Comparison of Tumor - Infiltrating Lymphocytes Between Primary and Metastatic Tumors in Her2+ and HER2-Breast Cancer Patients

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The impact of tumor infiltrating lymphocytes (TILs) on survival was confirmed in various cancer types. Our study aims to investigate the prognostic role of TILs on survival in patients with primary and metastatic tumors in breast cancer patients. We retrospectively identified 29 patients with human epidermal growth factor receptor - 2 (HER2+) and HER2 - early breast cancer diagnosed between 2012 and 2018 at Institute of Oncology Prof. Dr. Al. Trestioreanu Bucharest and who subsequently experienced regional or distant recurrence confirmed by tumor biopsy/resection.

Keywords: metastatic breast tumor, primary breast tumor, tumor-infiltrating lymphocytes, HER2, immune system

Breast cancer (BC) is the second leading cause of cancer death in women [1,2]. It has been estimated that half of the new worldwide BC cases and 60% of the BC deaths occur in developing countries [3]. It occurs more frequently in elderly women and in most cases, risk factors are linked to estrogen hormone that stimulates breast tumor growth [1,4-8]. Tumor development is a heterogeneous process, making it difficult to evaluate the relationship between the tumor and the tumor microenvironment [9]. In breast cancer, the adaptive immune response can easily be seen in the infiltration of breast lesions from the time of benign breast atypia and with the increase in density due to invasive malignancy [10]. The presence of tumor-infiltrating lymphocytes (TILs) is associated with favorable with good prognostic in breast cancer [11,12]. TILs play an important role in mediating the response to chemotherapy and in improving all clinical outcomes in most subtypes of breast cancer [10].

Previous studies have reported that immune activation at the baseline, as assessed by pathology or gene expression arrays, is associated with a higher likelihood of pathological complete response after neoadjuvant chemotherapy (NAC) [13,14], particularly in human epidermal growth factor receptor-2 (HER2)-positive and HER 2 negative breast cancers [15,16]. Furthermore, trastuzumab has been predicted to have beneficial effects. The presence of TILs in residual disease after neoadjuvant chemotherapy is associated with better prognosis breast cancers patients with HER2 negative [17-20]. This suggests that chemotherapy could convert low-TILs tumors into high-TILs tumors [21]. In the case of HER2 positive, even incremental increases in TILs inside and around the tumor were predictive in both the chemotherapy response and the improvement in patient survival rates [21-23].

This finding supports the concept that chemotherapy could partly exert its antitumor effect through the immune system [24]. Very little is known about the change in TILs during metastatic progression and the prognostic impact of TILs in metastatic sites [25,26].

Our study aims to investigate the prognostic role of TILs on survival in patients with primary and metastatic tumors in breast cancer patients.

Experimental part

We retrospectively identified 29 patients with HER2- and HER2+ breast cancer diagnosed between 2012 and 2018 at Institute of Oncology *Prof. Dr. Al. Trestioreanu*, Bucharest and who subsequently experienced a regional or distant recurrence confirmed by tumor biopsy/resection.

All the tumor specimens were prepared for H&E staining and immunohistochemistry (IHC) and were reviewed by a pathologist. Immunohistochemistry was carried out using the following primary antibodies: anti estrogen receptor (ER), PR receptor and proliferation marker (Ki67). The specimens were considered positive for hormone receptor if ≥1% of the cancer cells expressed ER. For the patients determined as $HER2^+$ by IHC, FISH was used to confirm $HER2^+$ disease.

The breast cancer subtypes were classified using IHC as previously described: HER2 positive or HER2 overexpressing ($HER2^+$). Hematoxylin–eosin stained slides for the paired match cases were evaluated for stromal TILs by a pathologist. The specimens were classified into three groups: low TILs (<10%), moderate TILs (10-60%), and lymphocyte predominant breast cancer (LPBC) (≥60%).

Statistical analyses

Associations of the percentage of between the primary and metastatic tumors were evaluated using Fisher's exact test for categorical variables and using the two-sided t-tests for continuous variables. The correlation between the percentages of TILs was calculated using Spearman's and Kendall's rank correlation coefficient test. In all the analyses, the differences were considered significant at $P < 0.05$.

The research meets the conditions of the ethical guidelines and legal requirements and was approved by each Ethical Committee of the Universities of Medicine

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and Pharmacy (see authors' affiliations). Informed consent was obtained from every patient included in the study.

Results and discussions

Medical files of 80 women diagnosed with breast cancer between 2010-2018 were retrospectively analyzed but only 29 breast cancer patients presented the inclusion criteria in the study. Median age at diagnosis was 59.78 years, stage distribution was 10%, IA, 14% in stage IIB, 14% stage II A, 10% stage IIIB, 14% stage IIIC, and 38% stage IV.

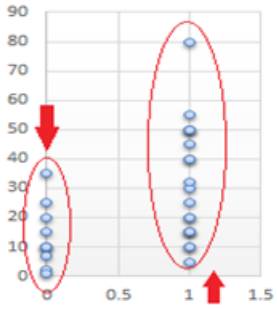

Fig1. Distribution of Ki67 for the sample of patients with regard to the HER groups

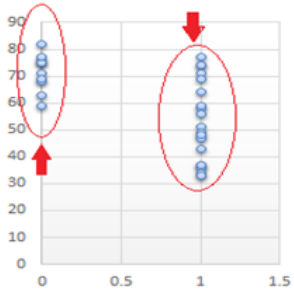

Fig 2. Distribution of age for the sample of patients with regard to the HER groups

Table 1
AGE DIFFERENCE BETWEEN HER GROUPS

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Age	Equal variances assumed	8.620	.007	3.604	27	.001	17.974	4.987	7.741	28.206
	Equal variances not assumed			4.438	26.803	.000	17.974	4.050	9.660	26.287

Table 2
CLINICOPATHOLOGICAL CHARACTERISTICS OF PRIMARY SURGICAL BREAST TUMOR SPECIMENS

Characteristics	Total patients (n=29)	HER2+ (n=11)	HER2- (n=18)
Age, years Mean (range)	59.78	71.5	53.53
T			
Tis	2	0	2
1	8	3	5
2	12	4	8
3	4	0	4
4	3	3	0
N			
1	14	7	7
2	2	1	1
3	5	2	3
Stage			
1	3	1	2
2	9	3	6
3	7	4	3
4	10	3	7
Histological grade			
1	0	0	0
2	17	8	9
3	12	3	9
ER			
+	24	11	13
-	5	0	5
Ki67	25.59%	135%	31.95%
Chemotherapy			
Neoadjuvant	19	8	11
Adjuvant	5	1	4
Trastuzumab			
YES	3		
NO	26		
Hormonal therapy			
YES	22	10	12
NO			

Of all breast cancer patients, 38% represented HER2 positive patients and 62% HER 2 negative patients, 5 patients are TNBC. Ki 67 percentage ranged between 1% and 80% (median was 25.59%). We didn't find any statistical correlation between age and Ki67 level (fig.1). As reported in table 1 young patients had HER2-. The characteristics of the 29 breast cancer patients at the time of diagnosis of the primary breast cancer are presented in table 2. We evaluated the core needle biopsy and surgical specimens before received neoadjuvant chemotherapy for excluding the possibility of alterations in the immune microenvironments of the tumors caused by the therapy.

Most of the patients received neoadjuvant systemic treatment (85% of patients received chemotherapy, 27% of HER2+ patients received trastuzumab). The first biopsy sites of the metastatic tumors were the skin (n=2), brain (n=2), lung (n=5), bone (n=1), and liver (n=3). The median follow-up time after the first biopsy of recurrent tumors was 12 months (range,2–36 months). One patient died of metastatic disease at the last follow-up.

Median TILs levels in the overall population was intermediate, with similar results in HER2- and HER2+ patients. Of the primary tumors, 7% were higher, 48% were intermediate TILs tumors, and 45% were low TILs tumors (table 3). Among the corresponding first metastatic tumors, 45% were intermediate TILs tumors and 55% were low TILs tumors. We found in our study that younger women showed lower levels of TILs than older patients. We have not found significantly different TILs levels between primary and metastasis in the 10 cases with available samples, we explored (table 4). In the group of patients studied, it found that tumors with intermediate TIL levels tended to have ER-negative /HER2-negative breast cancer.

The percentage of TILs in the primary tumors was not significantly different than in the metastatic tumors. This difference was similar in the human epidermal growth factor receptor 2 HER2+ and HER2 - breast cancer groups. TILs were not significantly different in cases where the patient has been received prior neoadjuvant systemic treatment.

Impact of TILS on survival are presented in table 6.

Table 3

DISTRIBUTION OF PATIENTS REGARDING TILs LEVELS FOR HER2- AND FOR HER2+

Tils	HER2+	HER2-
	%	
Low	40	42.1
Intermediate	50	47.4
High	10	5.3

Subtype	First site of biopsy	Primary tumor TILs	Metastatic tumor TILs
HER2+	Lung	low	low
		low	low
		intermediate	intermediate
	Liver	low	low
		intermediate	low
		intermediate	low
HER2-	Brain	low	low
	Brain	low	low
	Lung	low	low
	Lung	intermediate	intermediate
	Skin	intermediate	intermediate
	Skin	intermediate	intermediate
	Bone	intermediate	low

Table 4

TUMOR-INFILTRATING LYMPHOCYTES (TILs) BETWEEN PRIMARY AND METASTATIC BREAST CANCER TUMORS FOR EACH SUBTYPE

			varsta	TILS1	HER	KI67	ER	TILS2
Kendall's tau_b	varsta	Correlation Coefficient	**1.000**	-.057	**-.479****	-.213	-.190	-.112
	TILS1	Correlation Coefficient	-.057	**1.000**	-.101	-.094	.175	-.208
	HER	Correlation Coefficient	-.479**	-.101	**1.000**	**.423****	**.487***	.096
	KI67	Correlation Coefficient	-.213	-.094	.423**	**1.000**	.278	.132
	ER	Correlation Coefficient	-.190	.175	.487*	.278	**1.000**	.145
	TILS2	Correlation Coefficient	-.112	-.208	.096	.132	.145	**1.000**
Spearman's rho	varsta	Correlation Coefficient	**1.000**	-.068	**-.573****	-.270	-.227	-.137
	TILS1	Correlation Coefficient	-.068	**1.000**	-.106	-.115	.182	-.224
	HER	Correlation Coefficient	-.573**	-.106	**1.000**	**.497****	**.487****	.099
	KI67	Correlation Coefficient	-.270	-.115	.497**	**1.000**	.327	.169
	ER	Correlation Coefficient	-.227	.182	.487**	.327	**1.000**	.149
	TILS2	Correlation Coefficient	-.137	-.224	.099	.169	.149	**1.000**

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 5

CORRELATIONS BETWEEN ANALYZED VARIABLES

Descriptive Statistics						
TILS1		N	Minimum	Maximum	Mean	Std. deviation
1	Months	12	2	75	19.08	21.008
2	Months	13	3	62	16.15	16.921
3	Months	2	15	20	17.50	3.536

Table 6
DESCRIPTIVE STATISTICS FOR THE MONTHS INDICATOR BASED ON TILS LEVELS

Table 7
STATISTICAL DIFFERENCE FOR MONTHS VARIABLE BETWEEN TILS (1 AND 2) GROUPS

Independent Samples Test

	Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.342	.564	.385	23	.703	2.929	7.600	-12.793	18.652
Equal variances not assumed			.382	21.162	.706	2.929	7.668	-13.010	18.869

The mean follow-up of patients who had Tils low was 19.08 month, for Tils intermediate was 16.15 and for high was 17.5 months.

Although many adjuvant and neoadjuvant studies have assessed infiltrating lymphocytes and stromal lymphocytic infiltration has been found to constitute a robust prognostic factor in primary HER2+ tumors or HER2- breast cancers, whether lymphocytic infiltration in metastatic tumors could be a prognostic factor has not yet been evaluated [1,6,22,27]. In our study, only a patient with low TILs in metastatic tumors had a significantly lower OS than the patiences with intermediate TILs. Our results suggest that the patients with high or intermediate TILs may have a better, even in metastatic tumors.

This article reports the assessment of comparison of tumor infiltrating lymphocytes between primary and metastatic tumors.

It is now recognized that patients with HER2+ and HER2 negative early breast cancer with high levels of TILs on primary tumor have a lower recurrence rate, therefore suggesting that breast cancer recurrences might be enriched in low TILs tumors [1,28]. Moreover, previous reports showed lower TILs levels in secondary lesions as compared to primary tumors [1, 6,29, 30]. In our study, TILs were generally intermediate. We only found a nonsignificant decrease in TILs from primary to metastasis in the HER2 negative patients of our study, however, the sample size of patients with matched samples was small because it is difficult to detect TILs in nodes.

We observed that host-dependent factors are associated with TILs on metastasis. In particular, younger women showed lower levels of TILs than older patients. We found in our study that the characteristics of the tumor immune infiltrate may be different across metastatic sites. We observed the lowest TILs levels in lung and brain metastasis and the intermediate levels in skin secondary lesions and we did not observe any favorable impact of high TILs on OS in HER2+ breast cancer patients. We did not notice changes in metastasis TILs levels for patients receiving neo adjuvant or adjuvant therapy.

Conclusions

Tumor-infiltrating lymphocytes are associated with a better neoadjuvant chemotherapy response and prognosis in HER2+ and HER 2- breast cancers. Most of TILs levels on metastasis were low and did not differ between HER2- and HER2+ tumors. Younger patients showed significantly lower TILs. In TNBC patients, TILs were intermediate compared with HER2+ patients. The relationship between the immune system and HER2+ and HER2- breast cancer deserve further exploration in the metastatic settings.

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