Prevalence And Prognostic Value of (TILs) Tumor Infiltrating Lymphocytes in Triple Negative Breast Cancer Patients

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Abstract:

Background: Triple Negative Breast Cancer (TNBC) has a unique microenvironment of Tumor Infiltrating Lymphocytes (TILs). TNBC tumors are more likely to exhibit chromosomal instability and potential mutations than other subtypes.

Objectives: The study aims to evaluate the prevalence of CD8+ TILs biomarker by IHC in TNBC patients and its prognostic value. The greater number of TILs, the higher probability of response to chemotherapy. TILS suggest a likely option for immunotherapy in this disease.

Patients and Methods: CD8+ as a marker for TILs in the paraffin wax block of pre-treatment biopsies of 30 TNBC patients was evaluated, and its prognostic value by correlating it with OS and PFS was done. The study took place at the department of Clinical Oncology and Nuclear medicine Ain Shams university and Matarya teaching hospital.

Results: All patients (100%) were positive for CD8+, with a range of (1% to 60%), most of the patients (20 patients) had CD8 % between (10% to 20 %). High levels of CD8 + TILs were good prognostic indicators. Association of CD8+ TILs infiltrate status with longer PFS and OS in TNBC patients was recorded, but was not of statistical significance probably due to small sample size. Our study showed no correlation between CD8+ level and some clinical-pathological variables (tumor size, nodal status, tumor stage, menopausal status, age, family history).

Conclusion: All TNBC patients included in the study were positive for CD8+. High levels of CD8 + TILs are good prognostic indicators in TNBC. High CD8+ TILs infiltrate status was associated with longer PFS and OS in TNBC patients. Quantification of CD8 + TILs is feasible using routine immunohistochemical techniques.

Key words: CD8, TIL, triple negative, breast cancer, immunotherapy

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Introduction:

Breast cancer (BC) is the most generally perceived and basic disease in women [1], About 15% of cases are of the triple-negative breast cancer (TNBC) subtype [2]. TNBC is negative for Estrogen Receptor (ER), Progesterone Receptor (PR), and lacks overexpression of the human epidermal growth factor receptor 2 (HER2) [3]. As a subtype of breast cancer, TNBC is more aggressive with higher risk of recurrence [4]. TNBC has 6 different microscopic subtypes [5, 6].

Immune system plays a main role in cancer progression that was first observed by Rudolph Virchow over 150 years ago [7]. The concept of cancer immune-editing is widely accepted. [8]. Elimination, equilibrium and escape are the phases of immune-editing. [9]

Tumor-infiltrating lymphocytes include T cells and B cells and are part of the larger group of 'tumor-infiltrating immune cells' which compose of T cells, B cells, natural killer cells, among other cells in different proportions. Their increase correlated with improved survival and decreased metastatic spread [10]. The interaction of the immune system with tumor cells in breast cancer was reported in triple negative breast cancer (TNBC) [11]. Strong lymphocytic infiltrate (more than 60% infiltration by either stromal or

intratumoral lymphocytes) is defined as lymphocyte predominant breast cancers (LPBCs) [12]. Presence of CD8+ cells in the tumor before starting neoadjuvant chemotherapy proved to predict response to therapy in TNBC patients [11-13]. CD8+ cytotoxic T lymphocytes exhibit cytotoxic activity against tumor cells expressing tumor-associated antigen (TAAs) and induce tumor cell death directly upon their recognition [14]. High proportions of effector TILs have been associated with better clinical outcome of patients with TNBC but not in estrogen receptor positive breast carcinoma [15].

Aim of work:

To clarify the biological and prognostic function of TILs, which is a host factor in tumor microenvironment, this study focused on measuring effector CD8+ T cells as a marker for TILs with the aim of assessment of the prevalence of TILs (tumor infiltrating lymphocytes) by CD8+ biomarker, correlating its level to prognostic factors and to evaluate its prognostic value by assessing of (PFS) and (OS).

Patients and Methods:

This is a retrospective study, which included 30 TNBC patients where clinical data and paraffin wax blocks were collected from the breast cancer unit, department of clinical oncology and nuclear medicine, Ain Shams University and Matarya teaching hospital. Inclusion Criteria: Triple negative breast cancer patients proven histopathologically and by IHC studies, age >18 years, underwent surgical resection of the tumor. Exclusion Criteria: Bilateral breast cancer, concurrent with any other malignancy, male breast cancer cases, pregnant and lactating females, inflammatory breast cancer cases. Follow-ups were performed at clinical oncology department Ain shams university and Matarya teaching hospital.

IHC and CD8+ TIL quantification

The paraffin blocks were retrieved from the archives of the histopathology unit at Ain shams University and Matarya hospital.

- Then, 4-µm thick sections were cut from the biopsy. The sections were stained aiming at identifying the dense lymphocytic infiltration areas. Unstained sections were stained using an IHC method for CD8+ T cells.
- Then it was incubated for 24 min at 37°C with primary antibody, CD8 rabbit monoclonal antibody. Second incubation for ~20 min at 36°C was carried out using the secondary antibody from the kit. Visualization was carried out using the light microscopy and cells were counted positive only if expressing strong membranous and cytoplasmic staining for CD8+.
- If encountered within the tumor cell nests, CD8+ lymphocytic infiltration was interpreted as intratumoral otherwise it was classified as stromal.
- Intratumoral CD8+ lymphocytes expression levels were classified as: high, or low as per the candidate cut-off value.

Statistical analysis:

The collected data were coded, tabulated, and introduced to a PC using Statistical package for Social Science (SPSS 15.10.1 for Windows; SPSS Inc, Chicago, IL,2001).

Categorical variables were presented as numbers and percentages and continuous variables as median and range as appropriate. The significance of difference in the frequency of categorical variables between groups was determined using Chi-square test or Fisher's exact test when appropriate. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant at the level of <0.05. Survival analysis was performed using the Kaplan Meier method and the Log Rank test to determine the significance of difference in survival where Progression Free Survival (PFS) was measured as the time between the date of diagnosis and the date of disease relapse or the date of last follow-up if no relapse occurred patients who were not relapsed were censored. Overall Survival (OS) was measured as the time between the date of diagnosis and the date of the last follow-up or mortality. Patients who were still alive at time of analysis were censored**.**

Results:

Clinical data were collected from January 2015 to June 2018. All patients were observed from the date of their diagnosis till January 2020. Patient clinical data (table:1) revealed that age ranged between (25-72) years, with 19 patients (63.3%) below 50 years of age, twenty patients (66.7%) were premenopausal, and sixteen patients (53.3%) had family history of breast cancer. Regarding pathological data, 63.3% presented with T2 tumor size and 40% presented with N0 stage. Regarding the Prevalence of CD8+ as a marker to TILs in paraffin wax blocks; all patients (100%) were positive for CD8+, with a minimum range of 1% and a maximum range of 60 % (table:2). Most patients (20 patients) had CD8 % between (10% to 20 %). The mean of CD8+ in all samples was 15.63 %. Samples were then classified into high and low CD8+TILs by using Interquartile Range (The 75th percentile) as a candidate cut off value for CD8+ staining which was 20 %, patients presented with CD8+ of ≤ 20% were considered low, and patients presented with CD8+ of > 20% were considered high, we had 6 patients with high CD8+ TILs and 24 patients with low CD8+ TILs.

Level of CD8 was not affected by age, menopausal status, comorbidities, or family history. Also, it was not associated with tumor size, regional lymph nodes, or tumor stage (table:3).

Progression Free Survival (PFS) and OS for all patients was calculated and correlating CD8+ percentage with PFS and OS duration was done. Mean PFS for the whole group was 25.26 months CI (20.742 – 30.286). Fifteen patients (50%) progressed during the follow up period, among whom 13 (43.3%) had low CD8+, and only 2 patients (6.6%) had high CD8+, but it did not reach statistical significance (p=0.501). Patients

with baseline high CD8+ (>20%) did not reach 50% median survival probability, while Patients with baseline low CD8+ (≤20%) reached 50% median survival probability after about 22.3 months (Figure:1).

By the end of the study 27 (90%) patients were still alive, with 3 deaths in low CD8+ group, vs. zero deaths in high CD8+ group (p=0.579) but no significant statistical difference between high and low CD8+TILs (figure:2)

Table (1): Patients clinicopathological data.

	Characteristic	Number	Percent
Age	≤50	19	63.3%
	>50	11	36.7%
Menopausal status	Pre-Menopausal	20	66.7%
	Post-Menopausal	10	33.3%
Comorbidity	No	20	66.7%
	Yes	10	33.3%
Family History of breast cancer	+ve Family history	16	53.3%
	-ve Family history	14	46.7%
T tumor size	T1	2	6.7%
	T2	19	63.3%
	T3	7	23.3%
	T4	2	6.7%
Regional LNs	N0	12	40.0%
	N1	9	30.0%
	N2	7	23.3%
	N3	2	6.7%
Staging	IA	2	6.7%
	IIA	8	26.7%
	IIB	6	20.0%
	IIIA	5	16.7%
	IIIB	2	6.7%
	IIIC	7	23.3%
Surgery	BCS	8	26.7%
	MRM	22	73.3%
Radiotherapy	PORT	23	76.7%
	no PORT	7	23.3%

Categorical variables presented as numbers and percentages. BCS: Breast Conservative Surgery, MRM: Modified Radical Mastectomy, PORT: Post Operative Radiotherapy

Table (2): percentages of CD8 as a marker to TILs

CD8%	Mean	15.63
	Median	11.00
	Std. Deviation	13.283
	Minimum	1
	Maximum	60
	Interquartile Range	12

Data presented as mean, median, standard deviation, minimum, maximum, and interquartile range

Table (3): relationship between CD8+ and clinicopathological characteristics

		CD8+ Percentage				Total number	*P VALUE
		Low CD8+ ≤20%		High CD8+>20%			
		Number	%	Number	%		
Age	≤ 50	16	66.7%	3	50.0%	19	
	>50	8	33.3%	3	50.0%	11	0.641
Menopausal status	Pre-Menop	16	66.7%	4	66.7%	20	1.000
	Post-Menop	8	33.3%	2	33.3%	10	
Comorbidity	No	16	66.7%	4	66.7%	20	1.000
	Yes	8	33.3%	2	33.3%	10	
Family history	+ve FH	11	45.8%	5	83.3%	16	0.176
	-ve FH	13	54.2%	1	16.7%	14	
Tumor size (T)	T1&2	17	70.8%	4	66.7%	21	
	T3&4	7	29.2%	2	33.3%	9	1.000
Regional Lymph nodes	N0&1	16	83.3%	5	66.7%	21	
	N2&3	8	16.7%	1	33.3%	9	0.637
Staging	Stage I&II	12	50.0%	4	66.7%	16	0.657
	Stage III	12	50.0%	2	33.3%	14	

Categorical variables presented as numbers and percentages. The significance of difference in the frequency of categorical variables between groups determined using Chi-square test. The p-value considered significant at the level of <0.05. Pre-menop: premenopausal, Post-menop: postmenopausal, FH: family history

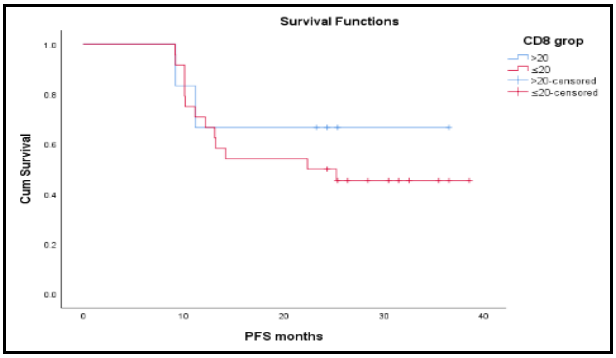

Figure (1): Correlation between CD8+TILS percentage & progression-free survival (PFS) duration.

Kaplan Meier PFS curve, PFS measured as the time between the date of diagnosis and the date of disease relapse or the date of last follow-up if no relapse occurred. Patients who were not relapsed were censored. Cumulative survival represented on the Y axis and duration of PFS in months on the X axis, the blue line; those with CD8+>20% and the red line are those with CD8+≤ 20%.

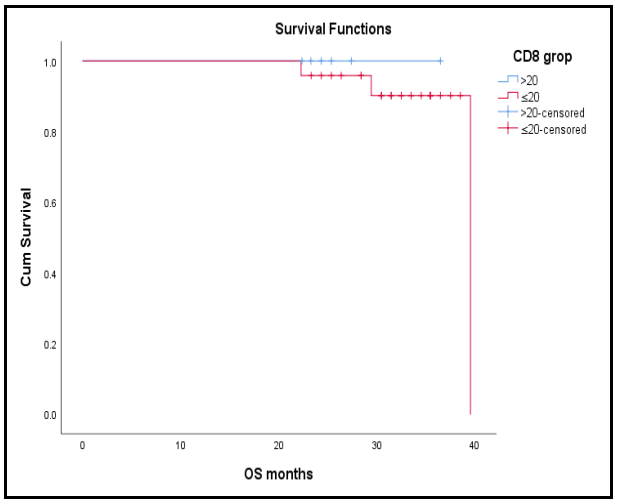

Figure (2): Correlation between CD8+TILS percentage & overall survival (OS) duration

Kaplan Meier OS curve. Overall Survival (OS) measured as the time between the date of diagnosis and the date of the last follow-up or mortality. Patients who were still alive at time of analysis were censored. Cumulative survival represented on the Y axis and duration of OS in months on the X axis, the blue line: those with CD8+>20% and the red line are those with CD8+≤ 20%.

Discussion:

In order to investigate the prognostic function of TILs as a host factor in tumor microenvironment, the current study was carried on effector CD8+ T cells as marker for TILs. CD8+ TILs is ranked as an integral component of the immune response. CD8+ cytotoxic T cells are important not only because of the critical role as cytolytic agents but also due to expected positive prognostic effect in breast cancer. The presence of CD8+ cells in the tumor before starting neoadjuvant chemotherapy predicted pathological complete response in TNBC [16]. Activated CD8+ T lymphocytes are known to kill cancer cells by several mechanisms [17]. This association has recently been reported in a number of large studies [18-20].

Regarding the prevalence of CD8+ Our study showed that All 30 patients (100%) were positive for CD8+, with a minimum range of 1% and a maximum range of 60 %, most of the patients had CD8 % between (10% to 20 %). Mori et al, 2019 [21] reported on CD8-positivity in TNBC of 52.5%. Other previous studies [22-25] showed that in all breast cancer subtypes CD8-positivity ranged from 47.5 to 79.1% and that CD8+infiltrates were seen in 60% of TNBCs.

We calculated the prognostic value of CD8+TILs as a marker of TILs by calculating, DFS and OS for all patient and then correlating CD8+TILs percentage with DFS and OS duration. Patients were segregated into high and low CD8+TILs by using Interquartile Range (The 75th percentile) as a candidate cut off value for CD8+ staining which was 20 %, Patients presented with CD8+ of ≤ 20% were considered low, and patients presented with CD8+ of > 20% were considered high, we had 6 patients with high CD8+ TILs and 24 patients with low CD8+ TILs. In NEOALTTO trial, TILs were classified into 2 categories based on (IQR) level of TILs, which was 12.5% with levels lower in hormonal receptor -positive vs hormone receptor negative tumors. They classified the cohort into 4 categories with LPBC at 30 % cut off point [26]. Hida et al. (2016) [27] classified TILs into three categories low, intermediate, and high (>50%)27. Ziai et al. (2018) [28] evaluated CD8+ T cell infiltration in breast cancer and classified patients as CD8-high or CD8-low depending on whether their expression was above or below this median cut-off value of 5.9%.

Regarding disease-free survival, 50% of patients progressed during follow up period, where 13 (43.3%) patients were with low CD8+, and only 2 (6.6%) patients with high CD8+. Our study showed that higher CD8+ TILs is associated with longer PFS, but it did not reach statistical significance (p=0.501) probably because of the small sample size. Patients with baseline high CD8+ (>20%) did not reach 50% median survival probability which means that by the end of the study more than 50% of patients did not show progression in their disease, while Patients with baseline low CD8+ (≤20%) reached 50% median survival probability after about 22.3 months. Vihervuori et al. 2019 [29] reported that, low frequency of CD8+ inflammatory cells (< 14% of TILs) predicted increased risk of mortality in TNBC.

Chen et al., 2014 [30] conducted a study on TNBC patients and reported on improved OS and DFS with more iCD8+ T cells. Herrero-Vicent et al., 2017 [31] found that, TILs had significant prognostic value for DFS. Median DFS was as low as 20 months in non-LPBC and as high as 97 months in LPBC. Denkert et al.,2018 [32] defined three TIL groups of low, intermediate, and high groups of (0-10%), (11-59%), and (> or equal 60%) respectively. Ten percent increase in TILs was associated with longer DFS in TNBC (p=0.011), the increased TILs was associated with better OS in TNBC. Anz et al., 2011 [33] found that although medullary breast cancer (MBC) strongly infiltrated by FOXP3+ Tregs, significantly good survival occurred if intratumoral CD8+ CTLs was higher. Ibrahim et al., 2014 [34] & Ali et al., 2014 [13] reported that low frequency of CD8+ TILs was associated with unfavorable outcome of TNBC. Rashidian et al. (2017) [35] reported that CD8+ TILs could predict response to immune checkpoint blocking therapy. Mahmoud et al., 2011 [23] also demonstrated improved prognosis with more CD8+ CTLs.

The current study found no significant difference between high or low levels of CD8 with age, menopausal status, comorbidities, and family history, also no significant difference between high or low levels of CD8 with tumor size, regional lymph nodes, tumor stage. Similarly, in NEOALTTO reported the same regarding age, menopausal status, involved lymph nodes, and tumor size [26].

Our preliminary work had some limitations such as retrospectively nature and small sample size.

Conclusion:

In the current study all patients were positive for CD8+. There was association of CD8+ TILs infiltrate status with longer PFS and OS in TNBC patients. Findings in the current study suggest that detecting CD8+ TILs is possible using routine IHC techniques.

Ethical committee approval:

The approval of research ethics committee to perform this study was taken. All consents were signed by patients and objectives of the study were explained to them.

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References:

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017 Jan;67(1):7-30.
2. Lin NU, Claus E, Sohl J, et al. Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer: high incidence of central nervous system metastases. Cancer. 2008 Nov 15;113(10):2638-45.
3. Jiang T, Shi W, Wali VB, et al. Predictors of Chemosensitivity in Triple Negative Breast Cancer: An Integrated Genomic Analysis. PLoS Med. 2016 Dec 13;13(12):e1002193.

4. Louie SM, Grossman EA, Crawford LA, et al. GSTP1 Is a Driver of Triple-Negative Breast Cancer Cell Metabolism and Pathogenicity. Cell Chem Biol. 2016 May 19;23(5):567-578.
5. Burstein MD, Tsimelzon A, Poage GM, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin Cancer Res. 2015 Apr 1;21(7):1688-98.
6. Salgado R, Denkert C, Campbell 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: A Secondary Analysis of the NeoALTTO Trial. JAMA Oncol. 2015 Jul;1(4):448-54.
7. Adams S, Diamond J, Hamilton E, et al. Safety and Clinical Activity of Atezolizumab (Anti-PDL1) in Combination with Nab-Paclitaxel in Patients with Metastatic Triple-Negative Breast Cancer. Cancer Res 2016;76(4 Supplement):P2-11-06-P2-11-06.
8. Garrido F, Romero I, Aptsiauri N, et al. Generation of MHC class I diversity in primary tumors and selection of the malignant phenotype. Int J Cancer. 2016 Jan 15;138(2):271-80.
9. Syn N, Wang L, Sethi G, et al. Exosome-Mediated Metastasis: From Epithelial-Mesenchymal Transition to Escape from Immunosurveillance. Trends Pharmacol Sci. 2016 Jul;37(7):606-617.
10. Gentles AJ, Newman AM, Liu CL, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med. 2015 Aug;21(8):938-945.
11. Garcia-teijido P,Luque C, Pelaez F, et al. Tumor infiltrating lymphocytes in triple negative breast cancer: the future of immune targeting. Clin Med Insights Oncol. 2016 Apr 5;10(Suppl 1):31-9.
12. Issa-Nummer Y, Darb-Esfahani S, Loibl S, et al. Prospective validation of immunological infiltrate for prediction of response to neoadjuvant chemotherapy in HER2-negative breast cancer--a substudy of the neoadjuvant GeparQuinto trial. PLoS One. 2013 Dec 2;8(12):e79775.
13. Ali HR, Provenzano E, Dawson SJ, et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. Ann Oncol. 2014 Aug;25(8):1536-43.
14. Melichar B, Študentova H, Kalábová H, et al. Predictive and prognostic significance of tumor infiltrating lumphocytes in patients with breast cancer treated with neoadjuvant systemic therapy. Anticancer Res. 2014 Mar;34(3):1115-25.
15. Solinas C, Fumagalli D, Dieci MV. Immune Checkpoint Blockade in HER2-Positive Breast Cancer: What Role in Early Disease Setting? Cancers (Basel). 2021 Apr 1;13(7):1655.
16. Liu S, Lachapelle J, Leung S et al. CD8+ lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer. Breast Cancer Res. 2012 Mar 15;14(2):R48.
17. Martínez-Lostao L, Anel A, Pardo J. How do cytotoxic lymphocytes kill cancer cells? Clin Cancer Res. 2015 Nov 15;21(22):5047-56.
18. Yu X, Zhang Z, Wang Z, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in breast-cancer: a systematic review and meta-analysis. Clin Transl Oncol. 2016 May;18(5):497-506.
19. Mao Y, Qu Q, Chen X, et al. The prognostic value of tumor-infiltrating lymphocytes in breast cancer: a systematic review and meta-analysis. PLoS One. 2016 Apr 13;11(4):e0152500..
20. Savas P, Salgado R, Denkert C, et al. Clinical Relevance of Host Immunity in Breast Cancer: From TILs to the Clinic. Nat Rev Clin Oncol. 2016 Apr;13(4):228-41.
21. Mori H, Kubo M, Kai M, et al. T-bet+ lymphocytes infiltration as an independent better prognostic indicator for triple-negative breast cancer. Breast Cancer Res Treat. 2019 Aug;176(3):569-577.
22. Liu F, Li Y, Ren M, et al. Peritumoral FOXP3(+) regulatory T cell is sensitive to chemotherapy while intratumoral FOXP3(+) regulatory T cell is prognostic predictor of breast cancer patients. Breast Cancer Res Treat. 2012 Sep;135(2):459-67.
23. Mahmoud SM, Paish EC, Powe DG, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011 May 20;29(15):1949-55.
24. Baker K, Lachapelle J, Zlobec I, et al. Prognostic significance of CD8+ T lymphocytes in breast cancer depends upon both oestrogen receptor status and histological grade. Histopathology. 2011 Jun;58(7):1107-16.
25. de la Cruz-Merino L, Barco-Sánchez A, Henao Carrasco F, et al. New insights into the role of the immune microenvironment in breast carcinoma. Clin Dev Immunol. 2013;2013:785317.
26. Salgado R, Denkert C, Demaria S, et al. 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.
27. Hida AI, Sagara Y, Yotsumoto D, 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 Jul;158(1):1-9.
28. Ziai J, Gilbert HN, Foreman O, et al. CD8+ Tcell infiltration in breast and colon cancer: Ahistologic and statistical analysis. PLoS One. 2018 Jan 10;13(1):e0190158.
29. Vihervuori H, Autere T, Repo H, et al. Tumor-infiltrating lymphocytes and CD8+ T cells predict survival of triple-negative breast cancer, - J Cancer Res Clin Oncol. 2019 Dec;145(12):3105-3114.
30. Chen Z, Chen X, Zhou E, et al. Intratumoral $CD8^+$ cytotoxic lymphocyte is a favorable prognostic marker in node-negative breast cancer. PLoS One. 2014 Apr 17;9(4):e95475.

31. Herrero-Vicent C, Guerrero A, Gavilá J, Gozalbo F, et al. Predictive and prognostic impact of tumour-infiltrating lymphocytes in triple-negative breast cancer treated with neoadjuvant chemotherapy. E cancer medical science. 2017 Aug 15;11:759.
32. Denkert C, von Minckwitz G, Darb-Esfahani S, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol. 2018 Jan;19(1):40-50.
33. Anz D, Eiber S, Scholz C, et al. In breast cancer, a high ratio of tumour-infiltrating intraepithelial CD8+ to FoxP3+ cells is characteristic for the medullary subtype. Histopathology. 2011 Nov;59(5):965-74.
34. Ibrahim EM, Al Foheidi ME, Al-Mansour M, et al. The prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancer: a meta-analysis. Breast Cancer Res Treat. 2014 Dec;148(3):467-76.
35. Rashidian M, Ingram JR, Dougan M, et al. Predicting the response to CTLA-4 blockade by longitudinal noninvasive monitoring of CD8 T cells. J Exp Med. 2017 Aug 7;214(8):2243-2255.