Case Report

Correlation between Tumor-Infiltrating Lymphocytes and Pathological Response in Locally Advanced Breast Cancer Patients Who Received Neoadjuvant Chemotherapy in H. Adam Malik General Hospital

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Keywords

Breast cancer · Neoadjuvant chemotherapy · Tumor-infiltrating lymphocytes · Pathological response

Abstract

Background: Tumor-infiltrating lymphocytes (TILs) are emerging as biomarkers mediating tumor response to treatments. Earlier studies have provided evidence that the level of TILs has prognostic value, particularly in triple-negative and human epidermal growth factor re-

ceptor-2-positive breast cancer. Moreover, the level of TILs has been associated with treatment outcome in patients undergoing neoadjuvant chemotherapy, and there is a strong correlation with pathologically complete response. In this study, we analyzed whether changes in TILs take place after neoadjuvant therapy and if they correlate with pathological response to treatment. **Patients and Methods:** We retrospectively analyzed the specimen slides from the Department of Anatomic Pathology of H. Adam Malik General Hospital during 2011–2015. We identified 51 patients fulfilling the inclusion criteria of this study. The histological sections had already been evaluated by hematoxylin and eosin slides. They were reassessed by our pathologist for the percentage of intratumoral and stromal TILs. The correlation with pathological response of the tumor after neoadjuvant therapy was also studied in these patients. Each case was also defined as high- or low-TIL breast cancer adopting previously validated cutoffs. **Results:** The mean age of the 51 patients was 49.22 years. The most frequent type of breast cancer histology was invasive ductal breast carcinoma in 49 (96%) patients, and there were 2 (4%) patients with lobular carcinoma. The histopathological grading for high TILs was grade 1 in 5 patients, grade 2 in 15 patients, and grade 3 in 3 patients. High TILs that had a pathologically complete response were found in 47.8% of patients, and low TILs were found in 28.8%. There was no significant correlation between TILs and pathological response in patients with neoadjuvant chemotherapy ($p = 0.157$). **Conclusions:** This research has not been able to demonstrate a significant correlation between TILs and pathological response in patients with locally advanced breast cancer who received neoadjuvant chemotherapy, but high TILs were more likely to have a complete response. Further information may prove useful for future biomarker trials.

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Introduction

The immune system is a key player in cancer progression. Preclinical data suggest that chemotherapy can trigger an antitumor immune response by causing immunogenic cell death that allows antigen cross-presentation and activation of dendritic cells and tumor-specific cytotoxic T cells [1]. The presence of a host antitumor immunity has been shown to influence the response to cytotoxic treatment. Denkert et al. [2] demonstrated that high tumor infiltration by lymphocytes at diagnosis is associated with a higher likelihood of a pathologically complete response after neoadjuvant chemotherapy. Tumor-infiltrating lymphocytes (TILs) at baseline are associated with highly proliferative, high-grade, and estrogen receptor-negative tumors and represent a strong prognostic factor for certain breast cancer subtypes, mainly for triple-negative breast cancer [3, 4].

Methods

An analytic retrospective study was performed. Prepared slides (pretreatment slides from incisional biopsy and posttreatment surgical specimens) of 51 locally advanced breast cancer patients who received neoadjuvant chemotherapy were collected based on inclusion criteria from 2011 to 2014. The prepared slides were reassessed by a pathologist at our

hospital. Pathological response to neoadjuvant chemotherapy was determined as reported previously [2]. Data were analyzed by the χ^2 test to find a relationship between the response TILs and pathology response in locally advanced breast cancer after neoadjuvant chemotherapy.

Pathology

Hematoxylin and eosin (HE)-stained slides of primary tumors were retrieved and evaluated for the percentage of intratumoral (IT) and stromal (STR) TILs according to predefined criteria [1]. Cases were defined as high TIL if IT TILs and/or STR TILs were $\geq 50\%$ and as low TIL if IT TILs and STR TILs were $< 50\%$, adopting already validated cutoffs [1, 2]. These cutoffs were defined before any statistical analyses. Pathological response was defined as assessed from the results of a postoperative pathological examination, complete response indicating the eradication of the entire tumor in both invasive and noninvasive breast cancer as well as the lymph nodes, and incomplete response indicating that there was still tumor or lymph node left in both invasive and noninvasive breast cancer [2].

Statistics

Statistical analysis was carried out using the project for statistical computing. During the study period, the data were taken from the subdivisions of the 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 TILs and pathological responses that met the inclusion criteria. Data were collected from 2011 to 2015. There were 51 patients who met the inclusion criteria. From the collected data, the characteristics of the sample, such as age, frequency, type of histopathology, and histopathological grading, were recorded. Then, the relationship between TILs and pathological response before and after chemotherapy in breast cancer patients was analyzed. A paired *t* test was used to evaluate the comparison between pretreatment and posttreatment TILs. The association between TILs and pathological response was calculated using the χ^2 test. All statistical tests were two sided and considered significant if the *p* value was ≤ 0.05 .

Results

In this reevaluation of 51 specimens from 2011 to 2015 (mean age of patients 49.22 years), the most frequent type of breast cancer histology was invasive ductal breast carcinoma in 49 (96%) patients, and there were 2 (4%) patients with lobular carcinoma (Table 1). The histopathological grading for high TILs was grade 1 in 5 patients, grade 2 in 15 patients, and grade 3 (the lowest percentage of high TILs [37.3%]) in 3 patients (Table 1). There was no significant difference between TILs in patients aged < 50 and ≥ 50 years (Table 1). In addition, no significant correlation was found between histopathological grading/histological type of breast cancer and pathological response in patients treated with neoadjuvant chemotherapy (Table 2, Table 3).

The data analysis also found no significant relationship between TILs and pathological response of patients with neoadjuvant chemotherapy. High TILs were more likely to be as-

sociated with a pathologically complete response (47.8%) when compared to low TILs (28.8%) (Table 4).

Discussion

The immune system serves as a protection to recognize and destroy abnormal cells before they become tumor cells or to kill them if the tumor has grown. The role of the immune system is called immune surveillance. Some evidence supports that the immune system plays a role in the defense against malignant tumors. Several studies support the following points: (1) many tumors contain an infiltration of mononuclear cells, consisting of T cells, NK cells, and macrophages; (2) tumors may regress spontaneously; (3) tumors develop more frequently in individuals with immunodeficiency or when the immune system does not function effectively, and even immunosuppression often precedes tumor growth; and (4) on the other hand, tumors often cause immunosuppression in patients [5].

In patients with breast cancer, systemic administration of neoadjuvant chemotherapy provides an opportunity for a rapid assessment of the success of the therapy regimen. This assessment does not only evaluate the role of predictive biomarkers, including TILs, but also allows the evaluation of the dynamics of changes in biomarkers before and after therapy [5]. In addition, several studies reported that a significant number of infiltrating lymphocytes in patients with neoadjuvant therapy had a pathologically complete response [6–8].

This study, according to Denkert et al. [2], reported that a pathologically complete response was found in 40% of patients with tumors characterized by high lymphocytic infiltration and in only 7.2% of patients with no lymphocytic infiltrates. This study also confirmed the role of prediction in patients with breast cancer who received neoadjuvant chemotherapy. In another study, Denkert et al. [9] also reported an increase in TILs after trastuzumab administration in breast cancer with HER2 expression and the administration of neoadjuvant chemotherapy with carboplatin. The researchers also found that nearly half of the patients (47.4%) with a high level of lymphocyte infiltration had a pathologically complete response to neoadjuvant chemotherapy treatment. For every 10% increase in the number of TILs, there is an increase of 16% to obtain a complete pathological response. A study by Ma et al. [10] also reported that in patients with rich TILs the number of complete responses was higher when compared to those with poor TILs (36.6 vs. 4.3%). Various factors may affect the evaluation of TILs, such as the sample smear technique, and the assessment of the results by the pathologist may also be an influential factor, but this difference bias is also caused by histological type of breast cancer, grading histopathology, hormone receptor, and overexpression of HER2. Another study by Yamaguchi et al. [11] found the number of TILs to be a significant predictor of complete pathological response both in univariate and multivariate analysis of the presence of different TILs according to breast cancer subtype. There is an increasing incidence of TILs associated with a 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 breast cancer, where high TILs are more likely to have a complete pathological response.

Conclusion

This research was not able to demonstrate a significant correlation between TILs and pathological response in patients with locally advanced breast cancer who received neoadjuvant chemotherapy, but high TILs were more likely to have a complete response. Further information may prove useful for future biomarker trials.

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Statement of Ethics

This study has been approved by the local ethics committee of University of Sumatera Utara. All patient consents were received prior to the study.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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Table 1. Characteristics of patients with breast cancer ($n = 51$)

	TILs before chemotherapy, n (%)		p
	high TILs	low TILs	
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)	
Histopathological grading			
IDC		49 (96)	0.492
ILC		2 (4)	

There was no significant association between age and TILs ($p = 0.762$), nor between histopathological grading and TILs ($p = 1.000$). TIL, tumor-infiltrating lymphocyte; IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma.

Table 2. Correlation between histopathological grading and pathological response

Response	Grade, <i>n</i> (%)			<i>p</i>
	grade 1	grade 2	grade 3	
Complete	6 (28.6)	12 (57.1)	4 (14.3)	0.835
Incomplete	4 (13.8)	20 (69.0)	5 (17.2)	
Total	10 (20)	32 (64.0)	8 (16.0)	

There was no significant correlation between histopathological grading and pathological response.

Table 3. Correlation between histological type and pathological response

Histological type	Pathological response		<i>p</i>	Ratio	95% CI
	complete	incomplete			
ILC	1	1	0.453	0.58	0.034–9.86
IDC	18	31			

There was no significant correlation between histological type and pathological response ($p = 0.453$). IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; CI, confidence interval.

Table 4. Relationship between TILs before neoadjuvant chemotherapy and pathological response

TILs	Pathological response, <i>n</i> (%)		<i>p</i>	Ratio	95% CI
	complete	incomplete			
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)			

There was no significant correlation between TILs before chemotherapy and pathological response ($p = 0.157$). TIL, tumor-infiltrating lymphocyte; CI, confidence interval.