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The Significance of Tumor-infiltrating Lymphocytes (TILs) in Histopathological Grading of Invasive Breast Carcinomas

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Abstract

Breast cancer is the leading cause of cancer death in women worldwide with high incidence rate. Invasive breast carcinoma is the most common form of breast cancer. Prognosis and survival rate of the patient is related with histopathological grading of the invasive breast carcinoma. Tumor-infiltrating lymphocytes (TILs) have been reported to be associated with patient clinical outcomes in a number of different malignant tumors. We studied the expression of TILs in invasive breast carcinoma using Hematoxylin Eosin (HE) staining. We studied the expression of are tumor-infiltrating lymphocytes (TILs) in paraffin block of tissue biopsy from breast tumor specimens. Immunohistochemistry was used to assess the expression of TILs in tumor breast tissue from 80 samples. Univariate and bivariate analyses assessed outcomes according to the expression of TILs in different histopathological grading. Of the 80 tumor specimens, 36 (45 %) of samples have high grade of TILs and 44 (55 %). On bivariate analysis, there were significant differences in the expression of TILs lymphocytes between well, moderately and poorly differentiated invasive breast carcinoma, respectively ($p < 0.0001$).

As a conclusion; we found that the severity of invasive breast carcinoma differentiation is directly related to the degree of TILs expression.

Keywords: TILs; histopathological grading; invasive breast carcinoma.

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1. Introduction

Breast cancer is the leading cause of cancer death in women worldwide and the most commonly diagnosed of cancer in women [1]. About 80% of all breast cancers are invasive ductal carcinomas. The invasive breast cancer was grading based on number of mitoses, nuclear pleomorphism, and glandular formation. Histopathological grading is quantitatively determined using the Patey & Scarff method, and Bloom & Richardson modified by Elston & Ellis. The importance of histopathological grading is as determining factor for prognosis [2].

Local anti-tumor defense mechanisms describe the development and organization of the tumor microenvironment. The composition of the cell population and the relative proportion of inflammatory cells present in these areas affect the quality and characteristics of the inflammatory response. Lymphocytes found directly in the tumor area are tumor-infiltrating lymphocytes (TILs) [3]. TILs consist of a subset of lymphocytes with different proportions, including CD8+ T cells, CD4+ T cells, Natural Killer (NK) cells, and B cells, where T lymphocytes are considered the most dominant part of the tumor microenvironment and have a significant role in tumor development. The T lymphocyte subtype as part of the TILs has its role in the tumor microenvironment [4–6].

Tumor-infiltrating lymphocytes (TILs) have been reported to be associated with patient clinical outcomes in a number of different malignant tumors, including breast carcinoma. Ali and his colleagues in their study found that the presence of T cell infiltrates in tumors was independently associated with a reduced relative risk of death [7]. This is in line with the research of Loi and his colleagues which stated that patients with high degrees of TILs have excellent survival [8].

2. Materials and method

Samples

80 paraffin block of tissue biopsy from breast tumors were collected from the Anatomical Pathology laboratory of Dr. Wahidin Sudirohusodo Hospital Makassar and Hasanuddin University Hospital Makassar for the period 2016-2020 which were diagnosed as invasive breast carcinoma Grade 1 (well differentiated), Grade 2 (moderate differentiated), and Grade 3 (poor differentiated) through HE staining. Samples that were collected and met the inclusion criteria were then re-evaluated by two Anatomical Pathology Specialists with a consistent diagnosis, and then assessed for the degree of tumor-infiltrating lymphocytes (TILs). This study was approved by the Ethical Committee of Faculty of Medicine Hasanuddin University.

Hematoxylin Eosin staining procedure

After the tissue blocks were collected, the tissue was cut with a 3 m thick microtome. The tissue from the microtome cut was put into a water bath at a temperature of 60°C. The pieces in the water bath were taken using an object-glass, drained to dry, and then placed on a slide warmer at a temperature of 60°C for 15 minutes. Tissue slides that are ready to be stained are immersed in 2 containers of xylol solution for 5 minutes, 2

containers of 95% alcohol solution for 2 minutes and followed by immersing the slides in 70% alcohol solution for 2 minutes. The slide was then rinsed with tap water for 5 minutes before immersed in Hematoxylin Mayer solution for 15 minutes and rinsed again with tap water until the slide turned blue. After that, the slides were immersed in 1% Eosin solution for 5 minutes, in 70% alcohol for 2-5 minutes then in 95% alcohol solution for 2-5 minutes. After the dehydration step, the slides were immersed in carbol xylol solution for 5 minutes, before immersed in xylol solution for 2-5 minutes. Finally, the slide was drained and closed using a cover-glass with mounting agent as an adhesive liquid. The slides are ready to be viewed under a light microscope.

Tumor-Infiltrating Lymphocytes (TILs) Scoring Method

The TILs scoring method was based on the recommendations of the International TILs Working Group, 2014 [9]:

1. TILs were reported in the stromal compartment (=% stromal TILs), areas infiltrated by mononuclear inflammatory cells throughout the tumor stromal area. Areas occupied by tumor cells and stroma in areas of normal tissue were not assessed. The reported percentages are the mean values of several representative stromal areas reflecting various densities of TILs (not focusing on hot spot areas).

2. Areas with regressive artifacts, necrosis, and hyalinization around the tumor area were not assessed.

3. All mononuclear inflammatory cells were assessed, including lymphocytes and plasma cells, while granulocytes and other PMN leukocytes were not assessed.

Statistical analyses

All data obtained from the results are recorded and grouped based on the purpose and type of data for analysis using univariate and bivariate analysis. Univariate analysis used to describe the characteristics of the data obtained in the form of frequency distribution, range and average value presented in tabular form. In bivariate analysis, X2 test (Chi square) was applied to compare ordinal variables between two or more unpaired groups. In this study, In this study, we compared the degree of TILs in well differentiated, moderately differentiated and poorly differentiated invasive breast carcinomas; with p value: $p < 0.05$.

3. Results

Patient Characteristics

From a total of 80 samples as described in Table 1, 10 samples (12.5%) were in <40 years old category and 70 samples (87.5%) were in >40 years old category, with mean age 48.73 years old. The numbers of samples with low grade TILs were 44 (55.0%) samples and 36 (45.0%) samples with high grades TILs.

Table 1: Patient characteristics (n=80)

Characteristics	N	Minimum	Maximum	*Mean*	SD
Age (year)	80	28	70	48,73	9,05

Characteristics	**N**	**%**
Age:		
< 40 years	10	12,5
≥ 40 years	70	87,5
Degree of TILS :		
Low	44	55
High	36	45

Grading of Invasive Breast Carcinomas

Determination degree of TILs is carried out by assessing the percentage of mononuclear inflammatory cells in the tumor stromal area according to the recommendations of the International TILs working group, 2014. In this study, the TILs assessed were stromal TILs. TILs were categorized into four groups, score 0 = none, score 1 = mild (<30% TILs), score 2 = moderate (30-60% TILs), score 3 = severe (>60% TILs).

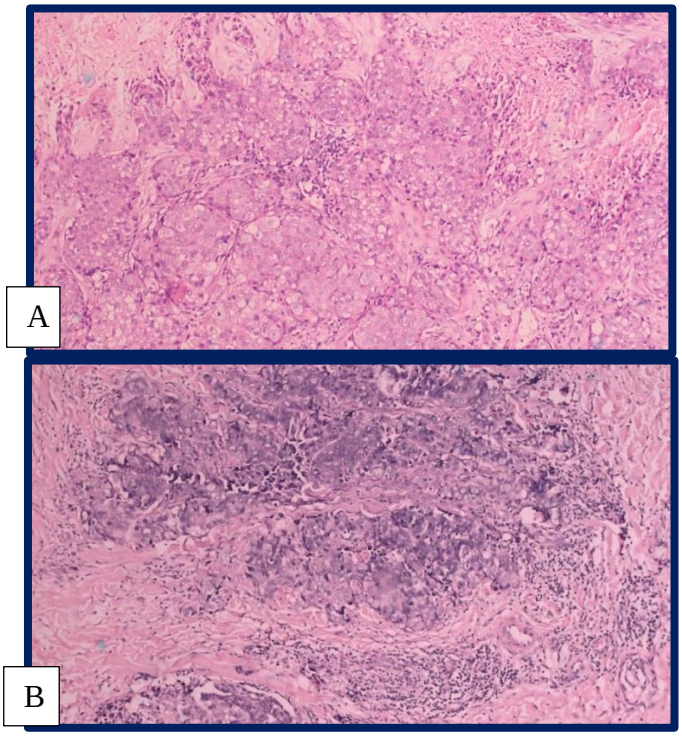

Figure 13: Mild. Stromal area with 5% of TILs,obj.10x (A) and stromal area with 20% TILs, obj.10x (B).

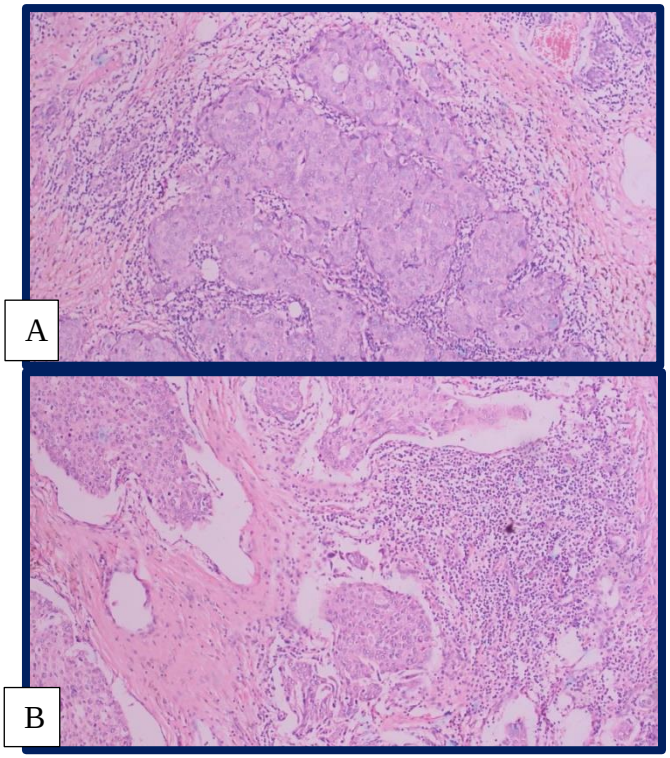

Figure 14: Moderate. Stromal area with 50% of TILs, obj.10x (A) and stromal area with 30% TILs, obj.10x (B)

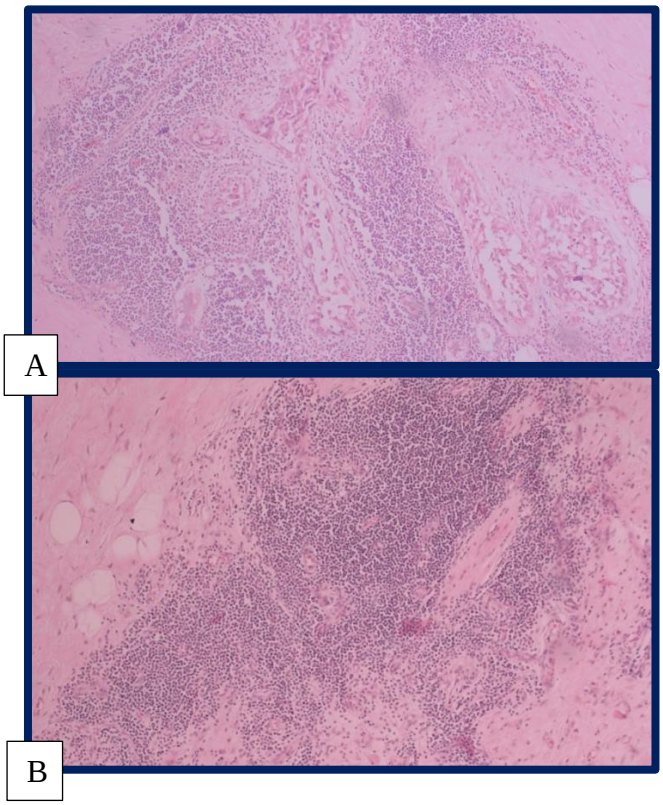

Figure 15: Severe. Stromal area with 70% of TILs, obj.10x (A) and stromal area with 80% TILs, obj.10x (B).

Analysis of Differences in the Degree of TILs Based on Histopathological Grade

Table 5: Degree of TILs by histopathological grade

Degree of TILs	Histopathological Grade (Differentiation)			Total	
	Good (%)	Moderate (%)	Poor (%)	(%)	
Low	1	15	28	44	
	(2,3)	(34,1)	(63,6)	(100)	
High	19	14	3	36	***p < 0,0001***
	(52,8)	(38,9)	(8,3)	(100)	
Total	20	29	31	80	
	(25,0)	(36,2)	(38,8)	(100)	

Based on Table 5, from a total of 80 samples, there were 44 (55.0%) samples with low TILs and 36 (45.0%) samples with high TILs. In the good differentiation degree group, from a total of 20 samples, 1 (5.0%) samples had low TILs, and 19 (95.0%) samples had high TILs. In the moderate degree of differentiation group, from a total of 29 samples, 15 (51.7%) samples had low TILs, and 14 (48.3%) samples had high TILs. As for the poor degree of differentiation group, out of a total of 31 samples, 28 (90.3%) samples had low TILs, and 3 (9.7%) samples had high TILs. Based on the Chi-square test, obtained $p < 0.0001$ ($p < 0.05$) so that it was concluded that there was a significant difference in the degree of TILs in good, moderate, and poor differentiation of invasive breast carcinoma samples.

4. Discussion

In this study, the assessment of TILs included TILs in the stromal area and the invasive area of the tumor margin, as the previous study by Iseki and his colleagues reported that the invasive frontal area of the tumor was the optimal area for assessing TILs [10]. Based on the Chi-square hypothesis test, in this study, the value of $p = 0.0001$ ($p < 0.05$) was concluded so that it was concluded that there was a significant difference in the degree of TILs in good, moderate, and poor differentiation of invasive breast carcinoma. In the good degree of differentiation group, there were more samples with high TILs than low, in the medium differentiation group, almost comparable between samples with low TILs and high TILs, while in the poor differentiation degree group, more samples with low TILs than those with low TILs. high TILs. So it can be concluded that the higher the degree of TILs, the better the degree of differentiation, conversely the lower the degree of TILs, the worse the degree of differentiation. T cells are part of adaptive immunity that plays a role in suppressing tumor growth. CD4 and CD8 T cells play a role in the destruction of tumor cells by producing IL-2 and together with IL-15 produced by host cells will help the function and viability of CD8 cells. In addition, CD8 T cells are also able to recognize tumor targets efficiently and will induce the destruction of tumor cells.

Tumor infiltrating lymphocytes (TILs) exhibit a localized immune response directed against tumor growth and metastasis. TILs have emerged as tools for assessing immune reactivity in various malignancies including colon, ovary, lung, bladder, breast and others as well as independent markers of good prognosis in many tumors. TILs in breast carcinoma play a role in tumor response to therapy in adjuvant and neoadjuvant settings.

The results of this study are in line with the study of Pujani and his colleagues who reported a significant correlation between stromal TIL with tumor grading, lymph node metastases, molecular subtypes and mitosis in breast carcinoma [11]. Similarly, in the study of Bjelobrk and his colleagues who found a positive correlation between the degree of TIL, tumor size, metastases in axillary lymph nodes, histological grade, stage and disease recurrence in breast cancer [12].

In conclusion, there was a significant difference degree of TILs in good, moderate, and poor differentiation of invasive breast carcinoma. The severity of invasive breast carcinoma differentiation is directly proportional to the lower expression of TILs.

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