ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Kocaeli Med J 2023; 12 (2): 269-275 doi:10.5505/ktd.2023.91298

Neoadjuvan Tedavi Alan Meme Kanserli Hastalarda Tümörü İnfiltre Eden Lenfositler Prognozu Öngörebilir

Tumor-Infiltrating Lymphocytes May Predict Prognosis in Breast Cancer Patients Receiving Neoadjuvant Chemotherapy

Seda Duman Öztürk[1], Çiğdem Öztürk[2], Oğuzhan Okçu[2], Gökçe Askan[3], Bayram Şen[4], Recep Bedir[2]

[1]Kocaeli Üniversitesi Tıp Fakültesi, Patoloji Ana Bilim Dalı, Kocaeli, Türkiye.

[2]Recep Tayyip Erdoğan Üniversitesi Tıp Fakültesi, Patoloji Ana Bilim Dalı, Rize, Türkiye.

[3]İstanbul üniversitesi Cerrahpaşa Tıp Fakültesi, Patoloji Ana Bilim Dalı, İstanbul, Türkiye.

[4]Recep Tayyip Erdoğan Üniversitesi Tıp Fakültesi, Biyokimya Ana Bilim Dalı, Rize, Türkiye.

ÖZ

Giriş: Meme karsinomlarında tedavi öncesi alınan iğne biyopsi örneklerindeki histopatolojik özellikler hastaların kemoterapi yanıtlarını öngörebilir ve böylece meme kanseri hastalarının prognozu hakkında bilgi sağlayabilir. Tümör infiltre eden lenfositler (TIL), birçok tümörde prognostik bir faktör olarak rapor edilmiştir. Bu çalışmada neoadjuvan tedavi alan hastalara ait tedavi öncesi iğne biyopsi örneklerindeki TIL ile klinikopatolojik parametreler ve hastaların sağkalım durumu arasındaki ilişkiyi araştırdık.

Yöntem: 2010-2021 yılları arasında kor biyopsi yapılan ve neoadjuvan kemoterapi sonrası opere edilen 74 meme karsinomlu hasta çalışmamıza dahil edildi. Olguların kor biyopsi materyallerinde TIL ile rezeksiyon materyallerinde tedaviye patolojik yanıt ve olguların klinikopatolojik özellikleri arasındaki ilişki değerlendirildi.

Bulgular: TIL ile tedavi yanıtı arasında anlamlı bir ilişki bulunamadı. ER ekspresyonunun yüksek olduğu durumlarda TIL düşüktü (p:0.012). Düşük TIL'li olguların çoğu Luminal A+B gruplarındaydı (p:0.013). Düşük TIL, kısa hastalıksız sağkalım (DFS) ile ilişkilendirildi ve TIL, çok değişkenli analizde DFS için prognostik bir faktördü.

Sonuç: Meme kanseri immünojenik bir tümördür ve neoadjuvan kemoterapi öncesi iğne biyopsi örneklerindeki TIL miktarı hastalığın prognozu üzerinde prediktif değere sahiptir.

Anahtar Kelimeler: meme neoplazileri, tümörü infiltre eden lenfositler, neoadjuvan tedavi

ABSTRACT

Objective: Investigation of the histopathological features in core biopsies can predict the chemotherapy responses and thus provide information about the prognosis of breast cancer patients. Tumor-infiltrating lymphocytes (TILs) have been reported as a prognostic factor in many tumors. We investigated the relationship between TILs and clinicopathological parameters and patients' survival status.

Method: 74 breast cancer patients who underwent core biopsy between 2010 and 2021 and were operated on after neoadjuvant chemotherapy were included in our study. The relationship between TIL in core biopsy materials of the cases and pathological response to treatment in resection materials and clinicopathological features of the cases were evaluated.

Results: No significant relationship was found between TIL and treatment response. TIL was low in cases with high ER expression (p:0.012). Most of the cases with low TIL were in the Luminal A+B groups (p:0.013). Low TIL was associated with short disease-free survival (DFS), and TIL was a prognostic factor for DFS in multivariate analysis.

Conclusion: Breast cancer is an immunogenic tumor, and TIL before neoadjuvant therapy has predictive value on disease prognosis.

Keywords: breast neoplasms; lymphocytes, tumor-ınfiltrating; neoadjuvant therapy

Gönderim Tarihi: 02.01.2023 **Kabul Tarihi:** 15.08.2023

Correspondence: Uzm. Dr. Çiğdem Öztürk, Recep Tayyip Erdoğan Üniversitesi Tıp Fakültesi, Patoloji Ana Bilim Dalı, Rize, Türkiye.
E-mail: dr.ozturkcigdem@gmail.com

Atıf/ Cite as: Duman Ozturk S, Ozturk C, Okcu O, Askan G, Sen B, Bedir R. Tumor-Infiltrating Lymphocytes May Predict Prognosis in Breast Cancer Patients Receiving Neoadjuvant Chemotherapy. Kocaeli Med J 2023; 12 (2): 269-275. doi: 10.5505/ktd.2023.91298

INTRODUCTION

Neo-adjuvant chemotherapy (NAC) is increasingly used in locally advanced breast cancers (BC) to protect the breast and improve survival (1). The molecular subtype is known to be an indicator of response to neoadjuvant chemotherapy, as triple-negative (TN) and Human epidermal growth factor receptor 2 (HER2) positive subtypes give a more complete response to chemotherapy than ER-positive luminal subtypes. Besides molecular subtype, no other clinical or pathological markers have been consistently proven to predict neoadjuvant therapy response. In addition, neoadjuvant treatment response provides information about the chemosensitivity of patients, making a major contribution to determining the post-treatment algorithm (2). Therefore, identification and validation of markers that can predict response to neoadjuvant therapy are necessary to guide therapeutic decisions.

The only material from which histopathological features of patients can be learned before neoadjuvant treatment is core biopsy specimens. Investigation of the histopathological features in these samples can predict the chemotherapy responses of the patients and thus provide information about the prognosis of the patients (3). In recent years, in addition to the known pathological parameters, the parameters of the tumor and the microenvironment of the tumor have been the subject of studies. One of these parameters is tumor-infiltrating lymphocytes (TILs), which represent the host immune response against the tumor. TILs have been reported as good prognostic markers in some solid tumors and especially in TN and HER2+ breast carcinomas with highly antigenic structures (4-6).

In our study, the relationship between TILs and survival was investigated, as well as a pathological response to neoadjuvant therapy and clinicopathological parameters such as nuclear grade, ER, PR, Ki67, Her2 expression to contribute to the prognosis of patients

MATERIALS AND METHODS

Patient selection and clinicopathologic evaluation: Our study was carried out retrospectively at Recep Tayyip Erdogan University Training and Research Hospital. The data of our study were obtained from the hospital database system between the years 2010-2021. Patients with breast carcinoma in our hospital and treated with NAC were included in the study. Patients who did not receive treatment in our hospital after diagnosis, did not have materials in the pathology archive, and could not be followed up were excluded from the study. A total of 74 patients who met the study criteria were identified. Age, gender and survival data of the patients were obtained from the hospital automation system.

ER, PR, Her2 and Ki-67 immunohistochemical staining statuses of the patients were obtained from the pathology reports. It was divided into molecular subtypes according to these stains (7).

Patients were classified as invasive ductal and invasive lobular carcinoma according to the World Health Organization (WHO) classification of breast cancers and graded according to the Nottingham histological grade scoring system (8).

The response of the patients to the treatment of resection materials after neoadjuvant therapy was classified into three categories according to the American Joint Committee on Cancer. Response to therapy was scored as complete, partial or no response according to the tumor percentage left in resection material (9). Since the number of our patients was small, patients with partial and unresponsive pathological response to treatment were included in a single category.

Assessment of TIL scores: TILs evaluation was standardized in the International TILs Working Group (ITILWG) 2014 (10). The ITILWG recommended first determining the tumor and stroma area at low magnification and then determining the TIL at 20x or 40x objective. All sections of invasive tumor were evaluated. Since tumor borders were not clear at the needle margins, imaginary borders were determined for each core, and the ratio of TIL to stroma was calculated, and the average TIL was determined from all cores of the cases. TIL scores were subclassified as low (<10%), intermediate (20%-40%) and high (50%-90%). Due to the limited number of patients, the low and intermediate TILs groups was considered as low TILs. All mononuclear cells, including lymphocytes and plasma cells, but not polymorphonuclear leukocytes, were scored (11). Histopathological samples of the TIL evaluation of cases are shown in Figure 1.

Areas outside the tumor border, around the intraductal component, normal lobules were excluded. TILs with crush artifacts, dense hyalinized areas, and necrosis within the tumor border were excluded. Two pathologists (ÇÖ, SDÖ) assessed the TIL score without knowledge of the other score or patient's information. A third pathologist's opinion (OO) was obtained for the scores that were inconsistent with each other and a common decision was reached.

Evaluations can be made on tumor stroma (sTIL) within the borders of tumor invasion or inflammatory cells (iTIL) within tumoral cell groups. However, sTIL evaluation is recommended primarily because it is easier, more reliable, and reproducible than iTIL (11). For that reason, our study focused on stromal TILs.

Statistical analysis: IBM SPSS Statistics, Version 23.0 (SPSS Inc., Chicago, United States) was used for statistical analysis. Descriptive statistics of patient groups are given as frequency and percentage (n, %). The relationship between categorical variables was evaluated with Pearson Chi-square and Fisher's Exact Test, considering the number of patients in the categories. Prognostic factors affecting survival of patients were determined by univariate Cox regression analysis. Relationships of these variables with survival were evaluated by Kaplan Meier survival analysis and log-rank test. A p-value of <0.05 was considered for statistical significance.

Ethics Approval: Ethics committee confirmation for our study was obtained from the ethics committee of Recep Tayyip Erdoğan Univesity Faculty of Medicine, non-interventional clinical research (E-40465587-050.01.04-538/2022-208). The study was managed under the Declaration of Helsinki and the ethical standards of the institutional research committee guidelines.

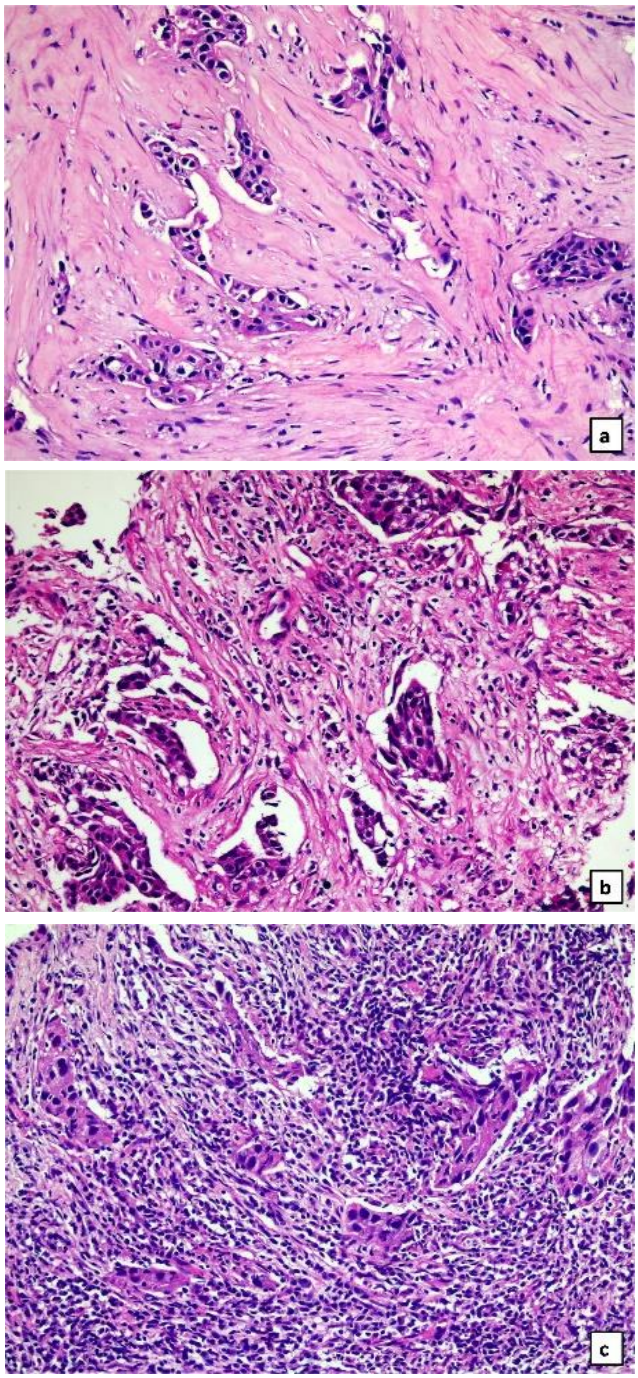

Figure 1. Low (A), intermediate (B) and high (C) tumor infiltrating lymphocytes samples of patients (Hematoxylin Eosin stained slidesx200).

RESULTS

General characteristics: All 74 patients were female and their ages ranged from 33 to 84, with a mean age of 57 years. Among the core biopsy samples examined in our study, 67 patients (90.5%) were diagnosed with invasive ductal carcinoma (IDC), while 7 patients (9.5%) were diagnosed with invasive lobular carcinoma (ILC). While 20 cases (27.0%) were Luminal A (LA), 35 cases (47.3%) were Luminal B (LB). 8 cases (10.8%) were Her2 +, 11 cases (14.9%) were TN. When the response to treatment was evaluated, no response was observed in 8 cases (10.8%), partial response was observed in 37 cases (50.0%), and complete response was observed in 29 cases (39.2%).

Clinicopathological parameters: No significant relationship was found between treatment response and TIL (P: 0.199). TIL tended to be low as ER expression increased (P: 0.012). Most of the cases with low TIL were in the Luminal A+B groups (P: 0.013). The relationship between TILs and clinicopathological parameters is shown in the table.

Table: Tumor Infiltrating Lymphocytes Ratio and Clinicopathological Parameters						
		Tumor infiltrating lymphocytes				
		0-40 %		50-90 %		
			N (%)		N (%)	P
Histological types	IDC	40	(88.9)	27	(93.1)	0.697
	ILC	5	(11.1)	2	(6.9)	
Treatment Response	No response/Partial response	30	(66.7)	15	(51.7)	0.199
	Complete Response	15	(33.3)	14	(48.3)	
Nuclear grade	Grade 1	13	(28.9)	4	(13.8)	0.319
	Grade 2	26	(57.8)	20	(69.0)	
	Grade 3	6	(13.3)	5	(17.2)	
ER expression	Negative	8	(17.8)	13	(44.8)	0.012
	Positive	37	(82.2)	16	(55.2)	
PR expression	Negative	13	(28.9)	13	(44.8)	0.161
	Positive	32	(71.1)	16	(55.2)	
Her2 expression	Negative	30	(66.7)	15	(51.7)	0.368
	Positive	13	(28.9)	11	(37.9)	
	Unknown	2	(4.4)	3	(10.3)	
Ki67 expression	Low	10	(24.4)	4	(16.0)	0.419
	High	31	(75.6)	21	(84.0)	
Molecular subtypes	Luminal A+B	38	(84.4)	17	(58.6)	0.013
	Her2+TN	7	(15.6)	12	(41.4)	
Anjiolymphatic invasion	Negative	28	(62.2)	20	(69.0)	0.553
	Positive	17	(37.8)	9	(31.0)	
Perineural Invasion	Negative	39	(86.7)	25	(86.2)	1.000
	Positive	6	(13.3)	4	(13.8)	
Lymph node metastasis	Negative	21	(46.7)	16	(55.2)	0.475
	Positive	24	(53.3)	13	(44.8)	
Metastasis	Negative	30	(71.4)	25	(89.3)	0.074
	Positive	12	(28.6)	3	(10.7)	
Death status	Alive with disease+No evidence of disease	36	(85.7)	27	(96.4)	0.230
	Death of disease	6	(14.3)	1	(3.6)	

IDC = Invasive Ductal Carcinoma; ER = Estrogen Receptor; PR =Progesterone Receptor ; Her2 = Human epidermal growth factor receptor 2); TN = Triple Negative; ILC = Invasive Lobular Carcinoma.

Prognostic outcome: 21 cases (28.4%) were in the low TIL group, 24 cases (32.4%) were in the intermediate TIL group, and 29 cases (39.2%) were in the high TIL group. Due to the small number of cases, low and intermediate TIL were studied as a single group and high TIL as the other group in the Cox regression analysis.

TIL was found as a prognostic variable for disease-free survival (DFS) in multivariate analysis (Hazard ratio, HR 4,683; 95% CI 1.035 - 21.198; P = 0.045). For overall survival (OS) (P: 0.222), the TIL was not an independent prognostic variable in cox regression analyzes.

Even when performing Kaplan–Meier curve for DFS showed a significant difference between TIL groups (P = 0.023), see Figure 2A. A trend was seen toward a worse OS for patients with low TIL compared to patients with high TIL in the Kaplan Meier curve (P = 0.122), see Figure 2B.

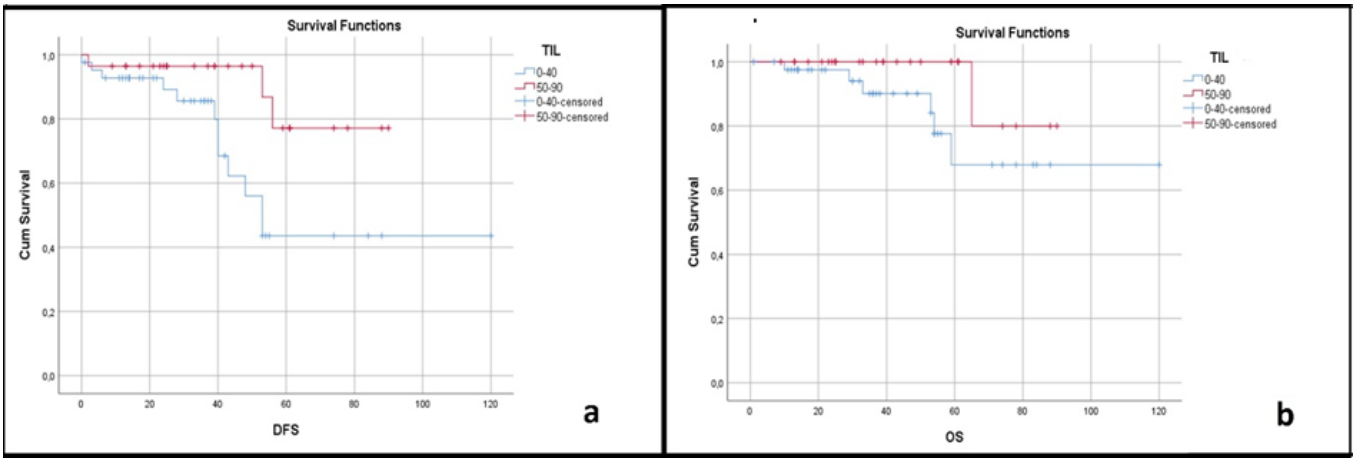

Figure 2. Kaplan-Meier curves for tumor infiltrating lymphocytes for the patients [For disease-free survival, DFS P: 0.023 (A), for OS P: 0.122 (B)]

DISCUSSION

In breast cancers, understanding the complex interactions between the tumor and the immune system, which constitutes the tumor microenvironment, may be useful in predicting the treatment response (11). Inflammatory cells forming the tumor microenvironment show a cytotoxic effect on tumor cells supported by Th1 CD4 and CD8+ T cells, B lymphocytes, and macrophages in the acute inflammation phase, whereas Th2 CD4+ and regulatory T cells reduce CD8+ cytotoxicity in the chronic inflammation phase and increase the release of IL-4, IL-10, IL-13, TGF-b and immunoglobulins from macrophages and B lymphocytes (11). It can be said that CD8 T and Th1 CD4 lymphocytes have antitumor properties, B and Th2 CD4 lymphocytes have protumoral properties. Genetic and epigenetic factors determine the relationship between the cancer cell and the host immune system.

Immune resistance mechanisms are regulated by immune checkpoints such as CTLA4, PD1, LAG3, and TIM3 and their ligands PDL1, PDL2, B7-H3, and B7-H4. As a result of mutations that may develop at these points, antitumor lymphocyte functions decrease and a tumor microenvironment with immunosuppressive character is formed (12). Cancer development and progression are affected as a result of these complex interactions between antitumoral and protumoral immunomodulators. Understanding these mechanisms may affect the choice of adjuvant and neoadjuvant therapy in many cancer types. Although lymphocytes forming the microenvironment of the tumor can be separated into specific types by IHC or molecular methods, since these methods require additional cost and time, ITILWG standardized the evaluation of TIL and recommended evaluation in routine HE stained sections with light microscopy without additional cost (12).

Some cancers, such as melanoma, lung, and bladder, contain high levels of genetic mutations and express tumor-associated antigens that induce an endogenous anticancer immune response. It is known that breast cancer (BC), which was considered immunogenically silent in previous years, is now in the middle group in terms of mutation (13). TILs, which indicates the immunity of the tumor, differs between different molecular subtypes in BC. Higher TILs levels are observed as a more antigenic expression is seen in Her2 and TN subtypes. In addition, the lack of expression of ER, which has an anti-inflammatory effect in these subtypes, may also cause high TILs. In our study, the relationship between ER expression and TILs was investigated, and as ER positivity increased, TILs level was found to be lower. This may be evidence of the anti-inflammatory effect of the ER. Therefore, there is a significant correlation between high TILs levels and good prognostic parameters, especially in Her2 and TN cases (14). On the other hand, the effect of TIL on survival in Luminal subtypes with ER expression is controversial (15,16).

In Denkert et al.'s study, TILs was found to be a good prognostic factor only in grade 3 cases and a poor prognostic factor in grade 1 and 2 cases in Luminal groups (17). In the study of Criasitieko et al., high TILs was found to be a poor prognostic marker in ER+ Her2- cases (18). In our study, the majority of our cases consisted of the ER+ luminal group, 11 patients were in the Triple-negative group and 8 patients were in the Her2+ group. Since the number of our cases was small, all molecular subtypes were evaluated together. In the survival analyzes of the cases, the DFS was significantly shorter in the presence of low TIL, and TIL was found to be a prognostic factor for DFS in the multivariate cox regression analysis. OS tended to be short in the presence of low TIL, but statistically significant results could not be obtained in OS due to the short follow-up times of the cases.

Morphological findings in core biopsy specimens, which are the only material of the cases before NAC, are very valuable, but there are also studies in the literature that argue that the TILs score in the core biopsy may be misleading while reflecting the TILs score in the resection material due to indefinite tumor borders, limited area of biopsy (19,20) Khan et al investigated the heterogeneity of TILs in tumor tissue by tissue microarray method and showed that TILs is highly heterogeneous in Her2+ cancers. In the same study, they showed that the effect of heterogeneity decreased in the presence of 4 or more cores (19). However, some studies argue that even a single core biopsy can reflect the entire tumor and show the TILs score accurately (20). In our study, the number of core biopsy samples of the cases varied widely (1-9 cores). The mean TILs value was determined by evaluating all core biopsy samples of the cases. However, since the only material before NAC is the core biopsy samples, even a single core is very

valuable in terms of histopathological features and the findings in the samples should be reported carefully.

Our study has different limitations. Our cases did not show a homogeneous distribution. In addition, we think that we could not obtain meaningful results due to the low number of different molecular subtypes, especially Her2 and TNBC cases.

The routine use of TILs in the clinical practice will help identify patients who will benefit from chemotherapy. It will also pave the way for the effective use of immunotherapy agents in the neoadjuvant treatment of breast cancer. In addition, unnecessary complications can be prevented by determining the patient groups to be treated with chemotherapy. For this reason, core biopsy materials, which contain valuable clues about patient prognosis, should be carefully examined and we think that reporting TILs in the routine pathology reports, will contribute to the prognosis of the patient.

Ethics Committee Approval: Ethics committee confirmation for our study was obtained from the ethics committee of Recep Tayyip Erdoğan Univesity Faculty of Medicine, non-interventional clinical research (E-40465587-050.01.04-538/2022-208).

Authors' contributions: All authors contributed to the article.

Conflict of Interest: There is no conflict of interest.

Funding: No financial support was received.

Informed Consent: Informed consent was not obtained as the study was retrospective.

REFERENCES

1. Yildirim N, Simsek M, Aldemir MN, Bilici M, Tekin SB. Relationship between 18-FDG-PET/CT and Clinicopathological Features and Pathological Responses in Patients with Locally Advanced Breast Cancers. Eurasian J Med. 2019;51(2):154-159.
2. Mozarowski P, Rasaiah B, Reed M, Lewis A, Walde N, Voutsadakis IA. Prognostic Role of Tumor Budding in Breast Cancer Patients Receiving Neo-Adjuvant Therapy. J Clin Med. 2021;10(4):827.
3. Gajdos C, Tartter PI, Estabrook A, Gistrak MA, Jaffer S, Bleiweiss IJ. Relationship of clinical and pathologic response to neoadjuvant chemotherapy and outcome of locally advanced breast cancer. J Surg Oncol. 2002;80(1):4-11.
4. Ravelli A, Roviello G, Cretella D, Cavazzoni A, Biondi A, Cappelletti MR, et al. Tumor-infiltrating lymphocytes and breast cancer: Beyond the prognostic and predictive utility. Tumour Biol. 2017;39(4):1010428317695023.
5. Wang S, Sun J, Chen K, Ma P, Lei Q, Xing S et al. Perspectives of tumor-infiltrating lymphocyte treatment in solid tumors. BMC Med. 2021;19(1):140.
6. Idos GE, Kwok J, Bonthala N, Kysh L, Gruber SB, Qu C. The Prognostic Implications of Tumor Infiltrating Lymphocytes in Colorectal Cancer: A Systematic Review and Meta-Analysis. Sci Rep. 2020;10(1):3360.
7. Bandyopadhyay S, Bluth MH, Ali-Fehmi R. Breast Carcinoma: Updates in Molecular Profiling 2018. Clin Lab Med. 2018;38(2):401-420.
8. Rakha EA, Allison KH, Ellis IO. Invasive breast carcinoma: General overview. In: Lokuhetty D, White VA, Watanabe R, Cree IA, editors. Breast Tumours, WHO Classification of Tumours. 5 ed. Lyon: IARC; 2019. p. 82-101
9. College of American Pathologists (CAP), "Protocol for the Examination of Biopsy Specimens From Patients With Invasive Carcinoma of the Breast," 2020. [Online]. Available: https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates.
10. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, Wienert S, Van den Eynden G, Baehner FL, Penault-Llorca F, Perez EA, Thompson EA, Symmans WF, Richardson AL, Brock J, Criscitiello C, Bailey H, Ignatiadis M, Floris G, Sparano J, Kos Z, Nielsen T, Rimm DL, Allison KH, Reis-Filho JS, Loibl S, Sotiriou C, Viale G, Badve S, Adams S, Willard-Gallo K, Loi S; International TILs Working Group 2014. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015 Feb;26(2):259-71.
11. 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. Adv Anat Pathol. 2017;24(5):235-251.
12. DeNardo DG, Coussens LM. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. Breast Cancer Res. 2007;9(4):212.
13. Criscitiello C, Esposito A, Gelao L, Kryukov GV, Cibulskis K, Sivachenko A, et al. Immune approaches to the treatment of breast cancer, around the corner? Breast Cancer Res. 2014 ;16(1):20413.
14. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013;499(7457):214-218.
15. Solinas C, Carbognin L, De Silva P, Criscitiello C, Lambertini M. Tumor-infiltrating lymphocytes in breast cancer according to tumor subtype: Current state of the art. Breast. 2017;35:142-150.
16. Mermut O, Inanc B, Gursu RU, Arslan E, Trabulus DC, Havare SB, et al. Factors affecting pathological complete response after neoadjuvant chemotherapy in breast cancer: a single-center experience. Rev Assoc Med Bras (1992). 2021;67(6):845-850.

17. Sønderstrup IMH, Jensen MB, Ejlertsen B, Eriksen JO, Gerdes AM, Kruse TA, et al. Evaluation of tumor-infiltrating lymphocytes and association with prognosis in BRCA-mutated breast cancer. Acta Oncol. 2019;58(3):363-370.

18. 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.

19. Criscitiello C, Vingiani A, Maisonneuve P, Viale G, Viale G, Curigliano G. Tumor-infiltrating lymphocytes (TILs) in ER+/HER2- breast cancer. Breast Cancer Res Treat. 2020;183(2):347-354.

20. Khan AM, Yuan Y. Biopsy variability of lymphocytic infiltration in breast cancer subtypes and the ImmunoSkew score. Sci Rep. 2016;6:36231.

21. Mani NL, Schalper KA, Hatzis C, Saglam O, Tavassoli F, Butler M, et al. Quantitative assessment of the spatial heterogeneity of tumor-infiltrating lymphocytes in breast cancer. Breast Cancer Res. 2016;18(1):78.