InaJCC The Indonesian Journal of Cancer Control

Original Article

Prognostic value of tumor-infiltrating lymphocytes among HER-2+ breast cancer patients receiving trastuzumab-based adjuvant therapy

Diah A Safitri[1], Ahmad Ghozali[2], Johan Kurnianda[1]*

p-ISSN 2797-4189
e-ISSN 2797-457X
DOI : 10.52830/inajcc.v1i2.47

Received: February 23, 2021
Accepted: November 25, 2022

Authors' affiliations:
1, Division of Hematology-Medical Oncology, Department of Internal Medicine, Faculty of Medicine Public Health and Nursing Universitas Gadjah Mada / Dr Sardjito Hospital Yogyakarta
2. Department of Anatomical Pathology, Faculty of Medicine Public Health and Nursing Universitas Gadjah Mada / Dr Sardjito Hospital Yogyakarta

Corresponding author:
Johan Kurnianda
E-mail: *johan.kurnianda@yahoo.com*

Abstract

Background: Tumor-infiltrating Lymphocytes (TILs) have prognostic value on HER2 positive breast cancer (BC). The absence of standardized method for evaluating TILs causes variations in results of previous studies. This study is performed to evaluate the prognostic value of TILs in HER2 positive BC treated with trastuzumab-based adjuvant therapy using standardized method recommended by the *International TILs Working Group*.
Aim: To analyze the prognostic value of TILs in HER2 positive BC patients receiving trastuzumab-based adjuvant therapy at Dr. Sardjito General Hospital, Yogyakarta and to analyze proportion differences between high TILs (≥30%) and low TILs (<30%).
Methods: This is a retrospective cohort study on HER2 positive, stage 1-3 BC patients who received trastuzumab-based adjuvant therapy. Histopathology slides from 6 hospitals/laboratories were analyzed by two pathologists.
Results: 73 data were available for analysis. TILs stroma <30% was 65,8% and most patients received combination of anthracyclines, taxanes and trastuzumab (67,1%). There was no difference of overall survival between high and low TILs (p log rank: 0,331).
Conclusion: The proportion of HER2 positive breast cancer with high TILs was lower than those with low TILs. HER2 positive BC with high TILs did not show better overall survival compared to those with low TILs. Our study did not support the theory that different TILs score has prognostic value in HER2 positive breast cancer. Since no formal recommendation for a clinically relevant TIL threshold has been given, further study with bigger samples and better concordance rate among pathologists should be done.

Keywords: *adjuvant, breast cancer, HER2, trastuzumab, tumor-infiltrating lymphocytes*

Abstrak

Latar Belakang: *Tumor-infiltrating Lymphocytes (TILs)* pada penderita kanker payudara HER2 positif mempunyai nilai prognostik. Belum adanya metode penilaian TILs yang terstandarisasi menyebabkan hasil penelitian sebelumnya bervariasi. Penelitian ini dilakukan untuk mengetahui nilai prognostik TILs pada kanker payudara HER2 positif yang mendapat terapi adjuvan berbasis trastuzumab dengan menggunakan metode yang telah distandardisasi International TILs Working Group.
Tujuan: Menganalisis nilai prognostik TILs pada penderita kanker payudara HER2+ yang mendapatkan terapi adjuvan berbasis trastuzumab di RSUP Dr. Sardjito Yogyakarta serta menilai perbedaan proporsi antara penderita kanker payudara HER2 positif dengan TILs tinggi (≥30%) dan penderita kanker payudara HER2 positif dengan TILs rendah (<30%).
Metode: Penelitian ini adalah penelitian kohort retrospektif yang dilakukan terhadap data pasien kanker payudara HER2 positif stadium 1-3 yang mendapat terapi adjuvan trastusumab, dengan slide histopatologi yang lengkap yang didapat dari 6 lokasi RS/laboratorium dan dianalisis oleh 2 ahli patologi anatomi.
Hasil: Dari 73 data yang dianalisis, terdapat 65,8% TILs stroma <30% dan sebagian besar pasien mendapat emoterapi dengan kombinasi antrasiklin, taxan dan trastuzumab (67,1%). Tidak terdapat perbedaan angka ketahanan hidup antara penderita dengan TILs tinggi dibanding TILs rendah (p log rank: 0,331).
Kesimpulan: Proporsi penderita kanker payudara HER 2 positif dengan TILs tinggi lebih kecil dibandingkan proporsi penderita kanker payudara HER 2 positif dengan TILs rendah. Penderita kanker payudara HER2 positif dengan TILs yang tinggi tidak memiliki angka ketahanan hidup yang lebih baik dibandingkan dengan penderita kanker payudara HER2 positif dengan TILs yang rendah. Saat ini belum ada ambang nilai TILs yang direkomendasikan, sehingga diperlukan penelitian lanjut dengan jumlah sampel yang lebih besar dan tingkat kesepakatan antar ahli patologi yang lebih baik.

Kata kunci: *ajuvan, HER2, kanker payudara, trastuzumab, tumor-infiltrating lymphocytes*

Background

Breast cancer (BC) is a heterogenous disease with multiple subtypes. Clinically, classification of BC is determined by expression of 3 biomarkers, i.e. estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth 2 (HER2). Breast cancer with negative ER, PR, and HER2 is called triple negative breast cancer (TNBC) and it accounts for 15-20% of all new cases.[1] In 20-30% of BC, there is amplification of HER2 resulting in overexpression of HER2. Long before an effective anti-HER2 treatment was discovered, this pattern was associated with more aggressive disease with worse prognosis.[2]

Trastuzumab-based adjuvant chemotherapy is still a treatment of choice for HER2 positive invasive BC, either with positive ER/PR or negative ER/PR. The recommended regimen usually contains of combination of trastuzumab and taxane, or trastuzumab, anthracycline, and taxane[3]. The use of anthracycline and taxane in adjuvant chemotherapy also give benefit to survival. The additional of trastuzumab in adjuvant chemotherapy regimen decreases risk in recurrence if it is administered both simultaneously and consecutively. Trastuzumab that is administered for a year is more effective than 6 months. Two years administration of trastuzumab is not more effective than one-year administration.[4]

Tumor infiltrating lymphocytes (TILs) plays the main role in response to cancer cells so that TILs might be the main marker of immune balance of host and tumor.[5] TILs consist all mononuclear cells (including lymphocyte and plasma cell) that are found in tumor tissue.[6] According to its infiltration site, TILs are classified into intratumor TILs and stroma TILs.[7]

TILs are related to improvement of survival in various kind of cancer. This shows that TILs play a role in antitumor immunity.[8,9,10] Some studies have also shown that high level of TILs in BC could predict better response after neoadjuvant chemotherapy.[6,10] High level of TILs is also able to give prognostic value. Triple negative BC and HER2 positive BC with high TILs show a better survival.[6,11]

However, the prognostic and predictive value of TILs are found inconsistent and there is no exact examination method for TILs.[5] Lately, there is a standardized approach that is recommended by international panel to assess TILs on routine histopathology slide as biomarker of BC.[12].

Methods

This is a retrospective cohort on HER2 positive breast cancer patients in Dr. Sardjito Hospital, Yogyakarta. Patients with stage 1-3 (T1-4/N0-3/M0) that received adjuvant trastuzumab and were diagnosed in 2007-2015 period with complete clinical and histopathological data were enrolled to this study while patient with stage 4 and incomplete data were excluded from this study. The ethical clearance of this study was obtained from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Gadjah Mada University – Dr. Sardjito Hospital with approval letter number: KE/FK/409/EC/2016.

Collection of clinical data was performed as the development of organ specific cancer registry in Dr. Sardjito Hospital for period of 2007-2015 and was continued by 1-year follow-up. Collection and selection of histopathology slide and parafin block were performed at Pathology Department of Dr. Sardjito Hospital, Faculty of Medicine Universitas Gadjah Mada, Waskitha Laboratorium Yogyakarta, Cito Laboratorium, Panti Rapih Hospital, and Bethesda Hospital. TILs calculation was performed at Pathology Department of Dr. Sardjito Hospital.

Minimum sample size is 60 samples. TILs were assessed with hematoxilin-eosin staining. The analysis of breast tissue was performed blindly by two pathologists from Pathology Department Dr. Sardjito Hospital. The mean of those assessment was analyzed. The expected coefficient of Spearman correlation is more than 0.60.

HER2 positive BC is diagnosed clinically and pathologically as breast cancer with HER2 +3 from immunohistochemistry examination. TNM staging is assessed based on American Joint Committee on Cancer (AJCC) criteria for patient's pathology report after surgery. The trastuzumab-based regimen consists of trastuzumab (H) in combination with anthracycline (A), cyclophosphamide (C), platinum (P), taxane (T), 5-fluorouracil (F), and methotrexate (M). The combination that had been used i.e., AC-TH, FAC-TH, CMF-TH, TCH, TPH, AT-H, AC-H, CAF-H, and TAC-H.

Numeric data is described in mean ± standard deviation (SD) or median with range of minimum to maximum value. Chi-square test or Fisher's exact test is used to analyze categorical data to compare all parameters to clinicopathological parameter. Overall survival is analyzed using Kaplan Meier method

continued by log-rank test to analyze the significance of difference. Univariate and multivariate analysis is performed with Cox's proportional hazards model to determine hazard ratio (HR) with 95% confidence interval.

Results

We collected 73 patients that met the inclusion criteria. The patient's age range was 27-74 years old with median age of 50 years old. Population of study subjects was dominated by those with age of ≥ 40 years old (87,7%), N0-1 (75%), negative ER and PR status (81,9%), degree of histology 3 (68%), and stroma TILs <30% (65,8%). The most administered chemotherapy regimen was combination of anthracycline, taxane, and trastuzumab (67,1%) (Table 1). The pathology slide that was eligible for assessment was from 2009-2015 period.

Table 1. Baseline characteristics of HER2+ breast cancer subjects

Parameter		N	(%)
Age (year), median, range	50 (27-74)		
Age when diagnosed (n=73)	• <40 years old	9	12,3%
	• ≥40 years old	64	87,7%
Tumor size (n=70)	• T ≤ 5 cm	31	44,3%
	• T > 5cm	39	55,7%
Nodal (n=60)	• N0-N1	45	75,0%
	• N2-N3	15	25,0%
ER/PR (n=72)	• negative	59	81,9%
	• positive (+/+, +/-, or -/+)	13	18,1%
Histological feature (n=71)	• Non-ductal	5	7,0%
	• Ductal	66	93,0%
Histological grade (n=50)	• 1-2	16	32,0%
	• 3	34	68,0%
TILs stroma (n=73)	• <30%	48	65,8%
	• ≥30%	25	34,2%
Chemotherapy type (in combination with trastuzumab) (n=73)	• antracycline and taxane combinatio	49	67,1%
	• other than antra cycline and taxane combination	24	32,9%

Coefficient of Spearman correlation from two pathologists showed that there was significant yet weak correlation (0,354; p=0,003) even though an international guideline to equalize technique and TILs reading had been used.

Most of study subjects were diagnosed as BC at the age of ≥ 40 years old (87,7%). Most of study subjects had T >5 cm and 75% of subjects had N0-N1. In this study most samples had poor histology profiles (68%) with invasive ductal carcinoma dominated the samples (93%).

The proportion of HER2 positive BC with TILs ≥30% in this study was higher than the previous study accounting for 34,2% of cases and the clinicopathological parameters based on TILs level is shown in Table 2. Some of TILs level in this study are shown in picture 1,2, and 3. Most of subjects in this study received combination of anthracycline, taxane, and trastuzumab.

Table 2. Clinicopathological parameters based on TILs level

Variables		TILs		p*
		<30%	≥30%	
Age when diagnosed (n=73)	• <40 years old	9	0	0,017*
	• ≥40 years old	39	25	
Tumor size (n=70)	• T ≤ 5 cm	19	12	0,329*
	• T > 5cm	27	12	
Nodal (n=60)	• N0-N1	29	16	0,093*
	• N2-N3	13	2	
ER/PR (n=72)	• negative	38	21	0,301*
	• positive (+/+, +/-, or -/+)	10	3	
Histological feature (n=71)	• Non-ductal	4	1	0,419*
	• Ductal	42	24	
Histological grade (n=50)	• 1-2	11	5	0,291*
	• 3	19	15	
Chemotherapy type (in combination with trastuzumab) (n=73)	• antracycline and taxane combination	35	14	0,116*
	• other than antracycline and taxane combination	13	11	

*Fisher exact test

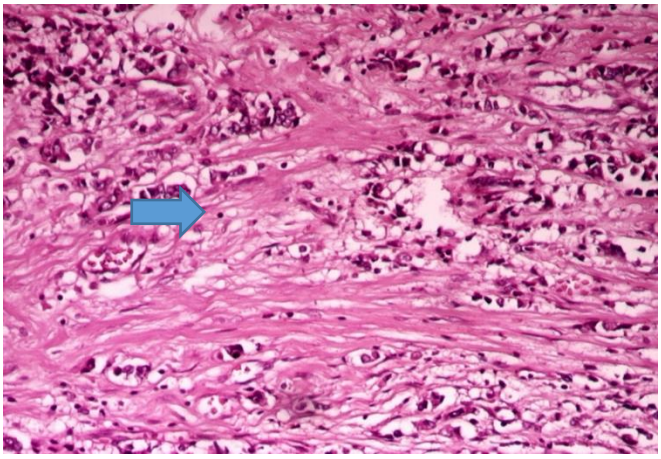

Figure 1. A hematoxylin and eosin (H&E) stained histopathology slide of a breast cancer under a microscope, showing 5% tumor-infiltrating lymphocytes (TILs) in the stromal area.

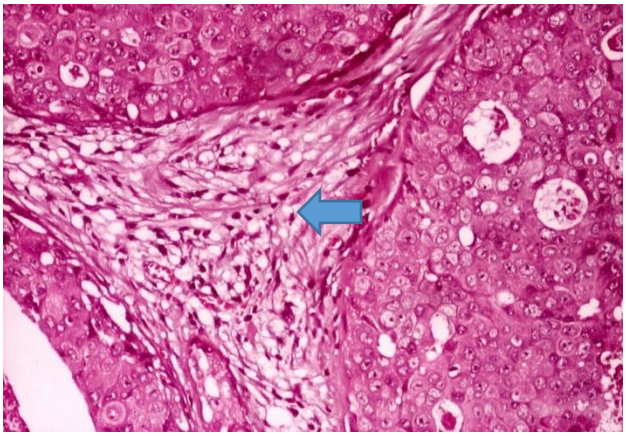

Figure 2. A H&E stained slide of breast cancer, showing 40% stromal tumor-infiltrating lymphocytes (TILs).

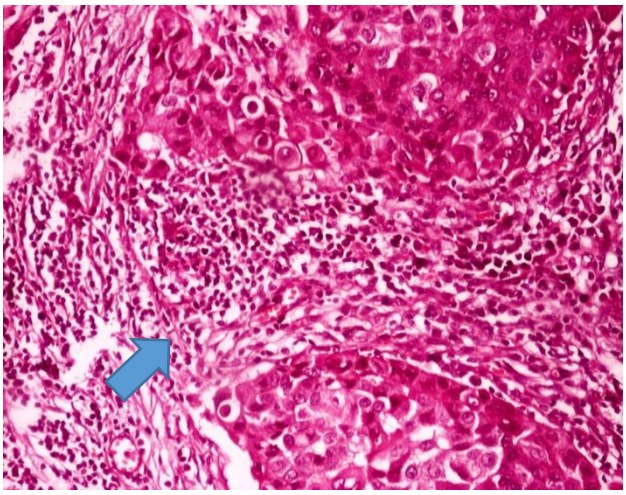

Figure 3. A H&E stained slide of breast cancer with high stromal TILs (80%). Almost all stroma was covered with inflammatory cells.

Follow-up was performed in range of 8,03 to 97,17 months with median of 38,52 months. Subjects with high TILs showed no difference in survival rate to low TILs (p log rank= 0,331) (Figure 4). This result is not consistent with this study's hypothesis.

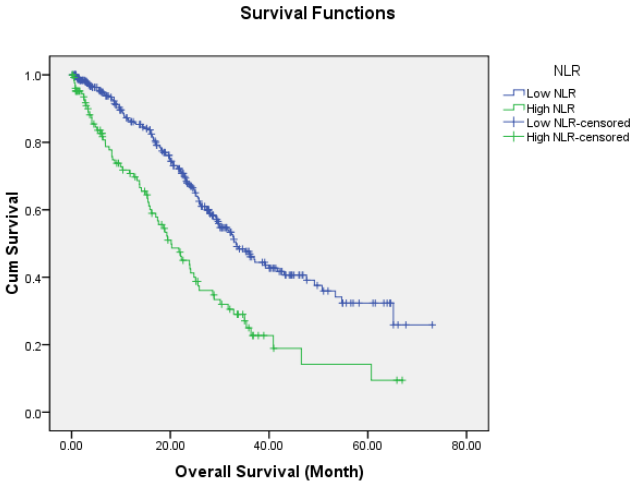

Figure 4. Overall survival of HER2+ breast cancer based on TILs level.

Discussion

The role of infiltrating immune cells is to control tumor growth and progression. However, infiltrating immune cells can also help to create an immunosuppressive environment in which the tumor can thrive.[13]

Even though there is a standardized approach on how to assess TILs on routine histopathology slide, heterogenous TILs' characteristics still might cause variability among assessors.[12,14]

Heterogenity in lymphocyte distribution, technical slide-related issues, scoring outside the tumor boundary, tumors with minimal assessable stroma, lymphocytes associated with other structures, and including other inflammatory cells also may lead to discordant sTIL assessment. [14]

There is no difference between high TILs and low TILs group based on clinicopathology factor (tumor size, nodal status, ER/PR, histology type, histology degree, and ductal or non-ductal carcinoma) and treatment regimen, except for age at first diagnosis.

Our study showed that most subjects were diagnosed at the age of of ≥ 40 years old and is consistent with previous studies that showed that BC at young age rarely occurred. Around 6,6% of BC was diagnosed in women at the age of < 40 years old, 2,4% at the age of < 35 years old, and 0,65% at the age of < 30 years old.[15]

Our study showed that invasive ductal carcinoma dominated the samples (93%) and supports a study done in Dr. Cipto Mangunkusumo Hospital which showed that 89,14% of 515 cases was invasive ductal carcinoma.[16]

The proportion of HER2 positive BC with TILs ≥30% in this study was higher than the previous study accounting for 34,2% of cases. The proportion of HER2 positive/ER negative BC and HER2 luminal B BC were 21% and 8% respectively on a study by Ohtani et al.[8] In another study by Liu et al. the proportion of HER2 positive BC patients was 31%.[17] However, there has not been any formal recommendation for a clinically relevant TIL threshold.[13]

Table 3. Univariate analysis for prognostic factors in HER2+ breast cancer

Parameters	*Hazard ratio*	CI 95%	p value
TILs stroma			
• <30%	1,597	0,615-4,143	0,336
• ≥30%			
Age			
• < 40 years old	0,994	0,227-4,351	0,993
• ≥ 40 years old			
Tumor size			
• T ≤ 5 cm	1,406	0,521-3,791	0,501
• T > 5cm			
Nodal			
• N0-N1	1,406	0,768-2,576	0,269
• N2-N3			
Hormonal status			
• negative	0,270	0,036-2,035	0,204
• positive			
Histological feature			
• non-ductal	0,646	0,147-2,845	0,564
• ductal			
Histological grade			
• 1-2	0,939	0,224-3,939	0,931
• 3			
Chemotherapy type (in combination with trastuzumab)			
• antracycline and taxane combination	1,125	0,411-3,074	0,819
• other than antra-cycline and taxane combination			

We carried out a univariate analysis for prognostic factors in HER2+ breast cancer to investigate the relationship between TILs stroma and prognosis. The result showed non-significant relationship (p: 0,336) (Table 3). Our study showed that higher TILs level (≥30%) did not relate to a better overall survival of HER2+ breast cancer subjects as shown in Figure 4.

Consistent to our study, some studies had shown that higher TILs level in stroma around tumor was not related to better prognosis in HER2 positive BC.[11,18-20] FinHER trial showed that associations between TILs and good prognosis was observed in the TNBC but not in luminal or HER2+ subtypes. However, in this study, TILs were measured as a continuous variable. Each 10% increase in TILs was associated with 13% reduction in the relative risk of distant recurrence, but there was no statistical significance observed for overall survival (OS) likely due to the small number of events observed.[11] The BIG 02-98 study showed that there was no significant prognostic effect in the global population, in those with ER positive/HER2 negative disease, or in the HER2 positive subgroup. In contrast, for the ER negative/ HER2 negative BC subtype, TILs were strongly for both disease free survival (DFS) and OS.[18] In a study by Hida et al., TILs score was classified as low (<10 %), intermediate (10–50 %), and high (>50 %) based on the area infiltrated by lymphocytes within the tumor itself plus the adjacent stroma. TILs proved to have significant prognostic value regarding relapse-free survival (RFS) in TNBC, but not among HER2+ BCs.[20]

A meta-analysis from 17 studies that evaluated level of TILs and prognostic parameter for BC revealed that high TILs were not correlated with clinicopathology of BC even though some subtypes could be correlated. Positive PD-1 T cell subtype was linked to high tumor grade, big tumor size, positive lymph node, negative hormonal receptor status, and HER2 status.[21]

Univariate analysis in various prognostic values showed that there was no significant correlation to death risk in stroma TILs ≥30% group compared to those with <30% of stroma TILs (HR 1,597; p=0,336; 95% IK 0,615-4,143). Age, tumor size, hormonal status, nodal status, histology type and degree were also not correlated with death risk (Table 3).

This result is not consistent with the previous studies probably due to the difference of setting (neoadjuvant vs adjuvant), TILs cut-off, molecular subtypes, tumor

microenvironment, the choice of general or subset TILs, and amplification level of HER2.[10,22,23]

However, this current study supports the study result of Hida et al. which was performed in both adjuvant and neoadjuvant setting. TILs proved to have significant prognostic value in TNBC, but not among HER2 positive BC.[20] The similar result was also stated by N9831 study in which the prognostic value of LPBC was only found in HER2+ breast cancer that received chemotherapy only instead of trastuzumab-based chemotherapy.[19] Gene expression analysis using FinHER sample showed that IDO1 and CXCL13 were strongly associated with TILs. Even though the immune gene that is related to HER2+ breast cancer prognosis has not been found yet, the high level of PD-1 and IDO1 that is associated to the benefit of trastuzumab for DFS showed that trastuzumab could modulate the micro-environment of immune system.[24]

Furthermore, CLEOPATRA study showed that anti-tumor immunity was still going on in advanced breast cancer setting (recurrent, unresectable, or metastatic). In advanced HER2+ breast cancer, high TILs is associated with increased survival.[25] On the other hand, Kotoula et al. showed that primary metastatic HER2+ breast cancer had low TILs and was not associated with the end result but it was enriched by mutations that changed the characteristic of amino acid. Metastatic breast cancer, especially de novo, with hydrophobic mutation did not show any benefit of trastuzumab administration.[26]

One study by Ohtani et al. involved all subtypes of breast cancer while our study only included HER2+ breast cancer.[10] Miyan et al. reported that every molecular subtype is generally characterized by T cell infiltration instead of specific phenotype infiltration. There is difference of immune response among the molecular subtypes of breast cancer. The luminal subtypes were marked by low immune response showing negative or weak presentation of lymphocytic host response (LHR). Non-luminal subtypes (ER -) showed a prominent immune response presented by either moderate or strong presentation of LHR.[27]

The quality and quantity of immune response could be influenced by a lot of factors in the microenvironment of tumor and lymph node, e.g. cytokines and chemokines that will affect the cell types through its function, nutrients availability, oxygen, and lactate.[25,26] Any difficulty in controlling microenvironment could affect study result. The difference of tumor microenvironment according to the molecular subtypes could be shown by the difference of stroma lymphocyte score marked by CD3 and the difference in CD8+ density, FOXP3+, *ζ–chain*+, and CD3+.[27,30]

TILs subset CD3+, CD4+, and CD8+ have prognostic value and high score is associated with better survival.[31-33] On the other hand, other immune cells, as of macrophage and FOXP3+ Tregs, facilitate and increase carcinogenesis and tumor growth. FOXP3+ Tregs is potential to suppress T cells by suppressing antitumor immunity that is caused by T cells CD4+, CD8+, dendritic cells, and NK cells.[23] The prognostic value of TILs FOXP3+ in breast cancer is affected by the expression status of ER, HER2, and the infiltration of T cell CD8+. TILs subset FOXP3+ is the bad prognostic indicator for ER+ breast cancer but in the HER2+/ER- subtype breast cancer, it acts as good prognostic indicator.[34] In this study, the analysis was performed on both ER/PR negative and positive breast cancer. Most of the subjects (80,8%) had negative ER/PR while the rest were ER+/PR+, ER+/PR-, and ER-/PR+.

The benefit of Trastuzumab in HER2+ breast cancer is not always achieved despite of its role as the standard treatment. Xu et al. showed that the amplification level of HER2 is correlated to pathological complete response (pCR).[35] However, TILs level is not associated with the response towards trastuzumab in locally advanced HER2+ breast cancer.[35]

Conclusion

Our study did not support the theory that TILs have prognostic value on HER2 positive breast cancer. The classification into high or low TILs did not show any difference in the survival of HER2 positive breast cancer patients. Since no formal recommendation for a clinically relevant TILs threshold has been given, further study with bigger samples and better concordance rate among pathologists should be done.

Conflict of Interest

The authors have no conflict of interest to declare.

References

1. Stagg J, Allard B. Immunotherapeutic approaches in triple-negative breast cancer: Latest research and clinical prospects. Ther Adv Med Oncol. 2013;5:169–81.

2. Brown-Glaberman U, Dayao Z, Royce M. HER2-targeted therapy for early-stage breast cancer: A comprehensive review. Oncology [Internet]. 2014;28(4). Available from: https://www.cancernetwork.com/view/her2-targeted-therapy-early-stage-breast-cancer-comprehensive-review
3. Gradishar WJ, Anderson BO, Balassanian R, Blair SL, Burstein HJ, Cyr A, et al. Breast Cancer. NCCN Guidelines. 2016
4. Anampa J, Makower D, Sparano JA. Progress in adjuvant chemotherapy for breast cancer: An overview. BMC medicine. 2015;13:195.
5. García-Martínez E, Gil G, Benito A, González-Billalabeitia E, Conesa M, García T, et al. Tumor-infiltrating immune cell profiles and their change after neoadjuvant chemotherapy predict response and prognosis of breast cancer. Breast Cancer Res. 2014;16:488.
6. Salgado R, Denkert C, Campbell C, Savas P, Nuciforo P, Aura C, et al. Tumor-infiltrating lymphocytes and associations with pathological complete response and event-free survival in HER2-positive early-stage breast cancer treated with lapatinib and trastuzumab. JAMA Oncol. 2015;1(4):448–55.
7. Mao Y, Qu Q, Zhang Y, Liu J, Chen X, Shen K. The value of tumor infiltrating lymphocytes (TILs) for predicting response to neoadjuvant chemotherapy in breast cancer: A systematic review and meta-analysis. PLOS ONE. 2014;9(12):e115103.
8. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348(3):203–13.
9. Kondratiev S, Sabo E, Yakirevich E, Lavie O, Resnick MB. Intratumoral CD8+ T lymphocytes as a prognostic factor of survival in endometrial carcinoma. Clin Cancer Res. 2004;10:4450–6.
10. Ohtani H, Mori-Shiraishi K, Nakajima M, Ueki H. Defining lymphocyte-predominant breast cancer by the proportion of lymphocyte-rich stroma and its significance in routine histopathological diagnosis. Pathol Int. 2015;65:644–51.
11. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: Results from the FinHER trial. Ann Oncol. 2014;25(8):1544–50.
12. Salgado R, Denkert C, Demaria N, Sirtaine F, Klauschen G, Pruneri S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: Recommendations by an International TILs Working Group 2014. Ann Oncol. 2015;26:259–71.
13. Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B, et al. Assessing Tumor-infiltrating Lymphocytes in Solid Tumors: A Practical Review for Pathologists and Proposal for a Standardized Method from the International Immunooncology Biomarkers Working Group: Part 1: Assessing the Host Immune Response, TILs in Invasive Breast Carcinoma and Ductal Carcinoma in Situ, Metastatic Tumor Deposits and Areas for Further Research. Vol. 24, Advances in Anatomic Pathology. Lippincott Williams and Wilkins; 2017. p. 235–51.
14. Kos Z, Roblin E, Kim RS, Michiels S, Gallas BD, Chen W, et al. Pitfalls in assessing stromal tumor infiltrating lymphocytes (sTILs) in breast cancer. NPJ Breast Cancer. 2020 Dec 1;6(1).
15. Assi HA, Khoury KE, Dbouk H, Khalil LE, Mouhieddine TH, El Saghir NS. Epidemiology and prognosis of breast cancer in young women. J Thorac Dis. 2013;5(S1):S2–8.
16. Tjahjadi G, Sakamoto G, Thindarbumi D, Watanabe S, Prihartono J, Ohno Y, et al. Pathological aspects of breast cancer in Indonesian females, applying a modified WHO classification. A joint study between Indonesia and Japan. Med J Indones. 1999;8:102–8.
17. Liu S, Duan X, Xu L, Xin L, Cheng Y, Liu Q, et al. Optimal threshold for stromal tumor-infiltrating lymphocytes: its predictive and prognostic value in HER2-positive breast cancer treated with trastuzumab-based neoadjuvant chemotherapy. Breast Cancer Res Treat. 2015 Nov 1;154(2):239–49.
18. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol. 2013;31(7):860–7.
19. Perez EA, Ballman KV, Tenner KS, Thompson EA, Badve SS, Bailey H, et al. Association of stromal tumor-infiltrating lymphocytes with recurrence-free survival in the N9831 adjuvant trial in patients with early-stage HER2-positive breast cancer. JAMA Oncol. 2016;2(1):56–64.
20. Hida AI, Sagara Y, Yotsumoto D, Kanemitsu S, Kawano J, Baba S, et al. Prognostic and predictive impacts of tumor-infiltrating lymphocytes differ between Triple-negative and HER2-positive breast cancers treated with standard systemic therapies. Breast Cancer Res Treat. 2016;158:1–9.
21. Yu X, Zhang Z, Wang Z, Wu P, Qiu F, Huang J. Prognostic and predictive value of tumor-infiltrating lymphocytes in breast cancer: A systematic review and meta-analysis. Clin Transl Oncol. 2016;18:497–506.
22. Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28(1):105–13.
23. Mao Y, Qu Q, Chen X, Huang O, Wu J, Shen K. The prognostic value of tumor-infiltrating lymphocytes in breast cancer: A systematic review and meta-analysis. PLOS ONE. 2016;11(4):e0152500.
24. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes (TILs) indicate trastuzumab benefit in early-stage HER2-positive breast cancer (HER2+ BC). Cancer Research [Internet]. 2013 Dec 15;73(24 Suppl):Abstract S1-05. Available from: http://cancerres.aacrjournals.org/content/73/24_Supplement/S1-05.abstract
25. Luen S, Salgado R, Fox S, Savas P, Eng-Wong J, Clark E, et al. Tumour-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab

or placebo in addition to trastuzumab and docetaxel: A retrospective analysis of the CLEOPATRA study. Lancet Oncol. 2017;18(1):52–62.
26. Kotoula V, Giannoulatou E, Alexopoulou Z, Karavasilis V, Kotsakis A, Dionysopoulos D, et al. Tumor infiltrating lymphocytes and hydrophobic amino acid changes in HER2-positive metastatic breast cancer. J Clin Oncol. 2016;34(15 Suppl):592.
27. Miyan M, Schmidt-Mende J, Kiessling R, Poschke I, de Boniface J, Ferlay J, et al. Differential tumor infiltration by T-cells characterizes intrinsic molecular subtypes in breast cancer. J Transl Med. 2016;14:227.
28. Dunn GP, Old LJ, Schreiber RD. The three es of cancer immunoediting. Annu Rev Immunol. 2004;22:329–60.
29. Hadrup S, Donia M, Thor Straten P. Effector CD4and CD8 T cells and their role in the tumor microenvironment. Cancer Microenviron. 2013;6(2):123–33.
30. Mohammed NA, Ali SA, Saleh SM, Abood AS. Breast cancer: CD3+ tumor infiltrating lymphocytes differences among molecular subtypes. Int J Sci Eng Res. 2014;5(7):790–3.
31. Rathore AS, Kumar S, Konwar R, Makker A, Negi MPS, Goel MM. CD3+, CD4+ & CD8+ tumour infiltrating lymphocytes (TILs) are predictors of favourable survival outcome in infiltrating ductal carcinoma of breast. Indian J Med Res. 2014;140:361–9.
32. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011;29:1949–55.
33. Ali HR, Provenzano E, Dawson SJ, Blows FM, Liu B, Shah M, et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12 439 patients. Ann Oncol. 2014;25:1536–43.
34. Liu S, Foulkes WD, Leung S, Gao D, Lau S, Kos Z, et al. Prognostic significance of FOXP3+ tumor-infiltrating lymphocytes in breast cancer depends on estrogen receptor and human epidermal growth factor receptor-2 expression status and concurrent cytotoxic T-cell infiltration. Breast Cancer Res. 2014;16:423.
35. Xu Q, Pan B, Wang C, Zhou Y, Mao F, Lin Y, et al. HER2 amplification level is not a prognostic factor for HER2- positive breast cancer with trastuzumab-based adjuvant treatment: A systematic review and meta-analysis. Oncotarget. 2016;7(39).
36. Masini C, Bisagni G, Ragazzi M, Bisagni A, Dallaglio K, Falco G, et al. HER2-amplification level and tumor-infiltrating lymphocytes in breast cancer patients treated with neoadjuvant trastuzumab. J Clin Oncol. 2016;34(15 Suppl):596.