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Locally advanced breast cancer: Tumor-infiltrating lymphocytes as a predictive factor of response to neoadjuvant chemotherapy

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ABSTRACT

Objective: To evaluate the pathologic response after neoadjuvant chemotherapy in patients with breast cancer according to the stromal tumor-infiltrating lymphocytes (TILs) as well as the evaluation of overall and disease-free survival according to TILs.

Methods: A six years (2008–2013) review was done including patients with locally advanced breast cancer that received neoadjuvant therapy and then surgery. An evaluation of the percentage of TILs was done in the pretreatment biopsies and a correlation analysis and survival curves were done.

Results: 187 patients were evaluated. The pathological complete response (pCR) in patients with TILs ≥30% was 58.5%, and in patients with TILs < 30% was 11% ($p < 0.001$). An Odds Ratio of 8.85 was obtained in patients with TILs ≥30% to achieve a pCR. This relationship was seen in patients with HER2-enriched and triple-negative subtypes. No correlation between TILs and survival was obtained (OS: log-rank; $p = 0.834$; DFS: log-rank; $p = 0.937$).

Conclusions: The study of TILs is important because they represent an additional tool to predict the response to neoadjuvant treatment mostly in HER2-enriched and triple-negative subtypes of breast cancer.

Introduction

Cancer has been considered a genetic disease. However, several recent studies have shown that not only a genetic condition could influence the development of cancer, their progression and their response to therapy, but also the tumor microenvironment, formed by benign cells and the extracellular matrix, can also be of paramount importance [1,2]. Therefore, immunology has gained renewed interest in the past few years due to emerging findings on mechanisms involved in tumoral immune evasion, a process called cancer immuno-editing [1,3,4].

The pathological complete response (pCR) is an objective useful tool used to evaluate which patients with locally advanced breast cancer could have a better prognosis after neoadjuvant treatment [5]. Thus, many authors have conducted studies to determine the stromal tumor infiltrating lymphocytes (TILs) as a predictive factor to neoadjuvant treatment [6–9].

In Venezuela, breast cancer is the most common cause of cancer death in women [10], and in our center at Luis Razetti Oncology Institute, patients with locally advanced breast cancer represent the most common subgroup, increasing from 33.16% to 58.14% in recent years [11]. That's why we conducted a study in order to evaluate the relationship between the TILs score and the response to neoadjuvant treatment in patients with locally advanced breast cancer, assessing its role as a predictive and prognostic factor as well.

Material and methods

This is a retrospective study, in which we included female patients with locally advanced breast cancer (no metastasis) from Luis

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Razetti Oncology Institute between 2008 and 2013. These patients had pretherapeutic biopsy confirmation of breast cancer, received neoadjuvant treatment and then surgical therapy with a definitive pathological assessment. Ethical approval was obtained for the Luis Razetti Oncology Institute ethical committee.

Pretreatment biopsies and pathology assessment

Paraffin-embedded pretherapeutic core biopsies from Luis Razetti Oncology Institute were used in this study. Patient's age, clinical tumor size, clinical nodal status, clinical TNM stage (AJCC 7ed), tumor histology, histological grade, hormonal receptors status (ER and PgR), HER2 receptor status, and Ki67 score had been routinely recorded in the pathology reports previously to this study. Hormone receptor positivity was defined as > 1% positive cells for ER and/or PgR. Only TILs was reassessed in the pretreatment biopsies.

Stromal TILs evaluation

Histopathological evaluation of percentage of TILs was done on hematoxylin and eosin sections of core biopsies according to the recommendations of the International TILs Working Group 2014 [12]. Thereby, the proportion of stromal area occupied by mononuclear cells, (including lymphocytes and plasma cells but not polymorphonuclear leukocytes) within the borders of the invasive tumor, not only on hotspots, were recorded as a percentage. TILs in tumor zones with crush artifacts, necrosis, and regressive hyalinization were excluded. In order to validate the scoring system, two pathologists with more than 10 years of experience and one pathologist with a work experience under 5 years participated independently and without knowledge of patient's outcomes. Based on the study made by Liu et al. [13] the threshold applied for the percentage of TILs was 30% in order to classify patients to have lymphocyte-predominant breast cancer (LPBC) or not (Fig. 1).

Pathological response evaluation

A pathological complete response (pCR) was defined as the absence of residual invasive or noninvasive tumor cells in breast and lymph nodes (ypT0 ypN0) [14]. So, only two groups were defined −patients with pCR and patients with non-pCR.

Neoadjuvant treatment

Based on the status of hormonal and HER2 receptors and Ki67 index, 4 clinical subtypes were classified − Luminal A group [ER+/PgR+/HER2-/Ki67 < 20%]; Luminal B group [ER+/PgR+/HER2-/Ki67 > 20% or ER+/PgR+/HER2+]; HER2-enriched group [ER-/PgR-/HER2+] and Triple negative group [ER-/PgR-/HER2-]. Patients with positive HER2 receptors were candidates to receive Trastuzumab plus cytotoxic chemotherapy as neoadjuvant treatment according to the availability of the drug. The others clinical subgroups received neoadjuvant therapy based on cytotoxic chemotherapy.

Statistical analysis

The percentages of TILs assigned for the two pathologists with more than 10 years of experience were compared each other using a Spearman correlation test in order to determine the level of concordance between the values. The Fisher's exact test and Chi-square test were used for categorical variables. Association between clinicopathological factors and the probability to obtain a pathological complete response (pCR) after neoadjuvant treatment were investigated with a logistic regression analysis. Odds ratios (ORs) and 95% CIs with two-sided p values were used. A p-value $\leq$ 0.05 was considered statistically significant. For the logistic regression analysis, the following clinical variables were used: age, $\leq$ *40 vs.* > *40*; clinical tumor size, *cT1-cT2 vs. cT3-cT4*; histological grade, *G3 vs. G1-G2;* Hormonal receptors, *negative vs. positive*; HER2 receptor, *negative vs. positive;* and TILs level, $\geq$ *30% vs.* < *30%.* The software SPSS v.22 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Results

There were included 187 patients, with a median of age of 50 years (range 59, min: 25 − max: 84). The majority of patients were classified as stage III (57.3%) and the stage IIIA was the most common, reaching 38% of the overall population. The most common histological type was ductal, representing 82.4% of the patients, with histological grade 2 (61.5%) being the most frequent one. The

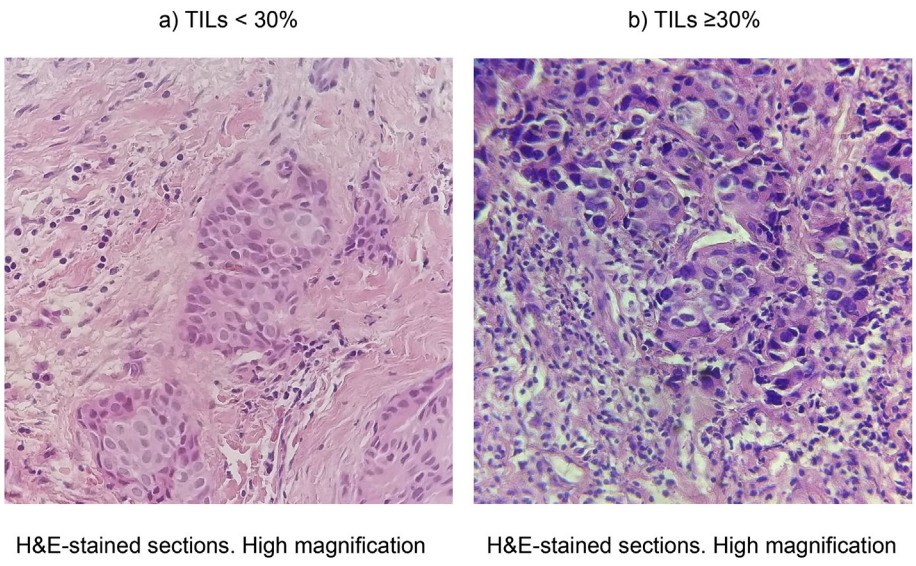

Fig. 1. Stromal tumor-infiltrating lymphocytes (TILs) histological representation.

Table 1
Clinicopathological characteristics of patients.

Characteristics	N° (%)
Patients (*n*)	187 (100)
Age (years): median (*range*)	50 (*25–84*)
Clinical stage TNM 7ed	
IIa	14 (7,5)
IIb	66 (35,3)
IIIa	71 (38,0)
IIIb	31 (16,6)
IIIc	5 (2,7)
Histological grade	
G1	9 (4,8)
G2	115 (61,5)
G3	63 (33,7)
Histological type	
Ductal	154 (82,4)
Lobular	18 (9,6)
Ducto-lobular	11 (5,9)
Papilar	1 (0,5)
Linfo-vascular invasion	
No	78 (42,2)
Si	108 (57,8)
Clinical subtype	
Luminal A	64 (34,2)
Luminal B Her2 -	36 (19,3)
Luminal B Her2 +	19 (10,2)
HER2-enriched	27 (14,4)
Triple negative	41 (21,9)
Surgical therapy	
MRM	135 (72,2)
BCS + ALND	34 (18,2)
BCS + SLNB	11 (5,9)
SM + SLNB	7 (3,8)
Neoadjuvant treatment	
AC	165 (88,2)
Taxanes	140 (74,9)
Trastuzumab	33 (17,6)
Adjuvant treatment	
Endocrine therapy	123 (65,8)
Trastuzumab	42 (22,5)
Radiotherapy	177 (94,7)

MRM: modified radical mastectomy; BCS: breast-conserving surgery; ALND: axillary lymph node dissection; SLNB: sentinel lymph node biopsy; SM: simple mastectomy; AC: doxorubicin/ cyclophosphamide.

most common clinical subtype was Luminal A (34.2%), followed by Luminal B, then the triple-negative group, and finally HER2-enriched. The most frequently surgical therapy done was modified radical mastectomy (72.2%) and the neoadjuvant chemotherapy scheme more commonly used was doxorubicin-ciclofosfamide followed by taxanes (68.4%). Moreover, 46 patients were included in the HER2-enriched clinical subtype, but only 33 patients (71.7%) did receive trastuzumab as part of their neoadjuvant treatment. The others clinicopathological characteristics of the patients are listed on Table 1.

A correlation of 0.90 was obtained between the levels of TILs assigned by the two experienced pathologists ($p < 0.001$). In the same way, there was a correlation of 0.87 between pathologists with more than 10 years and the pathologist with less than 5 years of experience ($p < 0.001$).

The pathological complete response was seen in 21.4% of the population of this study (40 of 187 patients). We identified 41 patients with TILs greater than or equal to 30%, and 24 of those (58.5%) obtained a pCR. Furthermore, there were 146 patients with TILs values less than 30% and only 16 of them (11%) reached a pCR ($p < 0.001$) (Fig. 2).

The logistic regression analysis included age, tumor size, histological grade, hormonal receptor status, HER2 receptor status, and level of TILs. In the univariate analysis, patients with negative hormonal receptors obtained a pCR 3.49 times more than patients with positive receptors (OR: 3.49; [CI 95%: 1.28–9.52], $p = 0.014$). Besides, a value greater than 30% of TILs was strongly associated with a better pathological complete response (OR: 8.85; [CI 95%: 3.62–21.66], $p < 0.001$) (Table 2).

According to the clinical subtype, patients in triple negative group presented a better pCR with greater values TILs ($p < 0.001$), behavior that was also observed in patients of the HER2-enriched group ($p = 0.001$). However, there wasn't an association between pCR and the level of TILs in patients of the Luminal group (Luminal A [$p = 0.953$]; Luminal B Her2 neg [$p = 0.695$]; Luminal B Her2 pos [$p = 0.373$]). (Fig. 2).

Regarding survival, a mean follow-up of 62.5 months was conducted. Bias due to loss during follow-up or missing data represented 17.11% (32 patients). Overall survival and disease-free survival graphs didn't show statistically significant differences between patients with TILs values greater than or less than 30% (OS: log-rank, $p = 0,937$; *DFS:* log-rank, $p = 0,834$) (Figs. 3 and 4).

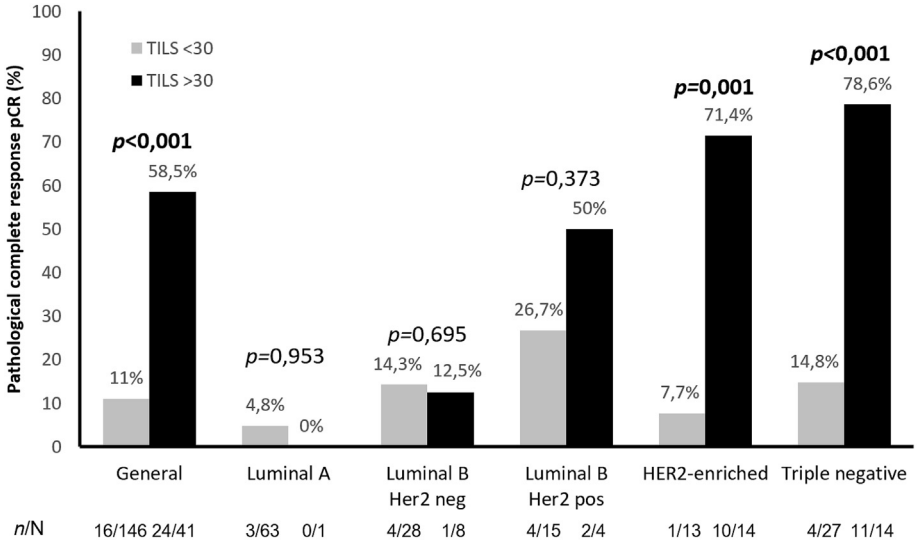

Fig. 2. Pathologic complete response (pCR) rates related to the stromal tumor-infiltrating lymphocytes (TILs).

Table 2
Logistic regression analysis of clinicopathological factors and pathological complete response after neoadjuvant treatment.

Factor	Univariate analysis			Multivariate analysis		
	OR	CI 95%	p	OR	CI 95%	p
Age (≤ 40 vs > 40)	1,45	0,51–4,16	0,487			
Clinical tumor size (cT1-cT2 vs cT3-cT4)	1,94	0,81–4,65	0,136			
Histological grade (G3 vs G1-G2)	1,65	0,64–4,25	0,299			
Hormonal receptors (Negative vs Positive)	3,49	1,28–9,52	**0,014**	3,29	1,48–7,31	**0,004**
HER2 receptors (Negative vs Positive)	1,44	0,43–4,89	0,557			
TILs level (≥ 30% vs < 30%)	8,85	3,62–21,66	<**0,001**	6,42	2,79–14,78	<**0,001**

OR: odds ratio; CI: confidence interval; TILs: Stromal tumor-infiltrating lymphocytes.
Values in bold are statistically significant at 95% of confidence level.

Discussion

Pathological complete response (pCR) was 21.4% in this study, which is correlated with several historical reports [5,13]. In fact, Spring et al. [15] recently published a meta-analysis investigating the pCR in more than 18.000 patients with breast cancer after neoadjuvant treatment, and the overall pCR rate was 21.5% (5.7–62%). Besides, higher rates were found in patients classified as HER2-enriched and triple negative.

It's well known that breast cancer patients with pCR after neoadjuvant treatment have better prognosis than those without [14]. For this reason, pCR has been emerging as an additional tool to be considered for decision making in terms of de-escalating treatment. Thus, Tadros et al. [16] in a prospective cohort study made at the MD Anderson Cancer Center suggest that after neoadjuvant treatment, a complete response corroborated by image-guided percutaneous biopsies in the tumor bed in the breast could avoid axillary surgery. However, clinical trials are necessary to make

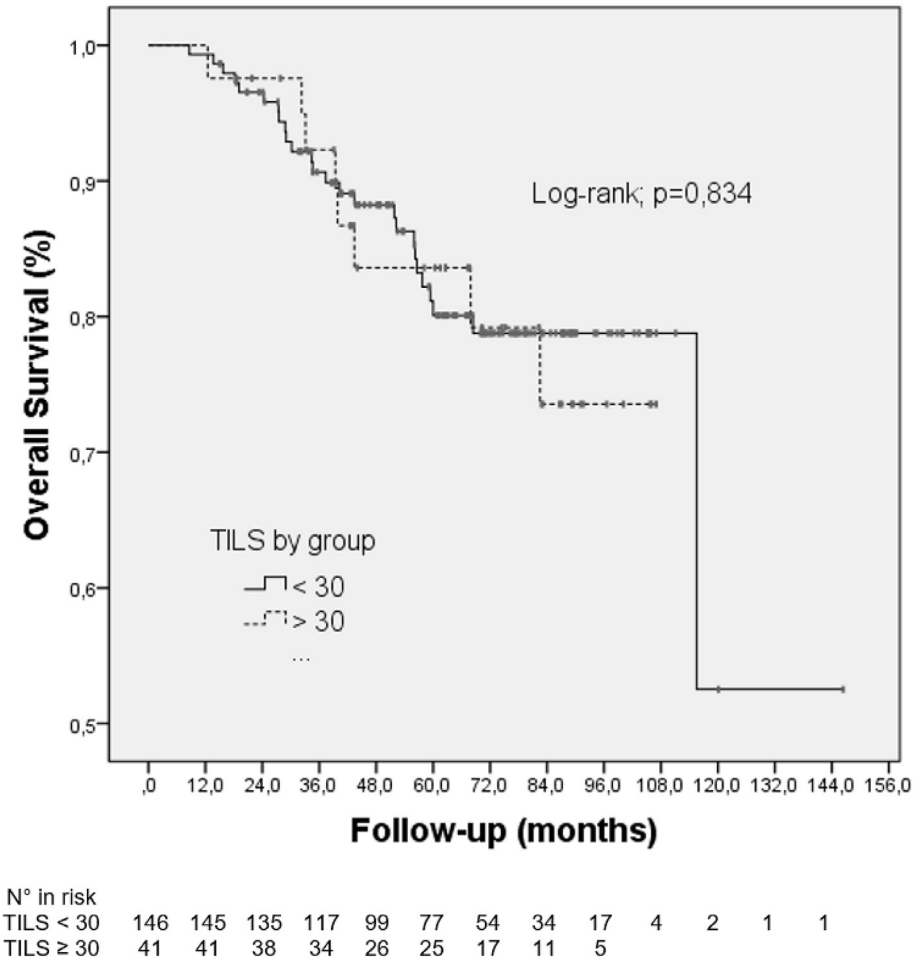

Fig. 3. Overall survival curve in patients with TILs <30 vs TILs ≥30.

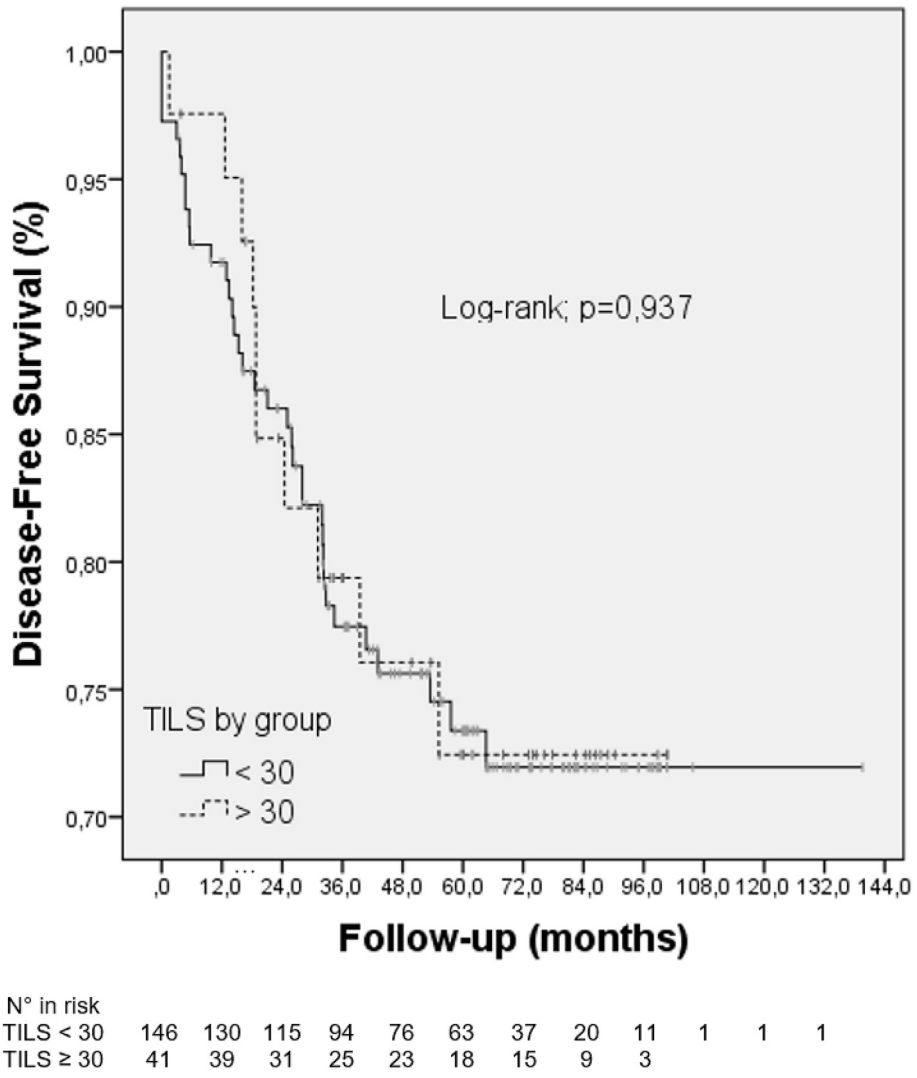

Fig. 4. Disease-free survival curve in patients with TILs <30 vs TILs ≥30.

definitive recommendations in the future.

On the other hand, it seems that the immunologic system is related with a better response to neoadjuvant treatment [17,18]. Loi S. et al. [6] in a study based on the results of the FinHer trial suggested that a greater value of TILs were related to better pCR rates. This is particularly accurate in patients classified under triple negative and HER2-enriched groups. These results are very similar to our findings [6,8,9,13].

We made a logistic regression analysis in which clinicopathological factors associated with a pCR were hormonal receptors status and TILs value. Others authors have also reported that age, tumor size, and histological grade are strongly related to pCR [9,13]. This could be due to a small sample size in our study.

In a previous research, overall and disease-free survival rates were determined according to TILs values in patients with breast cancer, and no correlation between these variables was found [19]. Despite this, a better prognosis, improved overall survival, disease-free survival and event-free survival was determined in several trials, specifically in patients belonging to HER2-enriched and triple negative groups as the value of TILs was higher [6,8,19,20].

The assignment of TILs values between pathologists with more than 10 years of experience and the pathologist with less than 5 years of experience had a good correlation, even more among pathologists with more experience. We can deduct that is very feasible the implementation of the TILs evaluation, regardless of the experience of the pathologist assessing the biopsy.

The major limitations of our study were the small population size, and the retrospective design. These characteristics decrease the statistical impact of the investigation, however, we believe that this research could contribute to add interesting data highlighting the role of TILs as another predictive factor to obtain a pCR after the neoadjuvant therapy.

It's important to note that there are new pathways in this area, such as the relationship between pre-invasive disease and TILs. Pruneri et al. [21] published an article in which they studied 1.488 patients with ductal carcinoma in situ in order to evaluate the clinical relevance of TILs in this scenario, shown higher TILs values in patients with positive HER2 receptors, although they did not find a significant association between TILs and the 10-year risk of ipsilateral breast event.

For all these reasons, we recommend the implementation of TILs score in breast cancer pretreatment biopsies as a routine, because is a feasible and helpful resource that serves as a predictor mainly in patients with clinical subtype HER2-enriched and triple negative.

Conclusions

We conclude that TILs can be used as a predictive factor for pCR after neoadjuvant treatment in patients with HER2-enriched and triple negative immunophenotypes.

Disclosure statements

The authors declare no conflict of interests. No funding was received for this study.

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