Correlations Between Known Prognostic Markers and Tumor - infiltrating Lymphocytes in Breast Cancer

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The presence of tumor-infiltrating lymphocytes (TILs) is associated with a favorable long-term outcome in breast cancer. The main objective of this study was to show that there is a correlation between known prognostic and predictive factors and TILs. We retrospectively identified 56 patients with human epidermal growth factor receptor-2 (HER2 +, n = 22) and HER2 negative (HER2-, n = 34) breast cancer diagnosed between 2012 and 2018 at the Institute of Oncology Prof. Dr. Alexandru Trestioreanu, Bucharest. Hematoxylin-eosin-stained slides of these paired samples were evaluated for stromal TILs. The present study reports that TILs can be used as a clinically useful biomarker that has the ability to stratify HER2 negative and HER2 positive patients in prognostic function, bringing relevant information in addition to the established prognostic factors.

Keywords: Immune microenvironment, immunohistochemistry, metastatic breast tumor, primary breast tumor, tumor-inûltrating lymphocytes

In Romania, breast cancer is the most common form of cancer in women, with an estimated incidence of 9629 new cases for 2018. As a consequence, breast cancer represents, in Romania, in 2018, 11.5% of cases (value very close to that of the world but lower than that recorded at the EU level), and the mortality rate dropped by half compared to the 2012 mortality rate, which represented about 6.6%, a value close to the one registered at the EU level. (2018, www.iarc.fr/ GLOBOCAN). [1] It is estimated that for Europe, including Romania, the situation will become even more critical as the population ages.

By the very high absolute values of incidence and, inevitably, of mortality, breast cancer is one of the forms of major concern, both for prevention as well as for treatment, and not for the sake of scientific research. Numerous efforts have been made over time to improve the survival rate by early diagnosis and multiple (combined) therapies. At present, there are new approaches regarding the diagnosis and therapeutic conduct for this form of cancer, but research should also focus on the identification and implementation of other individual prognostic factors, factors that could lead to an improvement in the clinical decision making regarding the patient in order to establish an individualized treatment.

The composition of lymphocyte infiltration in breast cancer has been extensively researched to date; however, although it is commonly accepted that the high presence of TILs is associated with improved prognosis, the correlation between various TILs subpopulations and experimentally observed responses is still characterized by a certain degree of controversy [2]. It is considered that 75% of TILs are T cells.

Experimental part

The study was conducted on a number of 56 patients diagnosed and admitted to the Institute of Oncology Prof. Dr. Alexandru Trestioreanu, Bucharest, between 2012 and 2018.

For the studied groups we performed in a first step the description of the inflammatory infiltration on standard lamellae stained with hematoxylin eosin (HE) or immunohistochemical (IHC) analysis.

For the histopathological analysis, all tumor blocks were fixed in 10% formalin and embedded in paraffin by preparing thick sections for HE staining and IHC staining. IHC is a histological method of identifying cellular or tissue by exploiting the principle of binding antibodies to antigens. Epitopes are visualized by applying a chromogenic substrate (typically diaminobenzidine - DAB).

The number of TILs was estimated by analysis of the mononuclear inflammatory infiltration in stromal tumors using 20x-40x lenses. The infiltrate level was calculated by reporting the area occupied by TILs in the total area of the tumor stroma and the results were divided into 3 categories:

-low inflammatory infiltration (0-10% of the stroma)

- moderate inflammatory infiltration (10-40% of stroma)

-inflammatory rich infiltrate (40-90% of stroma)

Of the 56 patients included in the study, 22 patients were HER-2 / neu positive representing 39.3% and the remaining 34 negative HER-2 / neu patients representing 60.7%.

The average age of patients at the time of diagnosis was 54.23 years, a minimum of 29 years and a maximum of 82 years.

The average follow-up period for patients was 32.14 months from the time of diagnosis.

For HER-2 / neu positive patients the median age was 59.64 years. In the case of HER-2 / neu negative patients, mean age at diagnosis was 50.74 years.

From tumoral inflammatory infiltrate (TILS) analysis in relation to HER-2 / neu status, we observed that of the total of 22 patients with positive HER-2 / neu , 11 patients had reduced TILs, 8 moderate, and 3 TILs.

In the group of 34 patients with negative HER-2 / neu, 14 had reduced TILs, 13 moderate, and 6 TILs-rich patients.

From a statistical point of view, there is no correlation between HER-2 / neu and TILs.

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			TILs1			Total
			reduced	moderate	rich	
HER	Positive	Count	11	8	3	22
		% within HER	50.0%	36.4%	13.6%	100.0%
	Negative	Count	15	13	6	34
		% within HER	44.1%	38.2%	17.6%	100.0%
Total		Count	26	21	9	56
		% within HER	46.4%	37.5%	16.1%	100.0%

Table 1
STATISTICAL CORRELATION BETWEEN HER-2 / NEU AND TILS

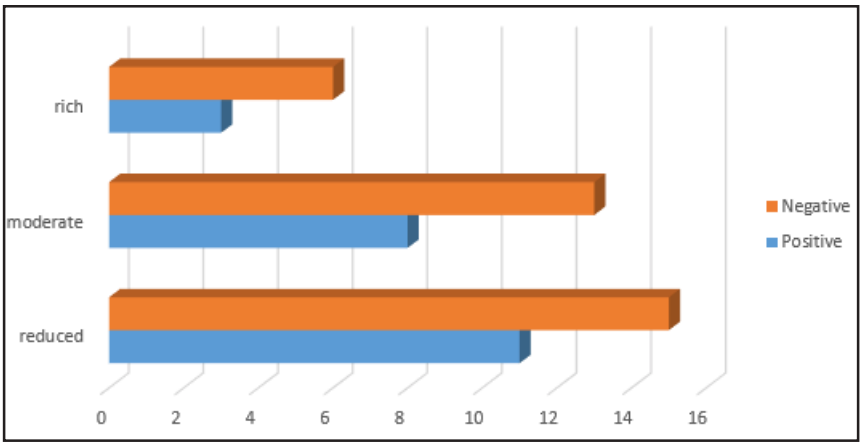

Fig. 1 Representation of TILs according to HER-2 / neu

The mean age of patients according to the presence of TILs was as follows:
-54.5 years for patients with reduced TILs
-57.10 years for patients with moderate TILs
-46.78 years for patients with rich TILs

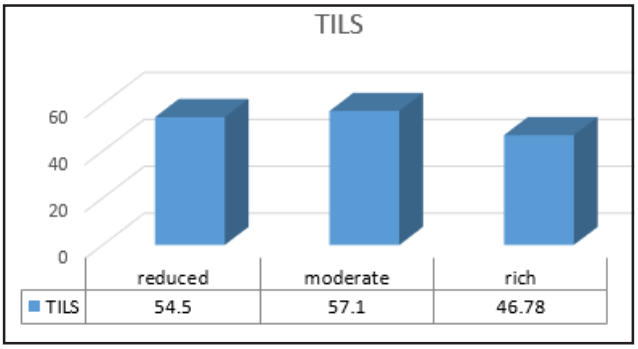

Fig. 2 Graphic representation of mean age according to TILs

The mean age of patients according to the presence of TILs was as follows:
-54.5 years for patients with reduced TILs
-57.10 years for patients with moderate TILs
-46.78 years for patients with rich TILs

Statistical age does not correlate with the presence of tumor TILs. Tumor infiltration of the lymph nodes is a prognostic factor and correlates with disease-free survival and overall survival in breast cancer. Of all patients, 38 had positive axillary lymph nodes and 18 negative nodes.

Correlations				
			varsta	TILs1
Kendall's tau_b	Age	Correlation Coefficient	1.000	-.085
		Sig. (2-tailed)		.426
		N	56	56
	TILs1	Correlation Coefficient	-.085	1.000
		Sig. (2-tailed)	.426	
		N	56	56
Spearman's rho	age	Correlation Coefficient	1.000	-.104
		Sig. (2-tailed)		.447
		N	56	56
	TILs1	Correlation Coefficient	-.104	1.000
		Sig. (2-tailed)	.447	
		N	56	56

Table 2
AVERAGE AGE CORRELATION ACCORDING TO TILS

nodes * TILs1 Crosstabulation						
			TILs1			Total
			reduced	moderate	rich	
nodes	+	Count	19	17	2	38
		% within nodes	50.0%	44.7%	5.3%	100.0%
	-	Count	7	4	7	18
		% within nodes	38.9%	22.2%	38.9%	100.0%
Total		Count	26	21	9	56
		% within nodes	46.4%	37.5%	16.1%	100.0%

Table 3
STATISTICAL CORRELATION BETWEEN TILS AND LYMPH NODES

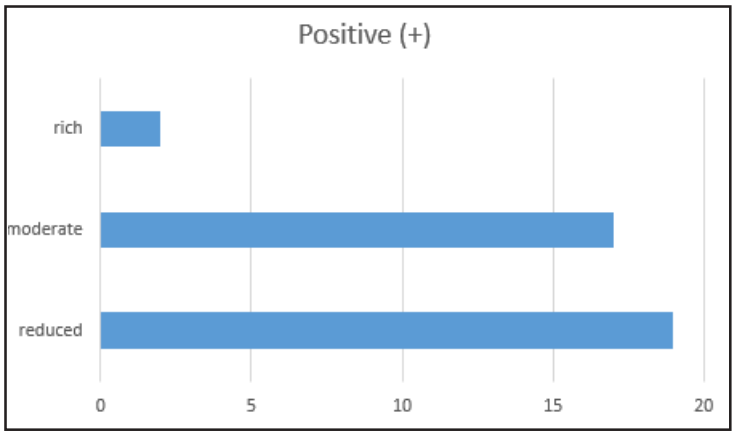

Fig. 3 Graphic representation of TILs by positive axillary lymph nodes

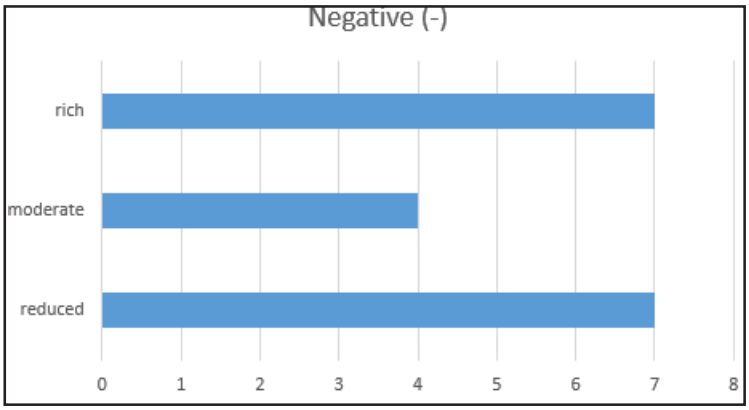

Fig. 4 Graphic representation of TILs according to negative axillary lymph nodes

Among patients with positive axillary lymph nodes, 19 patients had reduced TILs, 17 moderate and 2 TILs patients in increased amount. It is noted that the presence of positive axillary nodes is associated with low or moderate TILs, and the presence of negative axillary nodes is more frequent in patients with rich and low TILs.

It is noted that the presence of positive axillary nodes is associated with low or moderate TILs.

It was noted that the number of patients with low TILs negative lymph nodes was equal to that of patients with negative lymph nodes and TILs in a rich amount.

Grade * TILs1 Crosstabulation						
			TILs1			Total
			reduced	moderate	rich	
Grade	1	Count	1	1	0	2
		% within Grade	50.0%	50.0%	0.0%	100.0%
	2	Count	12	13	4	29
		% within Grade	41.4%	44.8%	13.8%	100.0%
	3	Count	13	7	5	25
		% within Grade	52.0%	28.0%	20.0%	100.0%
Total		Count	26	21	9	56
		% within Grade	46.4%	37.5%	16.1%	100.0%

Table 4
STATISTICAL CORRELATION BETWEEN HISTOLOGICAL GRADE AND TILs

ER * TILs1 Crosstabulation						
			TILs1			Total
			reduced	moderate	rich	
ER	positive	Count	19	16	6	41
		% within ER	46.3%	39.0%	14.6%	100.0%
	negative	Count	7	5	3	15
		% within ER	46.7%	33.3%	20.0%	100.0%
Total		Count	26	21	9	56
		% within ER	46.4%	37.5%	16.1%	100.0%

Table 5
STATISTICAL CORRELATION BETWEEN TILS AND ESTROGEN RECEPTORS

TILs analysis in relation to the degree of tumor differentiation concluded that tumors with low TILs are associated with a low differentiation degree (G3).

From the analysis of tumoral inflammatory infiltrate in relation to the estrogen receptor status, we identified that TILs in a reduced amount are higher in RE positive (46.3%).

Results and discussions

From the present study, the average age of the patients at the time of diagnosis was 54.23 years (minimum 29 years and a maximum of 82 years). For HER2 negative patients, the mean age was 50.74 years, and for HER2 positive it was 59.64 years. There is a statistically significant correlation between HER2 / neu expression and a higher age at diagnosis.

Patients aged 35 years younger at the time of diagnosis had a lower survival rate at 5 years (74.7% vs. 83.8% to 88.3% for women aged 35 to 69 years), even if they received treatment according to the stage of the disease, indicating the aggressiveness of the disease in younger patients. Women over the age of 65 at the time of diagnosis have increased mortality due to the fact that they present themselves to a physician in advanced stages of the disease, associated comorbidities and less aggressive therapies.

In 2013, Loi et. al. have documented a clear association between the presence of TILs in diagnosis and significantly better clinical outcomes among TNBC patients [3]. The analysis of a total of 2009 samples from the trial phase III adjuvant BIG 02-98, pointed out a higher linear correlation between the amounts of TILs and reduced risk of relapse and death, irrespective of the type of system chemotherapy (anthracyclines versus doxorubicin and docetaxel). Among the HER2-positive breast cancer population, TILs were also associated with improved outcomes for anthracycline-based treatment [4].

In breast cancer, the presence of TILs predicts the complete pCR response rate after neoadjuvant chemotherapy.

From the analysis of TILs in relation to the status HER-2 / neu we have observed that the 22 patients with HER-2 / neu positive, 11 patients had TILS low, 8 moderate and 3 patients TILS rich .

In the group of 34 patients with negative HER-2 / neu , 14 had reduced TILS, 13 moderate, and 6 TILS patients rich, with no statistically significant correlation between them.

Triple-negative breast cancer is a subtype of immunogenic cancer with a higher percentage of tumors containing TILs than other subtypes. TILs in triple-negative breast cancer have a prognostic role for recurrence and predictive response to treatment.

Statistically, in the present study, reduced TILs have been associated with the triple-negative cancer subtype.

From the TILs analysis in relation to estrogen receptors, we observed that 46.3% of the 46.3% positive patients had reduced TILs, 39% moderate, and only 14.6% rich.

The presence of axillary lymph node infiltration is one of the most important prognostic factors and has a role in the management of patients diagnosed with breast cancer, correlating both with disease-free survival and overall survival, as confirmed by Ismail Jatoi et all in 1999 [6], which concluded that the presence of lymph node metastases is not only a diagnostic marker and a marker of the presence of an aggressive phenotype. In the present study, we noticed a correlations between the presence of low TILs and the presence of positive axillary lymph nodes.

The analysis of TILs in relation to the histological differentiation (G) revealed the ascension between low TILs presence and poorly differentiated histological grade (G3), also supported by the study by Hee Jin Lee et al., 2015. [7]

The Ki-67 proliferation index is one of the major prognostic factors in breast cancer, independent of the status of the axillary lymph. [8-14] Its role as a predictive factor in establishing the neoadjuvant and adjuvant treatment is controversial due to the conflicting results obtained in various studies.

The average Ki-67 proliferation index was in our group of 27.86%. In the case of HER-2 / neu negative patients the mean value was 31.26% and for HER-2 / neu positive 22.59% (statistically significant association).

The presence of low TILs correlates with the Ki-67 proliferation index, as evidenced in the study by Maria Vittoria Dieci et al., 2015. [15] The presence of TILs correlates with a greater suppression of Ki-67 following neoadjuvant endocrine therapy, but appears to prevent a molecular response after HER2 + / HER2- chemotherapy.

Of all the patients who had the disease progression, 8 had been described in the histopathological TILs in reduced quotient and 7 TILs patients in a moderate amount, as described by Yuka Asano et al., 2018 in his study. It concluded that patients with triple-negative breast cancer and HER-2 positive who had relapsed had reduced TILs, although there were no changes in tumor subtypes, suggesting that a decrease in TILs may be involved in the recurrence of cancers TNBC and HER2 after effective neoadjuvant treatment [16-20]

There are studies that compared lymphocyte tumor infiltrate in the primary tumor with that of the secondary tumor, concluding that TILs secondary tumor is found in a reduced amount associated with an aggressive phenotype and reduced survival [21].

Conclusions

TILs are one of the best examples of the strict relationship between natural defence and carcinogenesis. In this scenario, evaluating TILs as a prognostic and predictive factor for neoadjuvant chemotherapy should become a routine analysis, especially with regard to the

most aggressive subtypes of breast cancer such as triple-negative molecular variants and HER2 positive.

This paper demonstrates that the presence of low TILs is associated with positive axillary lymph nodes and elevated Ki-67 values.

In this study, we studied the effect of TILs on clinical outcomes in a group of patients with HER2- and HER2 + breast cancer treated with Trastuzumab. These results suggest that trastuzumab is more effective in treating tumors with reduced TILs. Therefore, TILs assessment at initial diagnosis may be necessary for subdivision of HER2 + breast cancer patients who would benefit more from the additional use of trastuzumab. Developing strategies that can facilitate an effective immune response is also warranted.

The conclusion of this study is that TILs can be used as a clinically useful biomarker that has the ability to stratify HER2 negative and HER2 positive patients in prognostic function, bringing relevant information in addition to established prognostic factors.

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