Relationship between tumor infiltrating lymphocytes and tumor grading on the incidence of metastasis in breast cancer patients

Relación entre los linfocitos infiltrantes del tumor y la clasificación del tumor en la incidencia de metástasis en pacientes con cáncer de mama

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Abstract

Breast cancer is the most common cancer in women and the leading cause of death in Indonesia. Better breast cancer treatment strategies are needed to improve the prognosis of breast cancer, so it is important to know the nature of breast tumors. The immune system will respond to the presence of a tumor by the formation of TILs, while the nature of the tumor itself can be seen from the grading of the tumor. These two variables represent tumor suppressor and tumor progression respectively. The purpose of this study is to determine whether TIL and tumor grading can be used as predictors of metastatic events in breast cancer patients. A retrospective cohort analytic study research was conducted on 51 breast cancer patients who visited Ulin Banjarmasin Hospital during January - December 2023. Somers'd test was used with SPSS software to analyze the correlation between TIL and tumor grading on the incidence of metastasis with a confidence level value of α = 0.05. Given the results, there is a significant relationship between tumor grading and the incidence of metastasis (p value = 0.01) with weak relationship strength (r value = 0.296). In this study, there was no significant relationship between TIL and the incidence of metastasis in breast cancer patients at RSUD Ulin Banjarmasin (p value = 0.494). In conclusion, tumor grading can be used as a prognostic factor for the incidence of metastasis in breast cancer patients at RSUD Ulin Banjarmasin.

Keywords: TIL, Tumor Grading, Metastasis, Breast Cancer

Resumen

El cáncer de mama es el cáncer más común en las mujeres y la principal causa de muerte en Indonesia. Se necesitan mejores estrategias de tratamiento del cáncer de mama para mejorar el pronóstico del cáncer de mama, por lo que es importante conocer la naturaleza de los tumores de mama. El sistema inmunológico responderá a la presencia de un tumor mediante la formación de TIL, mientras que la naturaleza del tumor en sí puede verse a partir de la clasificación del tumor. Estas dos variables representan el supresor de tumores y la progresión del tumor, respectivamente. El propósito de este estudio es determinar si TIL y la clasificación del tumor pueden usarse como predictores de eventos metastásicos en pacientes con cáncer de mama. Se realizó un estudio analítico de cohorte retrospectivo en 51 pacientes con cáncer de mama que visitaron el Hospital Ulin Banjarmasin entre enero y diciembre de 2023. Se utilizó la prueba de Somers con el software SPSS para analizar la correlación entre TIL y la clasificación del tumor en la incidencia de metástasis con confianza. valor de nivel de $\alpha = 0,05$. Teniendo en cuenta los resultados, existe una relación significativa entre la clasificación del tumor y la incidencia de metástasis (valor de $p = 0,01$) con una fuerza de relación débil (valor de $r = 0,296$). En este estudio, no hubo una relación significativa entre TIL y la incidencia de metástasis en pacientes con cáncer de mama en RSUD Ulin Banjarmasin (valor de $p = 0,494$). En conclusión, la clasificación del tumor puede utilizarse como factor pronóstico de la incidencia de metástasis en pacientes con cáncer de mama en RSUD Ulin Banjarmasin.

Palabras clave: TIL, clasificación de tumores, metástasis, cáncer de mama.

Introduction

Breast cancer is the most commonly diagnosed cancer in women, and the second most common cause of death from cancer in the world[1]. According to Globocan 2020 data, there were 2,241,619 new cases and 684,996 deaths from breast cancer. In Asia, there were 1,026,171 new cases in 2020 and 346,009 deaths. In Indonesia, there were 65,858 new cases of breast cancer in that year and it is the first rank of cancer in Indonesia[2].

A better breast cancer treatment strategy is needed to improve the prognosis of breast cancer, for which it is important to know the nature of the breast tumor itself. In breast cancer, there are many cells and cytokines involved that form the tumor microenvironment (TME)[3,4]. The presence of specific killer lymphocytes in the TME is known as tumor infiltrating lymphocyte (TIL), which will direct the suppression or promotion of cancer cells to 3 phases, namely elimination, equilibrium or elimination[5].

The meta-analysis study from Gao *et.al*. explained that high TIL levels showed a better prognosis in both short-term and long-term assessments[6]. Invasive breast carcinoma is morphologically divided into three according to the growth pattern and degree of differentiation. Tumor grade assessment is important in determining the prognosis of breast cancer patients which can certainly be used as a basis for choosing patient therapy[7]. In a previous study, Hermansyah et al. found that high tumor grading was associated with the discovery of sentinel nodule metastases[8]. Another study by Kurozumi et al. also found that TIL grading is associated with hormone receptors that provide a better prognosis[9].

TIL and tumor grading appear to have a relationship that can theoretically be used to predict the incidence of metastasis in breast cancer patients. In this study, researchers wanted to prove the relationship between TIL and tumor grading with the incidence of metastasis in breast cancer patients in Banjarmasin.

Materials and methods

Observational analytic study with retrospective cohort design, secondary data were collected from the medical records of breast cancer patients who sought treatment at the oncology surgery clinic of RSUD Ulin Banjarmasin for the period January-December 2023. The research was carried out after obtaining an ethical clearance certificate from the Health Research Ethics Committee, Faculty of Medicine and Health Sciences, Lambung Mangkurat University.

The population in this study were breast cancer patients who had been diagnosed through clinical examination, imaging and histopathology at Ulin Hospital, Banjarmasin. Samples were obtained by non-random consecutively sampling method. The total sample obtained was 78 patients and after being selected according to the inclusion and exclusion criteria, a sample of 51 patients was included in the study.

Inclusion criteria in this study were breast cancer patients whose diagnosis was based on Histopathology results from incisional biopsy, excisional biopsy or core biopsy. Samples had complete examination data including evidence of metastasis from minimal imaging examinations of thoracic x-rays and liver ultrasound and had no history of other primary tumors recorded in medical records. Exclusion criteria in this study were if the sample had unrepresentative histopathology preparations to assess TIL and tumor grading based on anatomical pathologist examination. Somers'd test was used with SPSS software to analyze the correlation between TIL and tumor grading on the incidence of metastasis with a confidence level value of α 0.05.

Results

This study found a total of 51 breast cancer patients, with 31 (39.2%) patients belonging to the group with metastasis and 20 (60.8%) patients belonging to the group without metastasis. The age distribution of the sample was predominantly <50 years old, with an overall mean age of 51.09 years. Intermediate TIL was the most common (47.1%), followed by low TIL (45.1%). Grade 3 tumors were the most common (50.9%) followed by grade 2 (43.2%) samples. The most common site of metastasis was the lung and there was 1 sample that had 2 organs that experienced metastasis, namely the lung and contralateral **(Table 1)**

Table 1. Characteristics of research subjects

Variables	N	%	Mean±SD
Age			
<50	23	45.1	51.09 ±12.51
50-65	21	41.2	
>65	7	13.7	
TIL			
Low	23	45.1	-
Intermediate	24	47.1	-
High	4	7.8	-
Grade			
1	3	5.9	-
2	22	43.2	-
3	26	50.9	-
Metastasis			
Yes	20	39.2	-
No	31	60.8	-
Location of metastasis			
Lung	12	60	-
Bones	2	10	-
Contralateral	6	30	-
Brain	1	5	-

The correlation between TIL and the incidence of metastasis was performed using Somers'd correlation analysis. A p value of 0.494 was obtained, indicating that the correlation between TIL and the incidence of metastasis was not significant. The correlation value of 0.08 indicates a positive correlation with a very weak correlation strength and can be ignored **(Table 2)**.

Table 2. Correlation between TIL and incidence of metastasis

		Metastasis		Correlation coefficient (r)	P-value
		Yes	No		
TIL	Low	10 (19.6)	13 (25.5)	0.08	0.494
	Intermediate	9 (17.6)	15 (29.4)		
	High	1 (2.0)	3 (5.9)		
Total		20 (39.2)	31 (60.8)		
Somers'd Correlation Test Metastasis dependent					

The correlation between tumor grading and the incidence of metastasis was also performed and the p value = 0.010 indicated that there was a significant relationship between tumor grading and the incidence of metastasis. The correlation value of 0.296 showed a positive correlation with a weak correlation strength **(Table 3)**.

Table 3. Correlation between grading and incidence of metastasis

		Metastasis		Correlation coefficient (r)	P-value
		Yes	No		
TIL	Well	0 (0.0)	3 (5.9)	0.296	0.010*
	Intermediate	6 (11.8)	16 (31.4)		
	Poorly	14 (27.4)	12 (23.5)		
Total		20 (39.2)	31 (60.8)		

Notes: *significant at $p < 0.05$ on Somers'd metastasis dependent correlation test

Discussion

The above results indicate that TIL has a weak relationship to the incidence of metastasis so that it cannot be used as a prognostic factor. A similar study previously conducted by Santhi et al in 2019 on 53 breast cancer patients post *Modified Radical Mastectomy* at Sanglah General Hospital found that patients with *low* TIL (P=0.627), *intermediate* TIL (P=0.339) and MAI ≥ 25 /10 lp (P=0.531), MAI 13-24 /10 lp (P=0.531) did not find a statistically significant relationship with the incidence of axillary KGB metastases[10].

Our study shows that TIL cannot be used as a prognostic factor for the incidence of metastasis. This is related to the time of biopsy sampling, because to metastasize a cancer cell requires a process and time. Sudarsa and Aryanti shown that examination of TIL with a long period until neoadjuvant chemotherapy could not be a prognostic factor for the overall survival of breast cancer[11].

This is supported by several previous studies which mentioned that TIL mainly consists of T-cells, B-cells and NK cells. TIL activated in different periods have different phenotypes. Stanton et al. also showed that TILs are different in different subtypes of breast cancer. In TILs, most cells are CD3 positive. The proportion of CD25+ cells in freshly isolated TILs is low, and the proportion of CD25+ cells increases after adding IL-2 to the culture[12].

Low lymphocytes are associated with poor outcome[13]. The importance of lymphocytes has been investigated in several studies. Lymphocytes are associated with their cytotoxic function. The response is good when many lymphocytes infiltrate the tumor cells[14]. A study on peripheral serum measurements of high CD8 had better survival and in lymphocyte counts, there was a better outcome in high lymphocytes[15].

Peripheral serum lymphocytes are significantly lower in patients with short survival time. Lymphocytes may be a host factor that plays a role in breast cancer survival. One study described a positive correlation between cancer patient curability and lymphocyte levels and a negative correlation with neutrophil levels[16].

This study found an association between tumor grading and the incidence of metastasis in breast cancer patients. This is in accordance with the results of a study conducted by Ehinger *et al in* 2017 on 671 breast cancer patients who analyzed tumor histopathology for prognosis, finding that patients with ER-positive / HER2-negative / G1 breast cancer had a good prognosis, similar to 'Luminal A-like', while those with ER-positive / HER2-negative / G3 breast cancer had a worse prognosis, similar to 'Luminal B-like'[17]. Cserni *et.al's* research has shown that higher tumor grading in locally advanced breast cancer can progress from primary tumor to axillary metastasis or local or regional recurrence faster. Low tumor grading tends to grow more slowly than high tumor grading, and some may progress to high tumor grading, whereas others may not. The size of the primary tumor and subsequent axillary metastases or recurrences also show results in accordance with tumor grading and cancer stage[7,17].

This study found a weak association between tumor grading and the incidence of metastasis. This may be related to the timing of biopsy sampling, as metastasizing cancer cells requires time and process. The limited sample in this study may give different results for the strength of the relationship between the two variables.

There are limitations to this research, including that the research was only conducted in one hospital which could result in local bias and limited generalization. Retrospective study design cannot control time variables better when compared to prospective research with a longer period of time

Conclusions

TIL cannot be an indicator of prognostic factors for the incidence of metastasis in breast cancer patients, while tumor grading can be a prognostic factor for the incidence of breast cancer metastasis.

DISCLOSURE

Ethical Clearance

This study obtained permission from the Health Research Ethics Commission of the Faculty of Medicine and Health Sciences, Lambung Mangkurat University with letter number No.056/KEPK-FKIK ULM/EC/II/2024.

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Conflict of Interest

All authors declare no conflict of interest in this study.

Author's Contribution

All authors equally contribute to the study from the conceptual framework, methodology, validation, formal analysis, review and editing until reporting the study result trough publication.

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