Giorn. It. Ost. Gin. Vol. XXXIX - n. 2
Marzo-Aprile 2017

mini-review

Correlation between tumor-infiltrating lymphocytes to pathologic response in locally advanced breast cancer patients who receive neoadjuvant chemotherapy in Haji Adam Malik General Hospital

B.K. SIREGAR[1], S.P. JAMALUDDIN[2], R. SIBURIAN[3]

SUMMARY: **Correlation between tumor-infiltrating lymphocytes to pathologic response in locally advanced breast cancer patients who receive neoadjuvant chemotherapy in Haji Adam Malik General Hospital.**

B.K. SIREGAR, S.P. JAMALUDDIN, R. SIBURIAN

Background. *Tumor infiltrating lymphocyte (TILs) are emerging as biomarkers mediating tumor response to treatments. Earlier studied have provided evidence the level of TILs has prognostic value, particulary in triple negative and human epidermal growth factor receptor-2 positive breast cancer. More over the level of TILs has been associated with treatment outcome in patient undergoing neoadjuvant chemotherapy and strong correlation with pathologic complete response. In this study we analyzed whether changes in tumor infiltrating lymphocytes take place after neoadjuvant therapy and if they correlate with pathologic response to treatment.*

Patient and methods. *We retrospectively analyzed the specimen slide from Anatomic Pathology Department of H. Adam Malik General Hospital during 2011-2015. We identified 51 patients with the inclusion criteria of this study. The histological sections had already evaluated by hematoxillin and eosin slides. Its re-assessment with our pathologist for the extent of percentage intratumoral (it) and stromal (str) tumor infiltrating lymphocytes (TILs). The correlation with pathologic response in the tumor after neoadjuvant therapy was also studied in this patients. Each case was also defined as high TILs or low TILs breast cancer adopting previously validated cutoffs.*

Results. *From the 51 specimens, mean age was 49.22. The most types of breast cancer histology was invasive ductal breast carcinoma in 49 (96%) patients, and 2 patients (4%) with lobular carcinoma. The frequency of histopathological grading with high TILs: grade 1 encountered 5 patients, grade 2 was 15 and grade 3 was 3 patients. High TILs that had pathological complete response found in 47,8% patient and 28,8% in low TILs. There is no significant correlation between TILs to pathologic response of patients with neoadjuvant chemotherapy (P= 0,157).*

Conclusions. *This research has not been able to prove significant correlation between TILs with pathological response in patient with locally advanced breast cancer who received neoadjuvant chemotherapy but high TILs more likely to have complete response. Further information may prove useful to future biomarker trial.*

KEY WORDS: Breast cancer - Neoadjuvant chemotherapy - Tumor infiltrating lymphocites - Pathologic response.

Introduction

Immune system is a key player in cancer progression. Preclinical data suggest that chemotherapy can trigger an anti tumor immune response, by causing an immunogenic cell death that allows antigen cross presentation, activation of dendritic cells and tumor specific cytotoxic T cells (1). The presence of a host anti tumor immunity has been shown to influence the response to cytotoxic treatment. Dankert et al. demonstrated that high tumor infiltration by lymphocytes at diagnosis is associated with higher likelihood of pathological complete response after neoadjuvant chemotherapy. TILs at baseline are associated with high proliferative, high grade, and estrogen receptor (ER) negative tumors and represent a strong prognostic factor for certain breast cancer subtype, mainly for triple negative breast cancer (TNBC) (2, 3).

Methods

Analitic retrospective study was performed in this study. Preparat slides pretreatment from incisional biopsy and post treatment surgical specimen in 51 locally advanced breast cancer patient who receive neoadjuvant chemotherapy were collect based on inclusion criteria

[1] Consultant of Oncology Surgery Department of Surgery, North Sumatera University - H. Adam Malik General Hospital Medan, Nano Medicine and Stemcell Research USU, and Pathologist consultant Department of Pathologic Anatomic H. Adam Malik General Hospital Medan, Indonesia
[2] Consultant of Oncology Surgery Department of Surgery, North Sumatera University - H. Adam Malik General Hospital Medan, Indonesia
[3] Nanomedicine Center-Stem Cell, University of Sumatera Utara, Medan, Indonesia Chemistry Department, University of Sumatera Utara, Medan, Indonesia

Corresponding author: Rikson Siburian, e-mail: rikson@usu.ac.id

from 2011 to 2014. The preparat slides were reassessed with pathologist in our hospital. Pathological response to neoadjuvant chemotherapy was determined as reported previously (4, 5). Data were analyzed by chi square to find the relationship between the response tils with pathology response in local advanced breast cancer with neoadjuvant chemotherapy.

Pathology

Fullface hematoxillin and eosin stained (H&E) slides of primary tumors were retrieved and evaluated for the percentage of intratumoral (it) and stromal (Str) TIL, according to predefined criteria (1). Cases were defined as high TIL if It TIL and /or Str TIL ≥ 50%, and as low TIL if It TIL and Str TIL <50%, adopting already validated cutoffs (1, 4, 5). These cutoffs were defined before any statistical analyses. Pathological complete response was defined as assessed from the results of pathological examination postoperatively, consisting of complete response, namely the eradication of the entire tumor is both invasive and non-invasive breast cancer and the lymph nodes or incomplete are still met the tumor or lymph node both invasive and non-invasive (5).

Statistics

Stastistical analysis was carried out using the project for statistical computing. During the study period the data are taken from the Sub Division of Surgical Oncology and Pathology departments to collect tissue paraffin blocks of breast cancer before and after neoadjuvant chemotherapy and then in the return value of tumor infiltrating lymphocytes and pathological responses that met the inclusion criteria. Data were taken from 2011 to 2015. It was found the number of samples are 51 patients who met the inclusion criteria. From the data collected we obtained data on the characteristics of the sample by age, frequency, type of histopathology and histopathological grading. Then we analyzed to find the relationship TILs before and after chemotherapy in breast cancer patients with pathological response. Pair T test to evaluated comparison between pre-treatment and post-treatment of TILs was used. The association between TILs and status pathological response was calculated using either X^2 test. All statistical test was two sided and considered significant when P value ≤ 0.05.

Results

From Table 1 we do not found a significant association of age on TILs (P=0,762), and not found an association between the histopathologic grading with TILs (P=1.000).

Table 1 - CHARACTERISTIC PATIENTS WITH BREAST CANCER.

	TILs Before Chemotherapy		P
	High TILs	**Low TILs**	
Total	51		
Age			
< 50 Years	13 (43,3%)	17 (56, 7%)	0,762
≥ 50 Years	10 (47,6%)	11 (52,4%)	
Grade			
1	5 (50%)	5 (50%)	0,843
2	15 (46,9%)	17 (53,3%)	
3	3 (37,3%)	5 (62,5%)	
Histologic			
IDC	49 (96%)		0,492
ILC	2 (4%)		

Table 2 - CORRELATION BETWEEN HISTOPATOLOGIC GRADING PATHOLOGIC RESPONSE.

		Grade			P
		Grade 1	**Grade 2**	**Grade 3**	
Response	Complete	6 (28,6 %)	12 (57,1%)	4 (14,3%)	
	Uncomplete	4 (13,8 %)	20 (69,0%)	5 (17,2%)	0,835
Total		10 20%	32 64,0%	8 16,0%	

In the Table 2 we may not find any significant correlation between the histopathologic grading to pathologic response.

Table 3 - CORRELATION BETWEEN HISTOLOGIC TYPE AND PATHOLOGICAL RESPONSE.

Histology Type	**Pathologic Response**		**P**	**RP**	**CI 95%**
	Complete	**Uncomplete**			
ILC	1	1			
IDC	18	31	0,453	0,58	0,034-9,86

In the Table 3 there is no significant correlation between histologic type with pathological response (P=0,453).

From Table 4 we do not found significant correlation of TILs before chemotherapy with pathological response (p = 0.157).

Discussion

The immune system is one of them serves as protective to recognize and destroy abnormal cells before they be-

TABLE 4 - RELATIONSHIP OF TUMOR INFILTRATING LYMPHOCYTE WITH PATHOLOGICAL RESPONSE BEFORE NEOADJUVANT CHEMOTHERAPY.

		Pathological Response				P	RP	Confident Interval Lower-Upper
		Complete		Incomplete				
		N	%	N	%			
TILs	High	11	(47,8%)	12	(52,2 %)	0,157	2,292	0,720-7,298
	Low	8	(28,6 %)	26	(71,4%)			
	Total	19	(37,3%)	32	(62,7%)			

come tumors or kill them if the tumor has grown. The role of the immune system is called immune surveillance. Some evidence supports that there is a role in the immune system against malignant tumors derived from several studies, including that supports the theory that is: 1) many tumors contain the infiltration of mononuclear cells consisting of T cells, NK cells, and macrophages; 2) tumor may regress spontaneously; 3) tumor develops more frequently in individuals with immunodeficiency or when the immune system does not function effectively, even immunosuppression often precedes tumor growth; 4) on the other hand, tumors often cause immunosuppression in patients (6).

In patients with breast cancer systemic administration of neoadjuvant chemotherapy provides an opportunity for rapid assessment relative in the success of the therapy regimen is given, this arrangement is not only to evaluate the role of predictive biomarker, including tils, but also allow assessment of the dynamics of changes in biomarkers before and after (6). In addition, several studies reported that a significant number of infiltrating lymphocytes in patients with neoadjuvant therapy had a pathologic complete response (7-9).

Fullface hematoxillin and eosin stained (H&E) slides of primary tumors were retrieved and evaluated for the percentage of intratumoral (it) and stromal (Str) TIL in 51 patient, according to predefined criteria (1). Cases were defined as high TIL if It TIL and /or Str TIL ≥ 50%, and as low TIL if It TIL and Str TIL <50%, adopting already validated cutoffs (1).

In this research reevaluation in 51 specimens slide taken from 2011 to 2015, obtained a mean age of patients was 49.22, most types of breast cancer histology was invasive ductal breast carcinoma was 49 (96%) patients whereas lobular carcinoma as much 2 patients (4%) (Table 1). Frequency of histopathological grade: grade 1 with high TILs encountered 5, while grade 2 was 15 patients, grade 3 is the lowest percentage of high TILs (37.3%) patients (Table 1). There is no significant difference between the TILs in patients < 50 years and ≥ 50 years (Table 1). In addition, no significant correlation between histopathology grading and histologic type of breast cancer to pathological response in patients treated with neoadjuvant chemotherapy (Tables 2, 3).

The data analysis also found no significant relationship between TILs to pathologic response of patients with neoadjuvant chemotherapy, the percentage of high TILs more likely have pathological complete response (47,8%) when compared to low TILs that only 28,8% (Table 3).

This study according to Denkert et al. in 2010 reported that the pathologic complete response was found in 40% of patients with tumors characterized by high lymphocytic infiltration, and only 7.2% of patients with no lymphocytic infiltrates.This study also confirmed the role of prediction in patients with breast cancer who receive neoadjuvant chemotherapy. In a study of 68 patients treated with anthracycline and taxane-based regimens. In another study Denkert et al. in 2010 also reported an increase trantuzumab TILs after administration in breast cancer with HER2 expressed and the administration of neoadjuvant chemotherapy with carboplatin. The researchers also found that nearly half (47.4%) with a high level of lymphocyte infiltration had a pathologic complete response in patients with neoadjuvant chemotherapy treatment. For every 10% increase in the number of tumor infiltrating lymphocytes, there is an increase of 16% get a complete pathological response. A study by Yamin et al. in 2014 also reported patients with a rich TILs indicates the number of complete responses were higher when compared to the poor TILs 36.6 *vs* 14.3%. Various factors may affect the evaluation of the TILs, the sample smear technique and assessed of the results by the pathologist also be an influential factor, but this difference bias is also caused by histologic type of breast cancer, grading histopathology, hormone receptor and over expressions HER2. According to another study by Yamaguchi et al. 2012, found the number TILs be a significant predictor factor towards complete response pathological both univariate and multivariate analysis, the presence of different TILs by breast cancer subtype. The increasing incidence of TILs was associated with ductal histology, high grade, no hormone receptor expression and high expression of Ki-67 antigen proliferation. Lymphocytic infiltration has a significant correlation in patients with triple-negative, where high TILs more likely to be complete pathologic response.

Conclusion

This research has not been able to prove significant correlation between TILs with pathological response in patient with locally advanced breast cancer who received neoadjuvant chemotherapy but high TILs more likely to have complete response. Further information may prove useful to future biomarker trial.

References

1. Dieci MV, Mathieu MC, Guameri V, Conte P, Delaloge S, Andre F, Goubar A. Prognostic and predictive value of Tumor Infiltrating lymphocytes in two phase III randomized adjuvant breast cancer. Annals Of Oncology. 2015;26:1698-1704.
2. Demaria S, Volm MD, Shapiro RL, et al. Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. Clin Cancer Res. 2001;7:3025-3030.
3. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, Wienert S. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILsWorking Group 2014. Annals of Oncology. 2015;26:259-271.
4. Denkert C, Loibl S, Noske A, Roller M, Muller BM, Komor M, Buczies J, Darb-Esfahani S Kronenwett R, Hanusch C, von Torne C, Weichert W, Engels K, Solbach C, Schrader I, Dietel M, von Minckwitz G. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28:105-113.
5. Denkert C, Loibl S, Noske A, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28:105-113.
6. Kresno SB. Imunologi tumor dalam: Ilmu onkologi dasar. Balai penerbit FKUI. Jakarta. 2010:161-179.
7. Hornychova H, Melichar B, Tomsova M, Mergancova J, Urminska H, Ryska A. Tumor-infiltrating lymphocytes predict response to neoadjuvant chemotherapy in patients with breast carcinoma. Cancer Invest. 2008;26:1024-1031.
8. Loi S, Sirtaine N, Piette 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 doxorubicinbased chemotherapy: BIG 02-98. J Clin Oncol. 2013;31:860-867.
9. Loi S, Michiels S, Salgado R, et al. Tumor infiltrating lymphocytes is prognostic and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol. 2014;25(8):1544-1550.
10. Melichar B, Urbanek L, Kremova L, Kalabova H, Melichavora K, Malivora E, Hernychova H, Ryska A, Hyspler R, Solichova D. Urinary neupterin, hemoglobin and peripheral blood cell counts in breast carcinoma patient treated with desodense chemotherapy. Anticancer Res. 2008;28:2389-2396.
11. Ma Y, Kepp O, Ghiringhrlli F, et al. Chemotherapy and radiotherapy: cryptic anticancer vaccines. Semin Immunol. 2010;22:113-124.
12. Melichar B, Touskova M, Dvorak J, Jandik P, Kopecky O. The peripheral blood leukocyte phenotype in patient with breast cancer: Effect of doxorubicin/paclitaxel combination chemotherapy. Immunopharmacol Immunotoxicol. 2001;23:163-173.
13. Mahmoud SM, Paish EC, Powe DG, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011;29:1949-1955.