IJPRP

Indian Journal of Pathology: Research and Practice 8(5) September - October 2019
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.8519.9

Original Research Article

Association of Tumour Infiltrating Lymphocytes With Conventional Clinicopathological Prognostic Factors of Breast Cancer

Debjani Mallick[1], Sayan Kundu[2], Sudipta Chakrabarti[3], Prosun Gayen[4]

[1]Associate Professor, Department of Pathology, ESI PGIMSR, & ESIC Medical College, Joka, Kolkata, Diamond Harbour Road, Kolkata, West Bengal 700104, India, [2]Senior Resident, [3]Professor, Department of Pathology, ESI PGIMSR and ESI Hospital, Manicktala, 54 Bagmari Road, Kolkata, West Bengal 7000054, India. [4]Alumnus, Department of Applied Statistics and Informatics, Indian Institute of Technology, Bombay Powai, Mumbai, Maharashtra 400076, India.

Corresponding Author:
Sudipta Chakrabarti, Professor, Department of Pathology, ESI PGIMSR and ESI Hospital, Manicktala, 54 Bagmari Road, Kolkata, West Bengal 7000054, India.

E-mail: sudiptach@gmail.com

Received on 12.08.2019,

Accepted on 04.09.2019

Abstract

Morphological evaluation of tumor-infiltrating lymphocytes (TIL) is gaining importance as evidence strengthens its clinical relevance as immunological biomarker, in breast cancers. The significance of TIL also lies in the fact that its presence determines the immune response and thereby predicts improved disease outcome independent of chemotherapeutic regimen. The present study was conducted to establish the association of Tumour infiltrating lymphocytes (TIL) with other independent clinicopathological prognostic factors of breast cancer and find their statistical significance. A retrospective study was conducted with random 149 modified radical mastectomy specimens. Data was collected with respect to the following inclusion criteria: age, histological type, histological, grade, tumour size of primary breast cancers, lymph node status, lymphovascular invasion ER, PR, Her 2 neu status by immunohistochemistry. Univariate regression analysis was done for all the variables with TIL. Based on *p*-value and correlation coefficient these four variables: lymphovascular invasion, histological grade, Her 2 and Triple negative breast cancer (TNBC) showed high association with TIL. Multivariate logistic regression analysis emphasized that the four variables: histological grade, lymphovascular invasion, Her 2 and TNBC together are strongly associated with TIL. Hence the evaluation of TIL density in routine histopathological practice may prove to be of paramount relevance

Keywords: Tumour infiltrating lymphocytes (TIL); Breast cancer; Her 2; Triple negative breast cancer.

How to cite this article:
Debjani Mallick, Sayan Kundu, Sudipta Chakrabarti, *et al.* Association of Tumour Infiltrating Lymphocytes With Conventional Clinicopathological Prognostic Factors of Breast Cancer. Indian J Pathol Res Pract. 2019;8(5):575–582.

Introduction

Breast tumours are often infiltrated by leucocytes. These leucocytes comprises lymphocytes, macrophages, polymorphonuclear cells, dendritic cells or mast cells. Significance of these leucocyte population is in its role in innate as well as in adaptive immune response.[1] Significance of TIL evaluation

is due to its clinical relevance as predictive and prognostic biomarker in certain types of breast cancer. TIL scores can even predict the treatment response.[2] Genomic instability, characterized by huge number of tumour associated antigens, promotes anti tumour immune responses.[3] CD8+ and CD4+ TH1 produce cytokineslike gamma-interferon (IFN gamma) and interleukin 2 which leads to anti-tumor activity.[4] The significance of TILalso lies in the fact that its presence determines the immune response and thereby predicts improved disease outcome independent of chemotherapeutic regimen specially in triple negative breast cancers.[3] Tumour infiltrating leucocytes (TIL) are of two types: lymphocytes infiltrating into the tumour stroma or the stromal TILs and lymphocytes directly infiltrating tomour cells, also called intratumoral TILs.[5]

The present study was conducted to establish the association of Tumour infiltrating lymphocytes (TIL) with other conventional clinicopathological prognostic factors of breast cancer and find their statistical significance.

Material and methods

A retrospective study was conducted with random 149 modified radical mastectomy specimens from 2015 to 2017. Data was collected with respect to the following *Inclusion criteria:* age, histological type, histological, grade, tumour size of primary breast cancers,lymphnodestatus,lymphovascularinvasion ER, PR, Her 2 neu status by immunohistochemistry. *Exclusion criteria were*: core biopsy specimens, neo adjuvant chemotherapy received specimens, specimens where lumpectomy was previously done, recurrent tumours. As per institutional protocol all the specimens were fixed with 10% neutral phosphate-buffered formalin. Paraffin-embedded. 4 μm-thick sections of tumors from representative areas, stained with hematoxylin and eosin (H & E) stain, were studied. Immunohistochemistry was done with rabbit monoclonal clone antibody. Histological grading was done by Nottingham Bloom Richardson grading system.[6] Tumors were defined as triple negative as following: < 1% of ER and PR immunoreactivity, and absence of HER 2 protein overexpression.[7,8]

Tumour size and lymph node status determined the pathological tumour staging. TIL was scored based on the observations of two experienced pathologists according to the guidelines of International Working TIL group.[9] The area for assessment was selected at the invasive edge of the tumour. Necrotic areas, fibrotic areas, areas of hyalinization, ductal carcinoma in situ (DCIS) and normal lobules were excluded while TIL was assessed. Tumour bed was scanned and average TIL was scored in 400 × microscopic fields. Stromal TILs were included in the study comprising all mononuclear cells excluding polymorphonuclear leucocytes. Stromal TIL score was determined by percentage of stromal areas occupied by mononuclear inflammatory cells.

Data was tabulated and analyzed statistically. Univariate analysis was done for all the variables. Different statistical measures like correlation coefficient, chi square, *p*-value and information value were calculated for all the variables with TIL to test the significance level. *p*-value was calculated by Wald Chi-square test for each variable. A value of $p < 0.05$ was considered to indicate statistical significance. Only statistically significant variables were chosen to build multivariate logistic regression model with TIL as response variable. Receiver Operating Characteristic (ROC) curve was used to test the performance of the model. Data was analyzed employing SAS software.

Results

Out of 149 cases majority of the cases were of age >50 (58%), ductal morphology (83%), tumour size >5 cm (41%) and Nottingham Bloom Richardson grade 3 (64%). Lymphovascular invasion was present in 66% cases and axillary lymph node metastasis was present in 48% cases. ER, PR, Her 2 positive cases were 52%, 51%, 56% respectively. Triple negative breast cancer cases were 6% (Table 1), (Fig. 1).

Table 1: Characteristics of breast cancer patients with respect to prognostic variables.

Sl. No.	Variable	Number of cases	Percentage
1	**Age (yrs)**		
	<50	63	42%
	>50	86	58%
2	**Histological type**		
	Ductal	124	83%
	Others	25	17%

Sl. No.	Variable	Number of cases	Percentage
3	**Tumour size**		
	<2 cm	38	25%
	2–5 cm	50	34%
	>5 cm	61	41%
4	**Histological Grade**		
	1–2	54	36%
	3	95	64%
5	**Lymphovascular invasion**		
	Present	98	66%
	Absent	51	34%
6	**Lymph node**		
	Negative	77	52%
	Positive	72	48%
7	**ER**		
	Positive	78	52%
	Negative	71	48%
8	**PR**		
	Positive	76	51%
	Negative	73	49%
9	**Her 2**		
	Positive	84	56%
	Negative	65	44%
10	**Triple negative breast cancer (TNBC)**	9	6%

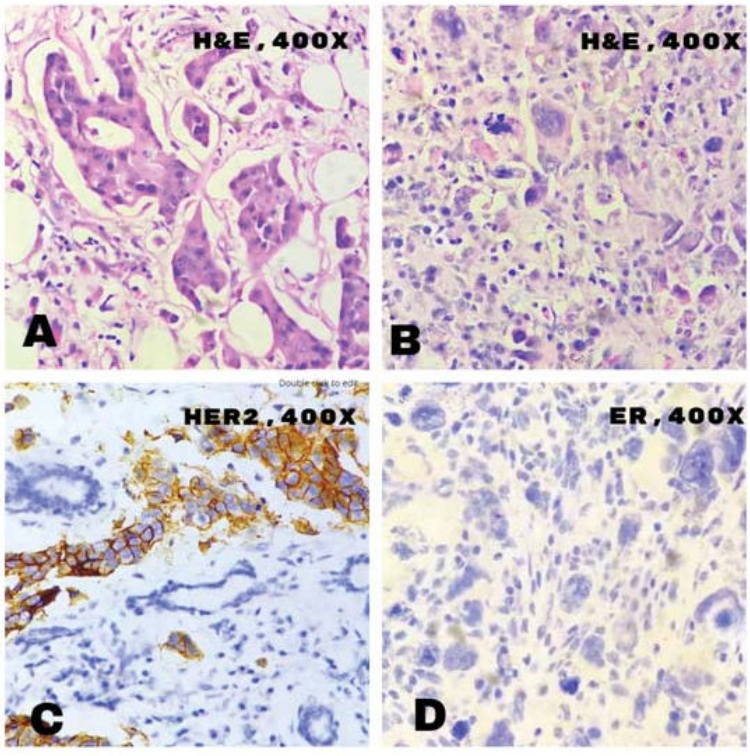

Fig. 1A: Section showing lymphovascular invasion and stromal tumour infiltrating lymphocytes in a case of breast cancer. (H&E, 400X)

B: Section showing Nottingham Bloom Richardson grade 3 invasive ductal carcinoma with stromal tumour infiltrating lymphocytes. (H&E, 400X)

C: Section showing Her 2 positive tumour cells and stromal tumour infiltrating lymphocytes in a case of breast cancer (Her 2, 400X)

D: Section showing ER negative tumour cells and stromal tumour infiltrating lymphocytes in a case of triple negative breast cancer (ER, 400X)

Based on correlation coefficient, chi-square and *p*-value, these four variables: lymphovascular invasion, histological grade, Her 2 and Triple negative breast cancer (TNBC) showed high association with TIL. TIL score was grouped into two groups: high (>50% score) and low (50% score). (Table 2) Information value was also calculated to test the significance of these four variables which are as follows: Lymphovascular invasion–1.89, Histological grade–1.07, Her 2 –0.42, TNBC – 0.15 (Fig. 2).

Table 2: Correlation coefficient of prognostic variables with TIL and their statistical significance

Sl. No..	Variable	TIL Score >50%	Percent	TIL Score <50%	Percent	*p*-value	Correlation with TIL
1	**Age (yrs)**					<.0001	8%
	<50	23	38%	40	45%		
	>50	38	62%	48	55%		
2	**Histological type**					0.1545	12%
	Ductal	54	89%	70	80%		
	Others	7	11%	18	20%		
3	**Tumour size**					0.2464	10%
	<2 cm	15	25%	23	26%		
	2–5 cm	16	26%	34	39%		
	>5 cm	30	49%	31	35%		
4	**Histological Grade**					<.0001	46%
	1–2	6	10%	48	55%		
	3	55	90%	40	45%		
5	Lymphovascular invasion					<.0001	54%
	Present	59	97%	39	44%		
	Absent	2	3%	49	56%		
6	**Lymph node status**					0.6116	4%
	Negative	30	49%	47	53%		
	Positive	31	51%	41	47%		
7	**ER**					0.7219	3%
	Positive	28	46%	43	49%		
	Negative	33	54%	45	51%		
8	**PR**					0.9697	0%
	Positive	30	49%	43	49%		
	Negative	31	51%	45	51%		
9	**Her 2**					0.0003	31%
	Positive	50	82%	46	52%		
	Negative	11	18%	42	48%		
10	**Triple negative breast cancer**	7	78%	2	22%	0.0362	19%

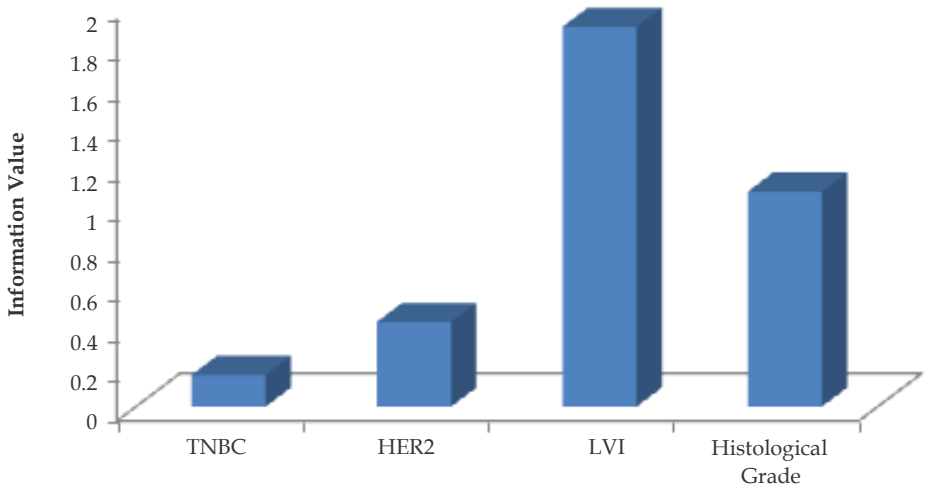

Fig. 2: The bar diagram showing the information value for all the four significant variables with TIL as response variable

The above mentioned four statistically significant variables were chosen to build multivariate logistic regression model with TIL as response variable. The model provided satisfactory classification result with 82.4% percent concordant pairs. ROC curve was used to ensure robust model performance. This analysis emphasized that the four variables: histological grade, lymphovascular invasion, Her 2 and TNBC together are strongly associated with TIL (Fig. 3).

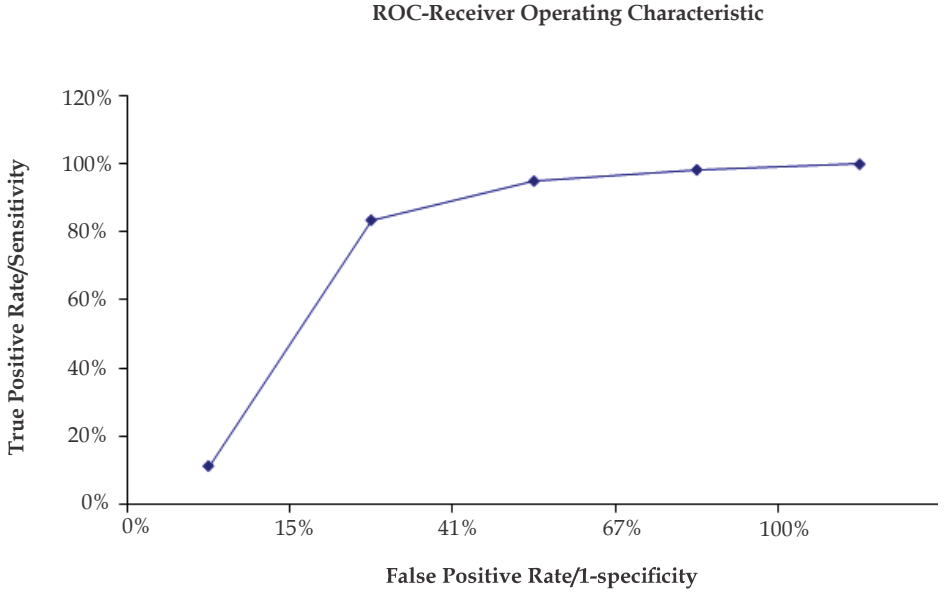

Fig. 3: The ROC curve reveals that if 1st 40% of the ranked population is targeted based on the model built on four variables: histopathological grade, HER 2, lympho vascular invasion and TNBC, it will be able to identify 84% of total high TIL score (True Positive) patient and 15% of total low TIL score (False Positive) patients.

Discussion

TIL influences immune tumor microenvironment and its association with other clinicopathological factors have been discussed in few studies.[10,11] Review of literature revealed a few studies from India regarding TIL and breast cancer, mostly from northern and western region of India.[12–14] The present study is probably the only study with this subject from eastern region of India.

The present study showed age as statistically significant variable in univariate analysis but not in multivariate analysis in presence of other more significant variables. Ruan *et al*. showed similar finding with significant *p*-value 0.04 in univariate analysis but not significant *p*-value 0.25 in multivariate analysis.[15]

Tumour size >5 cm were present in 61 cases but tumour size as a variable had poor correlation with TIL in the present study. Similarly Ruan *et al*. also did not find significant correlation between tumour size and TIL in cases of neoadjuvent treated TNBCs.[15] Some studies showed a significant correlation between TILs and lymph node status for patients that received neo adjuvant chemotherapy.[11,10] However others did not show statistical significant correlation with lymph node status like the present study.[15] 89% of the tumours in present study had ductal morphology with high TIL (>50%) but neither *p*-value was significant nor had high correlation coefficient. Miyoshi *et al*. also observed ductal histological type predominant in their early, late and non recurrent groups but neither had significant *p*-value[11]

Bloom HJ *et al*. found that both higher scores of stromal TILs and intratumoral TILs were associated with higher Ki-67 index and higher histological grade. Also intratumoral TILs scores were positively correlated with negative LVI in TNBCs.[6] Krishnamurti *et al*. found that TILs was significantly associated with histologic grade 3 in TNBCs.[16] The present study also showed high correlation of TIL with histological grade 3 but strong correlation with presence of lymphovascular invasion. This variation may be due to the fact that the present

study included stromal TILs only. Jana *et al*. found that stromal expression of CD10 revealed to be significantly associated with increasing tumor grade, increasing mitotic rate, and worsening prognosis. CD10 also plays important role in predicting treatment failure in breast carcinomas receiving neoadjuvant therapy.[13]

Studies showed that higher scores of TILs are present in triple negative breast cancers and they also indicates a better prognosis in TNBCs.[17,18] Stanton *et al*. showed that in the early stage HER 2-positive and in TNBCs, TILs are observed in up to 75% of tumors and lower amount of TILs in luminal subtypes.[19] The present study also showed association of TIL with TNBC and Her 2 positive cases. Miyoshi *et al*. observed recruitment of TILs in ER positive, HER 2 negative breast cancer and, higher TIL proportions tended to be more responsive to chemotherapy.[11] The present study revealed poor correlation of ER and PR positivity and TILs. This discordance may be due to the fact that recurrent tumours were excluded from the present study and Miyoshi *et al*. included them. Rathore *et al* studied the clinico-pathological characteristics and survival status of all patients and found that ER, PR, HER-2/neu, and stromal CD3+ and CD4+ counts showed insignificant association with the end outcome. But grading, stage and lymph node status showed statistically significant association with the end outcome.[12]

In relapse cases of TNBC who fail to achieve pathological complete response, TIL evaluation is useful. Higher levels of TILs indicate the use of immune checkpoint inhibitors which prevents tumour elimination. In such cases anti Programmed Cell death Ligand 1 (PDL1) therapy are targeted.[20] Clinical trial with pembrolizumab showed that tumours with pre existing CD8 T cells in the invasive tumour margin were more predictive of clinical response to anti PDL1.[21]

The role of TIL subpopulations and therapeutic response has been discussed in several studies. Garcia-Martinez *et al*. found that high ratio of CD8+/CD4+ was associated with higher pathological complete remission rate in pre neo adjuvant chemotherapy breast cancers.[22] CD8+ TILs was revealed to be a predictor for pathological complete remission irrespective of breast cancer subtypes in the study of Seo *et al*.[23] In the study by Rathore *et al*, the association of CD3+ positive TILs with other clinico-pathological factors like age, menstrual status, histological grade, and tumor stage was not statistically significant. However this discordance with the present study may be due to the fact that Rathore *et al*. included only CD3+ TILs and in one histological subtype of breast carcinoma i.e., infiltrating ductal carcinoma.[14]

Conclusion

Morphological evaluation of tumor-infiltrating lymphocytes (TIL) is gaining importance as evidence strengthens its clinical relevance as immunological biomarker, in breast cancers. The present study compared TIL scores with other conventional clinicopathological prognostic markers of breast cancer and found strong association of TIL with high histological grade, lymphovascular invasion, HER 2 and TNBC. Several studies revealed that TIL density is predictive for response to neoadjuvant chemotherapy (NACT). In some breast cancer subtypes TIL is a prognostic factor in patients treated with adjuvant chemotherapy. These indicates that treatment response and outcome varies with TIL levels. Hence theevaluation of TIL density in routine histopathological practice may prove to be of paramount relevance.

Conflicting Interest None

Acknowledgement Nil

References

1. Melichar B, Studentova H, Kalabova H, *et al.* Predictive and Prognostic Significance Of Tumor-Infiltrating Lymphocytes In Patients With Breast Cancer Treated With Neoadjuvant Systemic Therapy. Anticancer Research. 2014;34:1115–26. PMID: 24596349
2. Diecia MVB, Radosevic-Robinc ND, Fineberge SF, *et al.* Update On Tumor-Infiltrating Lymphocytes (Tils) In Breast Cancer, Including Recommendations To Assess Tils In Residual Disease After Neoadjuvant Therapy And In Carcinoma In Situ: A Report Of The International Immuno-Oncology Biomarker Working Group On Breast Cancer∗Seminars In Cancer Biology. 2018;52:16–25. http://dx.doi.org/10.1016/j.semcancer.2017.10.003
3. Loi S. Tumor-infiltrating lymphocytes, breast cancer subtypes and therapeutic efficacy. Onco Immunology. 2013;2:e24720. PMID: 24073365
4. Curiel TJ, Coukos G, Zou L *et al*. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10: 942–49. PMID:15322536. DOI:10.1038/nm1093

5. Hornychova H, Melichar B, Tomsova M, *et al.* Tumor-infiltrating lymphocytes predict response to neoadjuvant chemotherapy in patients with breast carcinoma. Cancer Invest. 2008;26:1024–31. PMID: 19093260 DOI:10.1080/07357900802098165
6. Bloom HJ, Richardson WW. Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. Br J Cancer. 1957;11:359–77. doi: 10.1038/bjc.1957.43.
7. Wolff AC, Hammond ME, Schwartz JN, *et al*. American Society of Clinical Oncology/ College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol. 2007;25:118–45. PMID: 19548375 DOI: 10.1043/1543–2165(2007)131[18:ASOCCO]2.0.CO;2
8. Hammond ME, Hayes DF, Wolff AC, *et al.* American society of clinical oncology/ college of american pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Oncol Pract. 2010;6:195–7. PMID: 21037871 doi: 10.1200/JOP.777003
9. Salgado R, Denkert C, Demaria S, *et al*., The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendationsby an international TILs working group 2014, Ann. Oncol. 2015;26(2):259–71. PMID: 25214542 PMCID: PMC6267863 DOI: 10.1093/annonc/mdu450
10. Caziuc A, Schlanger D, Amarinei G *et al.* Can Tumor-Infiltrating Lymphocytes (TILs) Be a Predictive Factor for Lymph Nodes Status in Both Early Stage and Locally Advanced Breast Cancer? J. Clin. Med. 2019;8:545. doi:10.3390/jcm8040545
11. Miyoshi Y, Shien T, Ogiya A, *et al.* Associations in tumor infiltrating lymphocytes between clinicopathological factors and clinical outcomes in estrogen receptorpositive/ human epidermal growth factor receptor type 2 negative breast cancer. Oncology Letters. 2019;17:2177–86. PMID: 30675282 PMCID: PMC6341802 DOI: 10.3892/ol.2018.9853
12. Rathore S, Kumar S, Konwar R, *et al.* CD3+, CD4+ & CD8+ tumour infiltrating lymphocytes (TILs) are predictors of favourable survival outcome in infiltrating ductal carcinoma of breast. Indian J Med Res. 2014 Sep;140:361-69. PMID: 25366203
13. Jana SH, Jha BM, Patel C, *et al.* CD10 a new prognostic stromal marker in breast carcinoma, its utility, limitations and role in breast cancer pathogenesis. Indian J Pathol Microbiol. 2014;57:530-36. DOI:4929.142639 0377/10.4103
14. Rathore AS, Kumar S, Konwar R, *et al.* Presence of CD3+ tumor infiltrating lymphocytes is significantly associated with good prognosis in infiltrating ductal carcinoma of breast. Indian Journal of Cancer. 2013;50:3. DOI: 10.4103/0019-509X.118744
15. Ruan M, Tian T, Rao J, *et al.* Predictive value of tumor-infiltrating lymphocytes to pathological complete response in neoadjuvant treated triple negative breast cancers. Diagnostic Pathology. 2018;13:66. PMID:30170605 DOI: 10.1186/s13000-018-0743-7
16. Krishnamurti U, Wetherilt CS, Yang J, *et al.* Tumor-infiltrating lymphocytes are significantly associated with better overall survival and disease-free survival in triple-negative but not estrogen receptor-positive breast cancers. Hum Pathol. 2017;64:7–12. PMID: 28153508 DOI: 10.1016/j.humpath.2017.01.004
17. Loi S, Sirtaine N, Piette F, *et al*. Prognostic and predictive value of tumorinfiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in nodepositive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicinbased chemotherapy: BIG 0298. J Clin Oncol. 2013; 31:860-867. PMID: 23341518 DOI: 10.1200/JCO.2011.41.0902
18. Kotoula V, Lakis S, Vlachos IS, *et al*. Tumor infiltrating lymphocytes affect the outcome of patients with operable triplenegative breast cancer in combination with mutated amino acid classes. PLoS One. 2016;11:e0163138. PMID: 27685159 DOI: 10.1371/journal.pone.0163138
19. Stanton SE, Adams S, Disis ML. Variation in the incidence and magnitude of tumor-infiltrating lymphocytes in breast cancer subtypes: a systematic review, JAMA Oncol. 2016;2(10) :1354–60. PMID:27355489 DOI: 10.1001/jamaoncol.2016.1061
20. Teijido P G, Cabal ML, Fernández IP *et al.* Tumor-Infiltrating Lymphocytes in Triple Negative Breast Cancer: The Future of Immune Targeting Clinical Medicine Insights: Oncology 2016:10(S1). PMID: 27081325 DOI: 10.4137/CMO.S34540
21. Tumeh PC, Harview CL, Yearley JH, *et al*. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515:568–71. PMID: 25428505 DOI: 10.1038/nature13954
22. Garcia-Martinez E, Gil GL, Benito AC, *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. PMID: 25432519 DOI: 10.1186/s13058-014-0488-5

23. Seo AN, Lee HJ, Kim EJ, *et al*. Tumour-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer. Br J Cancer. 2013;109:2705–2713.PMID: 24129232 DOI: 10.1038/bjc.2013.634