR. According to our results, the differences between neither ET or CT associated with HR, Her-2, grade, and ki-67 changes. Again, in our study, the difference between metastasized organs was not associated with HR, Her-2, grade, and ki-67 changes. Constanze V. et al. also sought the answers to these questions in their studies (17). In this study, the chemotherapy regimens were investigated separately as Taxanes and Anthracyclines, which are the most frequently used agents in the adjuvant period. As in our study, the chemotherapy regimens did not make a significant difference in HR and Her-2 changes. However, in this trial unlike our results patients who received adjuvant Tamoxifen had more statistically significant HR receptor changes. The variation in HR status was again higher in those using Letrozole, and this change was from positive to negative. These changes were found statistically significant. The difference between studies can be due to follows: First, in the present study, AI was examined as a group and not divided into Letrozole, Anastrazole, or Exemestane. Second, the duration of ET in both studies is unknown. However, CT had been completed for both Taxanes and Anthracyclines recipients, and these durations were probably similar in groups of the patient in both studies. Perhaps this is why both studies have produced similar results for CT, and conflicting results for ET. However, we need extensive studies, and so we can compare this information.

CONCLUSION

In our study, we found the rate of HR change between primary tumor and metastatic site similar to other published studies. However, the proportion of patients who later became positive when Her-2 was negative was significantly higher in our study. We found that the metastatic disease has more aggressive properties in tumor biology than the primary tumor. This result was a significant difference that distinguished our study from other studies in the literature. Again, we showed that CT or ET the location of metastasis did not make a significant difference in tumor biology. We also think that our study is essential for the data from our country and region.

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