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ORIGINAL ARTICLE

Tumor stromal infiltrating lymphocytes as a prognostic biomarker of survival in human epidermal growth receptor 2-positive early breast cancer

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Abstract

Background: *Anti-human epidermal growth receptor 2 (HER2) therapies have significantly improved the survival rates of patients with HER2-positive breast cancer. Achieving a pathological complete response (pCR) following neoadjuvant treatment correlates with better prognosis, underscoring the need to identify prognostic biomarkers such as tumor stromal infiltrating lymphocytes (TILs).* **Objective:** *To analyze the expression of TILs as a prognostic marker for disease-free survival (DFS) in patients with HER2-positive breast cancer treated with neoadjuvant chemotherapy with trastuzumab.* **Method:** *This retrospective, analytical, observational cohort study included patients with HER2-positive breast cancer treated at the oncology hospital between January 2018 and December 2021. Histopathological slides were reviewed to determine the proportion of TILs. DFS and overall survival (OS) were estimated using the Log-Rank test and Kaplan-Meier method. The association between categorical variables was assessed with Pearson's χ^2 test. A $p < 0.05$ was considered statistically significant.* **Results:** *One hundred and twenty-one patients were included. The DFS was 72.6 versus 61.9 months (hazard ratio [HR] 0.79, $p = 0.006$), and OS was 76.7 versus 66.1 months (HR 0.83, $p = 0.015$) in patients with TILs ≥ 10% compared to those with < 10%, respectively. The pCR rate was higher in the group with TILs ≥ 10% ($p = 0.03$).* **Conclusions:** *The presence of TILs ≥ 10% in patients with HER2-positive breast cancer treated with neoadjuvant chemotherapy and trastuzumab was associated with increased DFS, OS, and pCR rates.*

Keywords: *Breast cancer. Human epidermal growth receptor 2. Trastuzumab. Tumor stromal infiltrating lymphocytes.*

TIL como biomarcador pronóstico de supervivencia en cáncer de mama temprano HER2-positivo

Resumen

Antecedentes: *Las terapias anti-HER2 han incrementado la supervivencia de pacientes con cáncer de mama HER2 positivo. El impacto en supervivencia al lograr respuesta patológica completa (pCR) a la neoadyuvancia conduce a la necesidad de identificar biomarcadores pronósticos, como los linfocitos infiltrantes del estroma tumoral (TIL).* **Objetivo:** *Analizar la expresión de TIL como marcador pronóstico de supervivencia libre de enfermedad (SLE) en pacientes con cáncer de mama HER2 positivo tratadas con quimioterapia más trastuzumab neoadyuvante.* **Método:** *Cohorte retrospectiva, analítica, observacional. Se incluyeron pacientes con cáncer de mama HER2 positivo tratadas en el Hospital de Oncología entre enero 2018 y diciembre 2021. Se revisaron laminillas histopatológicas para*

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determinar la proporción de TIL. La SLE y supervivencia global (SG) se obtuvo por medio del Log Rank test y el método de Kaplan Meier; la dependencia entre variables categóricas, con el test de Chi cuadrada de Pearson (χ^2); tomando una p < 0.05 para la significancia estadística. **Resultados:** *Se incluyeron 121 pacientes. La SLE fue de 72.6 vs. 61.9 meses (HR: 0.79; p = 0.006) y la SG de 76.7 vs. 66.1 meses (HR: 0.83; p = 0.015) con TIL ≥ 10% vs. < 10, respectivamente. La pCR fue mayor en el grupo con TIL ≥ 10% (p = 0.03).* **Conclusiones:** *La expresión de TIL ≥ 10% en pacientes con cáncer de mama HER2 positivo tratadas con quimioterapia más trastuzumab neoadyuvante presentó un incremento en SLE, SG y pCR.*

Palabras clave: *Cáncer de mama. Receptor de crecimiento epidérmico humano tipo 2. Trastuzumab. Linfocitos infiltrantes del estroma tumoral.*

Background

The incorporation of anti-human epidermal growth receptor 2 (HER2) targeted therapies into neoadjuvant chemotherapy for HER2-positive breast cancer has successfully increased the rates of pathological complete response (pCR), defined as the absence of invasive tumor in the breast and lymph nodes (ypT0/is ypN0 or ypT0 ypN0)[1], to approximately 60-68%, with greater benefit observed in patients with hormone receptor-negative tumors[2-6]. Despite these advances, up to 50% of patients do not achieve this response. The impact of pCR as a surrogate marker for survival[7] has prompted a focus on new strategies based on the evaluation of immunological biomarkers capable of providing predictive and prognostic information, such as tumor stromal infiltrating lymphocytes (TILs)[8].

TILs are a diverse set of immune cells located within the tumor microenvironment, reflecting the host's immune activity against the tumor. They are predominantly composed of CD8+ T cells[9]. *In vitro* studies have demonstrated that these cells can proliferate up to 10 times more than normal immune cells, limiting tumor growth by 40-50% and achieving complete regression in 10-20% of patients undergoing oncological treatment[10]. The presence of TILs has been associated with high-grade tumors, HER2-positive or triple-negative breast cancers, and correlates with a better response to chemotherapy, as well as a reduced risk of relapse and mortality, with reductions of up to 10%[11-13].

This study aims to analyze the presence of TILs as a prognostic marker for disease-free survival (DFS) in patients with HER2-positive breast cancer treated with neoadjuvant chemotherapy combined with trastuzumab. In addition, the study seeks to evaluate the association between TILs and pCR. Our objective is to provide relevant data regarding the value of TILs in Mexican women, with the ultimate goal of optimizing therapeutic decision-making and promoting a more personalized and effective approach to HER2-positive breast cancer management.

Materials and methods

An observational, longitudinal, retrospective, and analytical study was conducted. Patients with a histopathological diagnosis of HER2-positive breast cancer, confirmed by immunohistochemistry or amplification by *in situ* hybridization (ISH), and treated at the Oncology Hospital of the Centro Médico Nacional Siglo XXI between January 1, 2018, and December 30, 2021, were included. The patients received neoadjuvant chemotherapy in combination with trastuzumab, had evaluable histopathological and immunohistochemical slides, and a complete surgical report. Patients with metastatic disease, a second primary cancer, incomplete treatment, or trastuzumab discontinuation due to toxicity were excluded. In addition, patients with non-evaluable slides were eliminated from the study.

In collaboration with the pathology department, the histopathological slides of each patient were evaluated to identify TILs, categorizing them as low (< 10%) and high (≥ 10%). In addition, variables such as age, neoadjuvant chemotherapy regimen, clinical stage, histological grade, lymphovascular invasion, hormone receptor expression, and Ki67 proliferation index were incorporated. For univariate analysis, measures of central tendency and dispersion were used for quantitative variables, whereas frequencies and proportions were employed for qualitative variables. Overall survival (OS) and DFS were calculated according to TIL levels using the Log-Rank test and the Kaplan–Meier method. The association between categorical variables was assessed using the Pearson χ^2 test, with a $p < 0.05$ considered statistically significant.

The Epi-Info 7 software facilitated the development of databases and data analysis through statistical methods, graphical representations, and epidemiological mapping. Data analysis was conducted using SPSS version 25.

The protocol received approval from the research and ethics committees, in accordance with the General Health Law and the Declaration of Helsinki. This

observational study was reported in accordance with the strengthening the reporting of observational studies in epidemiology guidelines. This is retrospective and non-risk, and does not require informed consent, as there was no direct contact with patients; data were obtained from clinical records and pathology materials. Information was handled confidentially and anonymously, with access restricted solely to the principal investigator, who held the encrypted data. Confidentiality agreements were signed, and a privacy notice was issued. No external support or funding was utilized, and the authors declare no conflicts of interest.

Results

Between January 2018 and December 2021, a total of 145 HER2-positive patients received neoadjuvant treatment with chemotherapy and trastuzumab. Of these, 24 patients were excluded due to the lack of adequate pathological material for analysis or incomplete follow-up data in their medical records. Consequently, 121 patients were included in this study, as depicted in the CONSORT diagram **(**Fig. 1). The median follow-up duration was 40 months. The mean age was 54.9 years (± 10.96). A total of 86% of patients (n = 103) presented with locally advanced disease (stages IIB-IIIC), and 40.3% (n = 49) expressed estrogen receptor positivity. Two-thirds of the cohort (67.8%, n = 82) exhibited TILs ≥ 10%. The clinical and pathological characteristics are summarized in table 1.

More than 90% of the patients received chemotherapy regimens including anthracyclines (AC-TH, EC-TH) (doxorubicin/epirubicin, cyclophosphamide, taxane, and trastuzumab). Radical surgery was performed in 90% of cases; 93.4% received adjuvant trastuzumab, and 94.2% underwent radiotherapy.

DFS

DFS at 2 years was 79.3%. A significant benefit was observed in patients with TILs ≥ 10%, with a median DFS of 72.6 months (95% confidence interval [CI], 68.8-74.7) compared to 61.9 months (95% CI, 53.8-69.9) in patients with TILs < 10%. The hazard ratio (HR) was 0.79 (95% CI, 0.73-0.97; p = 0.006), as shown in figure 2. Among the 20.7% of patients who experienced recurrence, 8.26% had local or nodal recurrence, followed by 3.3% with bone metastases and 3.3% with pulmonary metastases, which were the most frequent sites (Table 2).

OS

Patients with TILs ≥ 10% showed a median OS at 2 years of 76.7 months (95% CI, 74.0-79.4), compared to 66.1 months (95% CI, 60.2-71.9) in patients with TILs < 10% (HR 0.83; 95% CI, 0.81-0.98; p = 0.015), as shown in figure 3.

TILs

Univariate analysis demonstrated that the proportion of TILs was independent of histological type, clinical stage, or tumor grade; however, statistically significant associations were found regarding recurrence (p = 0.004), absence of progression during neoadjuvant chemotherapy (p = 0.03), pCR (p = 0.03), and lower breast cancer-specific mortality (p = 0.007) in the group with TILs ≥ 10%. This information is summarized in table 3.

pCR

Sixty-one point seven percent of patients achieved a pCR (n = 51). Of these, 78.4% had TILs ≥ 10%, while 21.6% had TILs < 10%. The pCR rate with TILs ≥ 10% was 60.8%, compared to 39.2% with TILs < 10% (p = 0.03) as shown in figure 4.

An exploratory analysis was conducted to evaluate DFS in relation to TIL expression and pCR. In patients with TILs ≥ 10% and pCR, DFS was 73.5 months versus 59.9 months in patients without pCR. In those with TILs < 10% and pCR, DFS was 58.0 months versus 52.4 months in patients without pCR (HR 2.07, 95% CI 1.35-3.17; p = 0.002), as illustrated in figure 5.

Discussion

The association between immune cell infiltration in breast cancer and its prognostic impact has been recognized over the past decade. TILs can enhance the efficacy of chemotherapy, induce tumor cell death, and play a role in controlling cancer progression. Although various studies have demonstrated that TILs are an independent factor for predicting the effectiveness of neoadjuvant therapy in breast cancer patients, these results vary due to tumor heterogeneity[14].

Regarding DFS, it has been shown that TILs are a favorable prognostic factor, meaning they decrease the risk of breast cancer recurrence, particularly in HER2-positive and triple-negative tumors. A meta-analysis by Li et al. evaluated 9,145 patients with

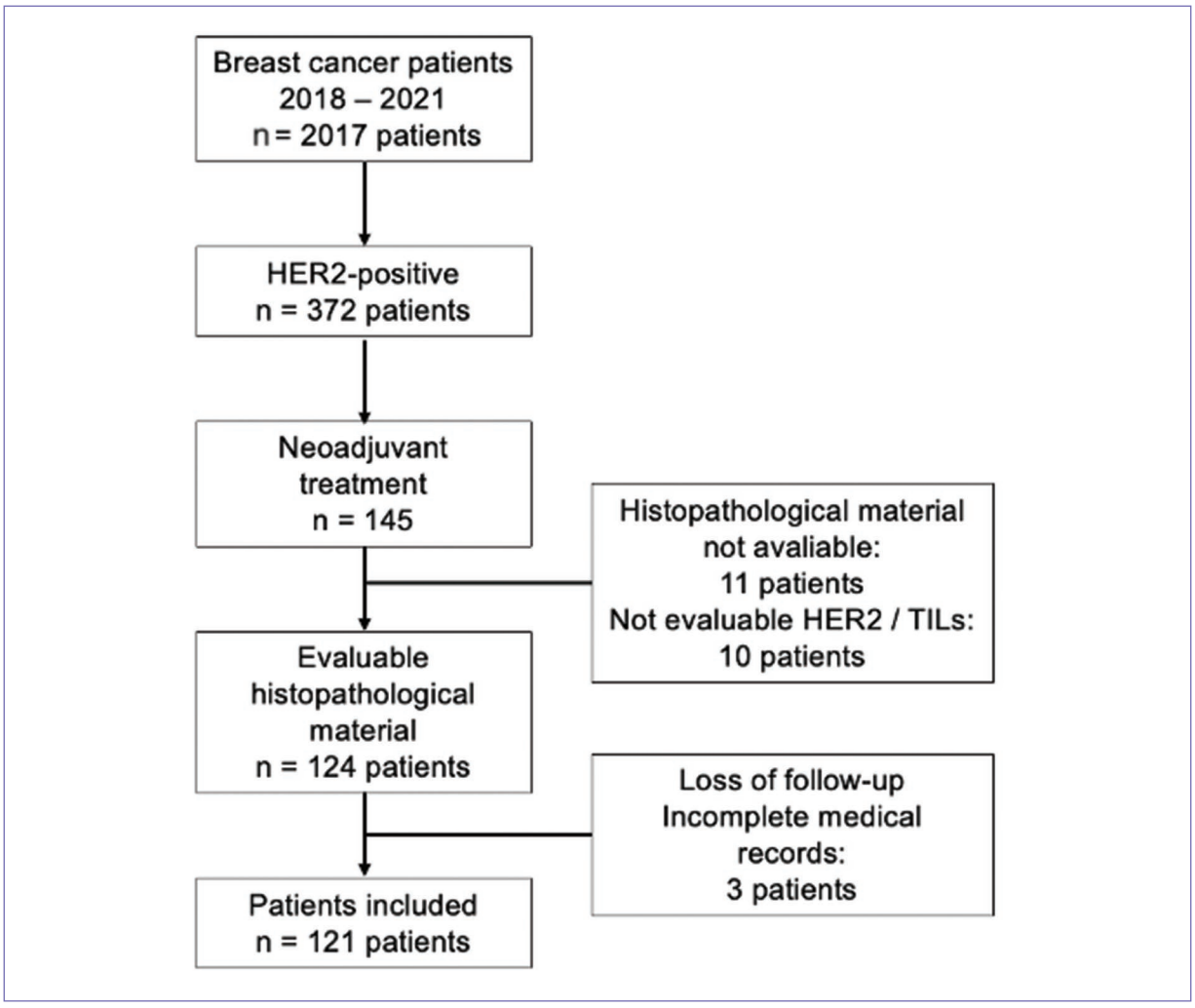

Figure 1. CONSORT diagram.

HER2-positive breast cancer. Elevated TIL levels were associated with greater benefit in DFS (HR 0.96) and OS (HR 0.90); in addition, a TIL threshold of 20% was most effective in predicting pCR in these patients (p = 0.035)[15].

In the Latin American population, specifically in Mexico, there are few relevant studies on this topic. Initially, research was conducted in patients with triple-negative breast cancer; in a study involving 62 patients, low TIL infiltration was associated with more advanced clinical stages, suggesting that TILs may serve as a useful prognostic factor. This study represents one of the first investigations in Mexico to evaluate this relationship, proposing the inclusion of TIL assessment as a routine parameter in pathology reports[16].

Subsequently, TILs were analyzed as a predictive factor for treatment response in 53 patients with HER2-positive breast cancer treated with neoadjuvant chemotherapy and trastuzumab. Although no statistical significance was observed for the primary endpoint, a trend toward better DFS and higher response rates was noted in patients with a higher percentage of TILs (> 25%). However, this study highlights the limited sample size[17].

In our study, we demonstrated that, similar to findings in Asian and European populations, in the Mexican population treated in a neoadjuvant setting with anti-HER2 therapy, TILs ≥ 10% serve as a prognostic factor. This is evidenced by a significant benefit in 2-year DFS, with a median of 72.6 months in patients with TILs ≥ 10% compared to 61.9 months in those with TILs < 10%, and a 21% reduction in recurrence risk (HR 0.79, 95% CI 0.73-0.97; p = 0.006). These findings support the role of TILs as a useful prognostic biomarker, as their presence reflects heightened immune activity

Table 1. Clinical and histopathological characteristics of the patients

Variable	Frequency (n = 121) No.	%
Age, mean±SD	54.9±10.96	
Histological type		
Ductal	112	92.56
Lobular	7	5.79
Others	2	1.65
Clinical stage		
I	2	1.6
IIA	15	12.4
IIB	25	20.7
IIIA	47	38.8
IIIB	16	13.2
IIIC	16	13.2
Histological grade		
G1	1	0.8
G2	60	49.6
G3	60	49.6
Tumor-infiltrating lymphocytes in the tumor stroma (TILs %)		
Low (TILs < 10%)	39	32.2
High (TILs ≥ 10%)	82	67.8
Neoadjuvant treatment		
AC-TH	67	55.4
EC-TH	45	37.2
TCb-HP	3	2.5
TCbH	6	5
Type of surgery		
Radical mastectomy	109	90
Breast-conserving surgery	12	10
Adjuvant Trastuzumab		
Yes	113	93.4
No	8	6.6
Adjuvant radiotherapy		
Yes	114	94.2
No	7	5.8

SD: standard deviation; G: grade; A: doxorubicin; C: cyclophosphamide; E: epirubicin; H: trastuzumab; T: docetaxel; Cb: carboplatin, P: pertuzumab; TIL: tumor stromal infiltrating lymphocytes.
Source: Medical Oncology Department, Oncology Hospital. Centro Médico Nacional Siglo XXI.

Table 2. Recurrence patterns

Site of recurrence	(n = 25) No.	%
Local/regional	5	4.13
Nodal	5	4.13
Bone	4	3.31
Pulmonary	4	3.31
Lymphangitic carcinomatosis	1	0.83
Liver	1	0.83
Central nervous system	1	0.83
Soft tissues/skin	3	2.48
Progression during neoadjuvant chemotherapy	2	1.65
Yes	119	98.35
No		

Source: Medical Oncology Department, Oncology Hospital. Centro Médico Nacional Siglo XXI.

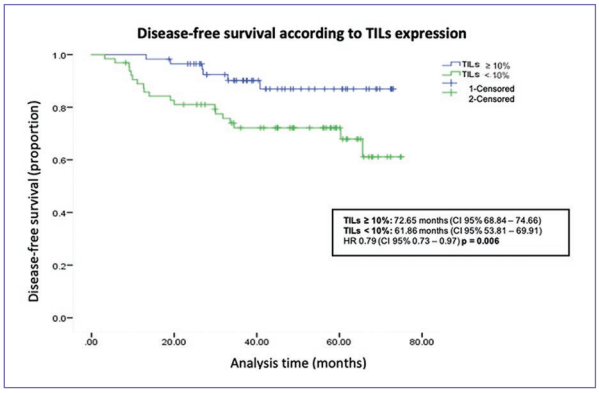

Figure 2. Disease-free survival according to tumor-infiltrating lymphocytes (TILs) expresión. Kaplan-Meier curve for DFS based on the percentage of TILs. A significantly longer DFS was observed in patients with TILs ≥ 10%, with a median of 72.65 months (95% confidence interval [CI], 68.84-74.66), compared to 61.86 months (95% CI, 53.81-69.91) in the group with TILs < 10%. The hazard ratio was 0.79 (95% CI, 0.73–0.97; p = 0.006).
Source: Medical Oncology Department. Oncology Hospital, Centro Médico Nacional Siglo XXI.

within the tumor microenvironment, which may restrict disease progression or eliminate residual disease post-treatment. Similarly, the low recurrence rate observed in patients with TILs ≥ 10% reinforces the premise that these patients could be more responsive to standard therapies, potentially avoiding exposure to more intensive treatments.

Regarding 2-year OS, patients with TILs ≥ 10% exhibited improved OS (76.7 months) compared to those with TILs < 10% (66.1 months), with a 17% reduction in the risk of death (HR 0.83, 95% CI 0.81-0.98; p = 0.015). The markedly low mortality rate in the TILs ≥ 10% group (2 of 82 patients, with no cancer-specific deaths) contrasts sharply with the TILs < 10% group, which experienced 10 deaths, 8 directly attributable to breast cancer.

Table 3. Univariate analysis for factors associated with TILs expresion

Cathegory	TILs				χ^2	p
	TILs ≥ 10%		TILS < 10%			
	n	%	n	%		
Histological type						
Ductal invasive	79	65.3	33	27.3	5.6	0.06
carcinoma	2	1.7	5	4.1		
Lobular carcinoma	1	0.8	1	0.8		
Others						
Clinical stage						
I	2	1.7	0	0.0	5.03	0.41
IIA	12	9.9	3	2.5		
IIB	17	14.0	8	6.6		
IIIA	27	22.3	20	16.5		
IIIB	12	9.9	4	3.3		
IIIC	12	9.9	4	3.3		
Histologic grade						
G1	1	0.8	0	0.0	0.51	0.77
G2	40	33.1	20	16.5		
G3	41	33.9	19	15.7		
Neoadjuvant treatment						
AC-TH	49	40.5	18	14.9	16.68	0.406
EC-TH	28	23.1	17	14.0		
EC-THP	2	1.7	1	0.8		
TCH	3	2.5	3	2.5		
Recurrence						
Yes	11	9.1	14	11.6	8.15	0.004
No	71	58.7	25	20.7		
Site of recurrence						
Local/regional	1	0.8	4	3.3	14.79	0.06
Nodal	2	1.7	3	2.5		
Bone	3	2.5	1	0.8		
Pulmonary	3	2.5	1	0.8		
Lymphangitic	0	0.0	1	0.8		
carcinomatosis	0	0.0	1	0.8		
Liver	0	0.0	1	0.8		
Centrall nervous system						
Soft tissues/skin	2	1.7	1	0.8		
Progression during neoadjuvant treatment						
Yes	0	0.0	2	1.7	4.27	0.03
No	82	67.8	37	30.6		
Site of progression						
Local	0	0.0	2	1.7	4.27	0.03
NA	82	67.8	37	30.6		
pCR						
Yes	31	60.8	20	39.2	4.58	0.03
No	26	37.1	44	62.9		
Cancer-specific mortality						
Death	4	3.3	8	6.6	7.23	0.007
Alive	78	64.5	31	25.6		

G: grade; A: doxorubicin; C: cyclophosphamide; E: epirubicin; H: trastuzumab; T: docetaxel; Cb: carboplatin, P: pertuzumab; pCR: pathological complete response; NA: not apply; TIL: tumor stromal infiltrating lymphocytes.
Source: Medical Oncology Department. Oncology Hospital, Centro Médico Nacional Siglo XXI.

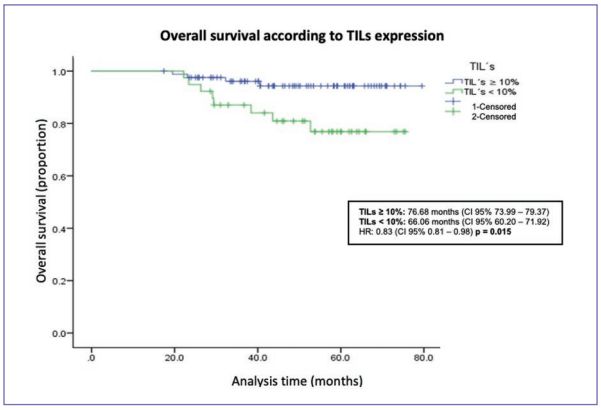

Figure 3. Overall survival according to tumor-infiltrating lymphocytes (TILs) expression. Kaplan-Meier survival curve for OS according to the percentage of TILs. Patients with TILs ≥ 10% demonstrated a median overall survival (OS) of 76.7 months (95% confidence interval [CI]: 73.99-79.37), which was superior to that of the group with TILs < 10%, who exhibited a median OS of 66.1 months (95% CI: 60.20-71.92). The difference between the groups was statistically significant (Hazard ratio 0.83, 95% CI: 0.81-0.98; p = 0.015).
Source: Medical Oncology Department. Oncology Hospital, Centro Médico Nacional Siglo XXI.

In terms of treatment response, we observed a significant association between elevated TILs levels (≥ 10%) and higher rates of pCR. Of the patients achieving pCR, 78.4% had TILs ≥ 10%, compared to 21.6% with TILs < 10% (p = 0.03). This difference further underscores the utility of TILs not only as a prognostic marker but also as a predictive indicator of treatment response.

These findings are consistent with international literature. The meta-analysis by Li et al. demonstrated that TILs are a prognostic biomarker, particularly in triple-negative and HER2-positive breast cancers–subtypes where immunogenicity is more pronounced–and identified TILs as an independent predictor of pCR. An immunologically active tumor microenvironment appears to enhance the efficacy of chemotherapy by promoting tumor cell destruction. The nearly 11-month difference in DFS and OS between the groups suggests a dynamic interaction between the tumor and the host immune system, which can significantly influence disease progression and survival. A tumor microenvironment rich in TILs confers better disease control, possibly due to a more effective immune response or residual sensitivity to subsequent treatments, as previously demonstrated in the NeoALTTO trial[13].

Recently, the ShortHER trial compared the standard sequential regimen of four cycles of anthracyclines

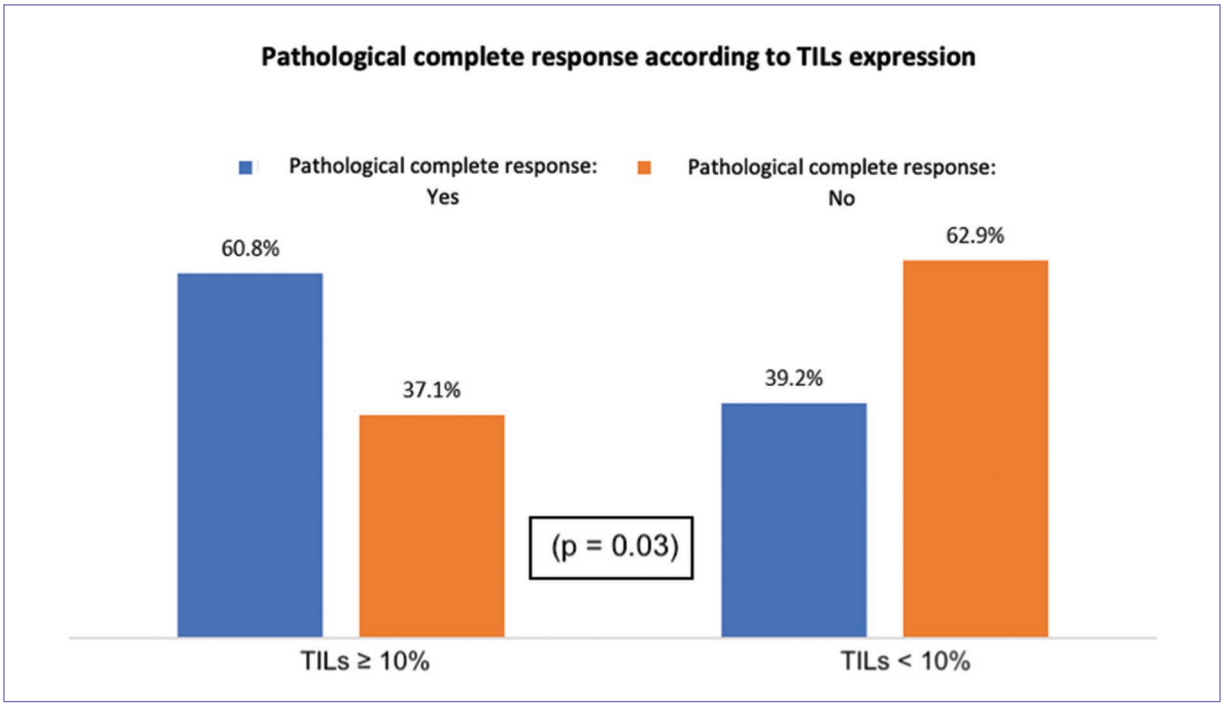

Figure 4. Pathological complete response (pCR) according to tumor-infiltrating lymphocytes (TILs) expression. Patients with a higher proportion of TILs achieved a pCR of up to 60.8%, compared to 39.2% in those with TILs < 10%.
Source: Medical Oncology Department. Oncology Hospital, Centro Médico Nacional Siglo XXI.

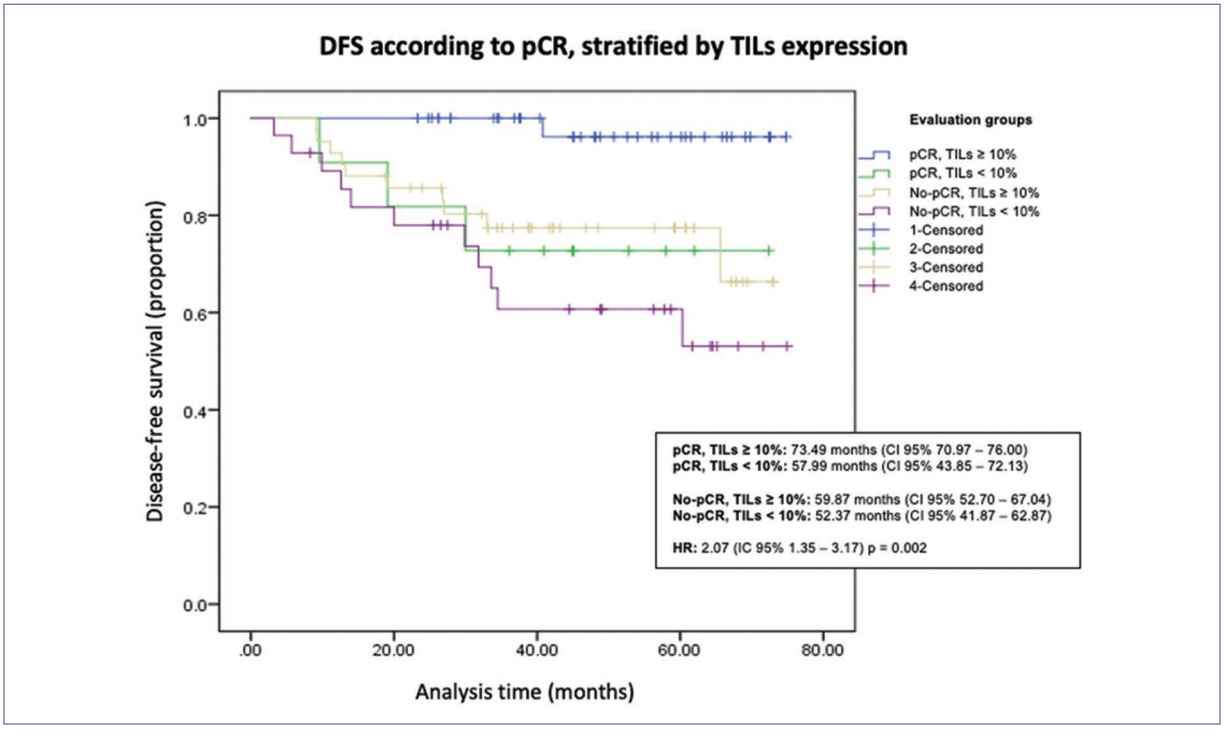

Figure 5. Disease-free survival according to pathological complete response (pCR), stratified by tumor-infiltrating lymphocytes (TILs) expression. Kaplan–Meier curve of EFS according to pCR and TILs percentage.Patients with TILs ≥ 10% and pCR had a longer EFS (73.49 months) compared to 57.99 months in patients with TILs < 10% and pCR. In patients with non-pCR, EFS was 59.87 months for TILs ≥ 10% and 52.37 months for TILs < 10%, hazard ration 2.07, 95% confidence interval, 1.35-3.17; p = 0.002.
Source: Medical Oncology Department. Oncology Hospital, Centro Médico Nacional Siglo XXI.

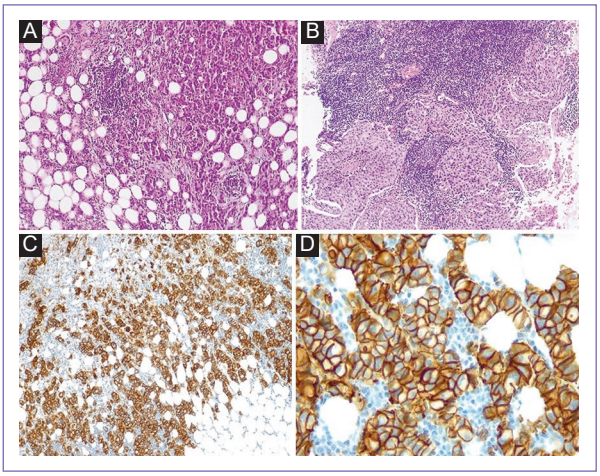

Figure 6. Photograph of tissue sections from a patient with human epidermal growth receptor 2 (HER2)-positive breast cancer, with an 80% tumor-infiltrating lymphocytes (TILs) report. **A:** invasive ductal carcinoma with a non-special pattern, grade 3 according to the Scarff-Bloom-Richardson grading system, accompanied by TILs. (HE10×). **B:** invasive ductal carcinoma with a non-special pattern, grade 3 according to the Scarff-Bloom-Richardson grading system, accompanied by intratumoral and peritumoral infiltrating lymphocytes (HE10×). **C:** HER2-positive breast cancer and TILs, exhibiting HER2 overexpression (3+) (HE10×). **D:** (HE40×). *Source: Medical Oncology Department. Oncology Hospital, Centro Médico Nacional Siglo XXI.*

followed by four cycles of taxanes and trastuzumab for 1 year with a shorter regimen consisting of three cycles of anthracyclines followed by three cycles of taxanes and a total of nine doses of trastuzumab in an adjuvant setting. With a 10-year follow-up, DFS was 91.3% in patients with TILs ≥ 20%, 93.3% in those with TILs ≥ 30%, and 98% in patients with TILs ≥ 50%, with statistically significant differences. Patients with TILs < 20% experienced greater benefit from the extended treatment (DFS at 10 years: 88.7 vs. 81.0%), whereas those with TILs ≥ 20% showed better DFS with the shorter regimen (87.1 vs. 92.2%; p = 0.01)[18].

These findings suggest that TILs could be useful for risk stratification, aiding in the selection of patients who may benefit from more intensive treatments or, conversely, from treatment de-escalation–particularly in contexts with limited access to targeted therapies.

It is noteworthy that, in the exploratory analysis conducted in our study, patients with TILs ≥ 10% and pCR had a median DFS of 73.5 months, compared to 59.9 months in patients without pCR. Conversely, patients with TILs < 10% exhibited a DFS of 58.0 months versus 52.4 months in those with and without pCR, respectively (p = 0.03). This underscores the expression of TILs as a more relevant prognostic factor than pCR in patients with HER2-positive breast cancer.

Overall, the interaction between TILs and pCR not only enhances DFS but could also be utilized to identify patients with a favorable prognosis even in the presence of residual disease. This supports their integration into personalized prognostic models.

These findings also highlight the need to standardize cutoff points in the assessment of TILs. Although a threshold of ≥ 10% proved useful in our study–primarily based on Asian studies–other research has proposed values such as 20 or even 30%, indicating methodological heterogeneity that currently limits universal application[19,20].

Among the limitations identified in this work is the retrospective nature of the study, where TILs measurement on slide review was performed by a single pathologist with specialized expertise in breast cancer. Consequently, there was no double verification or assessment of interobserver concordance. However, evaluating TILs through optical microscopy on hematoxylin and eosin-stained sections **(**Fig. 6) is feasible within routine clinical practice at secondary and tertiary care levels. Nonetheless, specific training in TILs interpretation is necessary for general pathologists reviewing breast cancer specimens. The reproducibility of TILs reporting is achievable, but heterogeneity remains a source of bias. It would be advisable to automate this assessment universally, as results may vary depending on the experience of different pathologists interpreting TILs.

Another significant limitation of our study is that it was based solely on the use of trastuzumab as an anti-HER2 therapy, due to limited access to dual blockade regimens with pertuzumab in our hospital unit. Therefore, we consider it ideal to replicate this methodology using these treatment schemes to compare results.

At the oncology hospital, approximately 600 breast cancer patients are treated annually, of whom 20% are HER2 positive. This highlights the necessity of having reliable biomarkers to categorize patients according to their risk, thereby guiding (de)escalation of therapies. The measurement of TILs expression does not require the implementation of additional equipment or materials.

Prospective trials are needed to validate the utility of TILs with longer follow-up periods and updated therapies, with the aim of incorporating their assessment into routine histopathological reports and establishing their use as an accessible, reproducible, and clinically relevant prognostic biomarker.

Conclusions

The expression of TILs ≥ 10% in patients with HER2-positive breast cancer treated with neoadjuvant chemotherapy plus trastuzumab was associated with improved DFS, OS, and complete pathological response.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that no experiments involving humans or animals were conducted for this research.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from patients, and received approval from the Ethics Committee. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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