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Stromal Tumor Infiltrating Lymphocytes (TIL) as a potential prognostic biomarker for recurrence in Locally Advanced Breast Cancer (LABC) patients

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ABSTRACT

Background: Tumor-infiltrating lymphocytes (TIL) serves as the host adaptive immune response in breast cancer. TIL has the potential to be an independent prognostic factor in breast cancer patients. In this study, we aimed to examine the association between stromal TIL and recurrence in locally advanced breast cancer patients.
Methods: A cohort retrospective study was conducted using medical records of female breast cancer patients with locally advanced breast cancer. We collected patients' data, including demographic data from the medical record. Stromal TIL was examined following the recommendations of the International TIL Working Group 2014.
Results: 75 samples were included with an average age of 49.5±8.4. Ductal carcinoma was the most common type histologically (88.0%). Luminal B Her2-negative was the predominant breast cancer subtype (32.0%). There was a significant association between the breast cancer subtype and disease-free survival (P – 0.014). The optimal cut-off to determine the recurrence of breast cancer was 15%. The sensitivity, specificity, PPV, NPV, and accuracy of TIL to predict the 24-month disease-free survival (DFS) were 80.5%, 82.4%, 84.6%, 77.8%, and 81.3%, respectively. Every 10% increase in TIL percentage could raise the DFS by 5.45 months (p=0.001). Patients with high TIL values had higher survival than those with low TIL values.
Conclusion: There was a significant correlation between the stromal TIL and the recurrence rate. The pre-therapy stromal TIL percentage can be employed as a potential biomarker to predict breast cancer recurrence, particularly in the first two years.

Keywords: locally advanced breast cancer, prognostic, stromal tumor-infiltrating lymphocytes, recurrence.
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INTRODUCTION

Breast cancer is the most common cancer among women worldwide. According to Global Burden Cancer (GLOBOCAN) statistics, there were 2.261.419 new breast cancer cases worldwide in 2020, or 24.5% of all women's cancer cases worldwide.[1] In developing countries like Indonesia, breast cancer treatment is proven to be challenging and relatively inadequate due to various factors, such as limited access to comprehensive breast cancer treatment and poor patient compliance to treatment. These lead to advanced breast cancer being one of the major issues in Indonesia.[2,3]

According to the previous data, 40–80% of individuals with breast cancer who seek medical attention are in stages III and IV or in advanced stage.[4,5] Locally advanced breast cancer (LABC) is characterized by large tumor size and infiltration into the chest wall or skin and regional lymph nodes but without distant metastases. The comprehensive treatment of locally advanced breast cancer has undergone significant change over the past three decades, shifting from neoadjuvant chemotherapy, the primary therapy and the foundation of breast cancer treatment, to surgery and radiotherapy.[6] The advantage of neoadjuvant chemotherapy in LABC is to decrease the size of the tumor and its stage until a full pathological response is attained, increasing the likelihood of resection, disease-free survival, overall survival, and HER2/Neu+ status.[7,8] However, several recent studies indicate that not all breast cancer patients with residual tumors will experience recurrence and that a rise in complete histological response does not always predict a better prognosis. This suggests the need for a more thorough examination of malignancies following neoadjuvant treatment.[9]

Research conducted in the past ten years has revealed a strong correlation between tumor regression and the body's immunity. This has been demonstrated by lymphocyte infiltration, particularly cytotoxic CD8+ T cells, which can diagnose long-term prognosis in breast cancer.[9] The distribution of lymphocytes in the intratumor and stroma, known as tumor-infiltrating lymphocytes (TIL), serves as the host adaptive immune response in breast cancer.[10] Previous studies by Denkert C et al. discovered that a high TIL value and a positive clinical outcome were present in 59.6% of patients with LABC of the triple-negative subtype. Another prospective cohort study discovered that a 10% increase in TIL was not associated with luminal HER-2 negative patients but with TNBC and HER2 positive patients'

longer disease-free survival.[11]

Data on the proportion of TIL in all subtypes of breast cancer is still scarce in Indonesia. Due to the simplicity and relatively easy procedure, TIL examination can become a routine examination in primary and secondary healthcare facilities and a basis for evaluating adjuvant therapy like immunotherapy. It is believed TIL has the potential to be an independent prognostic factor in breast cancer patients. In this study, we aimed to examine the association between stromal TIL and recurrence in locally advanced breast cancer patients.

MATERIAL AND METHOD

Study design and participants

A cohort retrospective study was conducted using 75 medical records of female breast cancer patients with LABC. Samples were taken by consecutive sampling based on inclusion and exclusion criteria. The inclusion criteria were breast cancer patients with LABC who underwent a mastectomy and additional therapy, including adjuvant chemotherapy, hormonal therapy, and radiotherapy, following the protocols at Dr. Soetomo General Hospital Surabaya. We also included the patients who received neoadjuvant therapy before having anatomic pathology examinations and paraffin preparations of their open biopsy or core biopsy samples, who had been clinically, radiologically, and pathologically proven to have recurred following mastectomy. We excluded patients with incomplete medical record data.

Data collection

We reviewed the medical records of female patients with LABC who met the inclusion and exclusion criteria of the study. We collected the name, gender, age, history of mastectomy surgery, pathological examination result, clinical features consisting of tumor size, lymph node status, histopathological type, histopathological grade, history of neoadjuvant chemotherapy, adjuvant chemotherapy, adjuvant radiotherapy, and hormonal therapy. Data on disease-free survival (DFS) period, recurrence, and confounding variables were gathered based on patient medical records. All the data were obtained from paper-based and electronic medical records. The DFS analyzed in this study was the 24-month DFS or 2-years DFS with consideration that the relapse is the highest in the first two years after initial treatment.[12]

Tumor-infiltrating lymphocytes (TIL) assessment

Tumor-infiltrating lymphocytes examined in this study were stromal TIL, e.g., leukocytes and cytoplasmic cells, excluding granulocytes and other multinucleated cells in the stroma from open histopathological biopsy or core biopsy specimens. This study did not examine the distribution of lymphocytes to cancer cells (intratumoral TIL). Department of Anatomical Pathology, Dr. Soetomo General Hospital (Surabaya, Indonesia) has reviewed the TIL following the recommendations of the International TIL Working Group 2014.

In brief, the percentage of TIL was determined using haemoxylin and eosin (HE) staining on the paraffin block from an open biopsy or core biopsy sample before neoadjuvant therapy. TIL was evaluated from the stromal compartment, a lymphocyte scattered in the stroma between carcinoma cells and not in direct contact with these cells. The TIL value is calculated as a percentage. The denominator used to determine the percentage of stromal TIL is the area of stromal tissue, which is the area occupied by mononuclear cells above the stromal area, not the number of stromal cells. The TIL value should be evaluated within the borders of the invasive tumor. In addition, we exclude the TIL outside of the tumor border, normal lobules, and tumor zones with necrosis areas and artifacts. In this study, the TIL was assessed by one expert pathologist (DF) to ensure no bias in determining TIL percentage.

Statistical analysis

The data were analyzed using the SPSS version 23.0 (IBM Corp., Armonk, NY, USA). The data are presented in frequency distribution tables and cross-tabulations. Independent and dependent variables were tested using logistic regression and the Chi-square test. Receiver operating characteristic (ROC) curve analysis was performed to calculate the optimal cut-off value for the TIL to predict the recurrence of cancer. We also calculated the likelihood of survival as a function of time using Kaplan-Meier analysis. The survival of LABC patients was measured based on the TIL percentage value.

RESULTS

Characteristics of subjects

From January 2017 to December 2021, we collected 75 samples that met the inclusion and exclusion criteria. The average age of the sample was 49.5 years old, with a median of 50.0 years old. The maximum value of the sample age is 69.0 years old, and the minimum value is 35.0 years old, with a standard deviation of 8.4 years old. The median tumor size in our study was 4.0 cm, with a mean tumor size of 4.6 cm. The largest tumor size was 10.0 cm, and the smallest tumor size was 1.0 cm, with a standard deviation of 2.2 cm. The average sample has a BMI of 25.8 and a median value of 25.6. The TIL levels of the samples obtained had an average of 21.5% with a median value of 20.0%. The highest TIL level obtained was 60.0%, and the lowest TIL level was 1.0%, with a standard deviation of 17.6%. The mean of the sample DFS was 28.2 months, with a median value of 26.0 months. Patients with the longest DFS was 66.0 months, and the shortest DFS was 9.0 months, with a standard deviation of 13.6 months.

We found that lymph node N2 status was the predominant lymph node status (26/75; 34.7%). Based on histopathology examination, the luminal B Her2-negative was the most common breast cancer subtype (32.0%), followed by luminal B Her2-positive patients (26.7%). Patients with grade II and grade III tumors were this study's predominant grade (42.7% for both grades). The characteristics of the research subjects are shown in Table 1.

The association between patients' characteristics and the DFS

We comprehensively analyzed the association between various patients' characteristics with the DFS, as shown in Table 2. Based on the age parameter, we divided the patients into two different groups: ≤ 40 years old and > 40 years old.

Table 1. The characteristics of subjects in this study.

Characteristics	*n* (%)
Age	
Average	49.5 ± 8.4
≤ 40 years	14 (18.7%)
> 40 years	61 (81.3%)
Tumor size	
Average (cm)	4.7 ± 2.2
< 2 cm	9 (12.0%)
2 – 5 cm	39 (52.0%)
> 5 cm	27 (36.0%)
Stadium	
Stadium IIIA	39 (52.0%)
Stadium IIIB	31 (41.3%)
Stadium IIIC	5 (6.7%)
Histopathology	
Ductal	66 (88.0%)
Lobular	9 (12.0%)
Lymph node metastasis	
N0	19 (25.3%)
N1	23 (30.7%)
N2	26 (34.7%)
N3	7 (9.3%)
Subtype	
Luminal A	6 (8.0%)
Luminal B Her2-negative	24 (32.0%)
Luminal B Her2-positive	20 (26.7%)
Her2 Overexpression	8 (10.7%)
TNBC	17 (22.7%)
Grade	
Grade I	11 (14.7%)
Grade II	32 (42.7%)
Grade III	32 (42.7%)
TIL	
Low TIL (<20%)	36 (48%)
High TIL (≥20%)	39 (52%)
Average	21.5% ± 17.6%
DFS	
DFS < 24 months	34 (45.3%)
DFS ≥24 months	41 (54.7%)
Average	28.2 ± 13.6

We found a total of 61 patients (81.3%) aged more than 40 years old and 14 samples (18.7%) aged less than equal to 40 years old. We found 6 patients (42.9%) with DFS of less than 24 months and 8 patients (57.1%) with DFS of more than equal to 24 months among patients aged ≤ 40 years old. Among the patients aged older than 40 years old, we found 28 patients (45.9%) with DFS of less than 24 months and 33 patients (57.1%) with DFS of more than equal to 24 months. We found no statistically significant correlation between age and DFS (p= 0.837).

We categorized the breast tumor size into three categories: less than 2 cm, between 2 and 5 cm, and greater than 5 cm. The majority of patients had a tumor between 2 and 5 cm in size, with a total of 39 patients (52.0%), followed by tumors over 5 cm in size, with a total of 27 patients (36.0%), and tumors less than 2 cm in size, with a total of 9 patients (12.0%). In patients with tumor size less than 2 cm, there were only 2 patients (22.2%) with DFS of less than 24 months, and there were 7 patients (77.8%) with DFS of ≥ 24 months. In patients with tumor size 2 - 5 cm, there were 20 patients (51.3%) with DFS of less than 24 months, and there were 19 patients (48.7%) with DFS of ≥ 24 months. In patients with tumor size greater than 5 cm, there were 12 patients (44.4%) with DFS of less than 24 months, and there were 15 patients (55.6%) with DFS of ≥ 24 months. There was no statistically significant correlation between tumor size and DFS (p= 0.286).

Stage IIIA, IIIB, and IIIC are categorized based on the characteristics of the tumor stage. In total, there were 39 patients (52.0%) who had stage IIIA, 31 patients (41.3%) who had stage IIIB, and 5 patients (6.7%) who had stage IIIC. In patients with stage IIIA, DFS was < 24 months in 19 patients (48.7%) and ≥ 24 months in 20 patients (51.3%). In the stage IIIB sample, DFS was < 24 months in 12 patients (38.7%) and ≥ 24 months in 19 patients (61.3%). Three patients (60.0%) had DFS < 24 months, and 2 patients (40.0%) had DFS ≥ 24 months in patients with stage IIIC. There was no statistically significant correlation between tumor stage and DFS in this study (p=0.559).

Based on the histopathological aspect, the tumor was classified into ductal and lobular carcinoma. Specifically, 9 patients (12.0%) of the sample had a lobular type, and 66 patients (88.0%) had a ductal type. Further, we associate histopathology and the DFS. There were 30 patients (45.5%) of patients with the ductal type who had DFS < 24 months, while 36 patients (54.5%) had DFS ≥ 24 months. In patients with lobular carcinoma, there were 4 patients (44.4%) with DFS < 24 months, and there were 5 patients (55.6%) with DFS ≥ 24 months. Histopathology was not significantly correlated with DFS (p=0.954).

We also identified the N0, N1, N2, and N3 groups of regional lymph node metastases. We identified 19 patients (25.3%) with N0 lymph node status, 23 patients with N1 status (30.7%), 26 patients with N2 status (34.7%) and 7 patients with N3 status (9.3%). In patients with N0 status, 9 patients (47.4%) had DFS < 24 months, and 10 patients (52.6%) had DFS ≥ 24 months. In the sample with N1 status, 12 patients (52.2%) had DFS < 24 months, and 11 patients (47.8%) had DFS ≥ 24 months. Most of the patients with N2 status had DFS ≥ 24 months, with a

total of 17 patients (65.4%), and only 9 patients (34.6%) had DFS < 24 months. In the sample with N3 status, 4 patients (57.1%) had DFS < 24 months, and 3 patients (42.9%) had DFS ≥ 24 months. The analysis showed that the regional lymph node metastatic status did not have a statistically significant relationship with the DFS (p=0.559).

Tumor subtype characteristics are divided into five categories: Luminal A, Luminal B Her2-negative, Luminal B Her2-positive, Her2 overexpression, and TNBC. A total of 24 patients (32.0%) had the luminal B Her2-negative subtype, and 20 (26.7%) had the luminal B Her2-positive subtype making up most of the patients. TNBC subtype was found in 17 patients (22.7%), the Her2 Overexpression subtype in 8 patients (10.7%), and the Luminal A subtype in 6 patients (8.0%). In the Luminal A patients, DFS < 24 months was found in 2 patients (33.3%), and DFS ≥ 24 months was found in 4 patients (66.7%). Thirteen patients (54.2%) of the Luminal B Her2 sample had DFS < 24 months, while 11 patients (48.8%) had DFS ≥ 24 months. Eleven patients (55.0%) of the Luminal B Her2-positive sample had DFS < 24 months, while 9 patients (45.0%) had DFS ≥ 24 months. A total of 6 patients (75.0%) in the Her2 overexpression sample had DFS < 24 months, while only 2 patients (25.0%) had DFS ≥ 24 months. In contrast, only 2 patients (11.8%) of the TNBC patients had DFS < 24 months, while 15 patients (88.2%) of the TNBC patients had DFS ≥ 24 months. We revealed a statistically significant correlation between tumor subtypes and DFS (p=0.014).

Our study classified the tumor grades into Grade I, which had a total of 11 patients (14.7%); grade II, which had a total of 32 patients (42.7%); and Grade III, which had a total of 32 patients (42.7%). Six patients (54.5%) of the grade I sample had DFS < 24 months, while 5 patients (45.5%) had DFS ≥ 24 months. In the Grade II sample, it was found that 15 patients (46.9%) had DFS < 24 months, and 17 patients (53.1%) had DFS ≥ 24 months. 13 patients (40.6%) of the Grade III sample had a DFS < 24 months, while 19 patients (59.4%) had a DFS ≥ 24 months. We found no statistically significant correlation between tumor grade and DFS (p=0.707). Based on the multivariate analysis using logistic regression, we found that the TIL value was the only significant independent risk factor for a recurrence of fewer than 24 months (p=0.001).

Table 2. The association between patients' characteristics and the 24-month DFS.

Variable	*n*	DFS		P-value
		< 24	≥ 24	
Age				
≤ 40 years old	14	6 (42.9%)	8 (57.1%)	0.837
> 40 years old	61	28 (45.9%)	33 (54.1%)	
Tumor size				
< 2 cm	9	2 (22.2%)	7 (77.8%)	
2 – 5 cm	39	20 (51.3%)	19 (48.7%)	0.286
> 5 cm	27	12 (44.4%)	15 (55.6%)	
Tumor stage				
IIIA	39	19 (48.7%)	20 (51.3%)	
IIIB	31	12 (38.7%)	19 (61.3%)	0.559
IIIC	5	3 (60.0%)	2 (40.0%)	
Histopathology				
Ductal	66	30 (45.5%)	36 (54.5%)	0.954
Lobular	9	4 (44.4%)	5 (55.6%)	
Lymph node metastasis				
N0	19	9 (47.4%)	10 (52.6%)	
N1	23	12 (52.2%)	11 (47.8%)	0.559
N2	26	9 (34.6%)	17 (65.4%)	
N3	7	4 (57.1%)	3 (42.9%)	
Subtype				
Luminal A	6	2 (33.3%)	4 (66.7%)	
Luminal B Her2-negative	24	13 (54.2%)	11 (45.8%)	
Luminal B Her2-positive	20	11 (55.0%)	9 (45.0%)	0.014
Her2 Overexpression	8	6 (75.0%)	2 (25.0%)	
TNBC	17	2 (11.8%)	15 (88.2%)	
Grade				
Grade I	11	6 (54.5%)	5 (45.5%)	
Grade II	32	15 (46.9%)	17 (53.1%)	0.707
Grade III	32	13 (40.6%)	19 (59.4%)	
TIL*				
High TIL	39	6 (15.4%)	33 (84.6%)	0.001
Low TIL	36	28 (77.8%)	8 (22.2%)	

*The determination of high and low TIL in this analysis was using the cut-off 20% reported in the previous study.[13,14]

The cut-off value of TIL to determine the DFS

We analyzed the receiver operating characteristic (ROC) curve to calculate the optimal cut-off from the TIL percentage value to determine the 24-month DFS. Based on the analysis result, we examined two cut-off values, as seen in Table 3. First, the cut-off value used in a previous study and the optimal cut-off value calculated in this study. The previous study utilized a cut-off value of 20%. By using the cut-off value of 20%, we divided the TIL values of the

subjects into high TIL and low TIL groups. We found that 36 patients (48.0%) had low TIL values, and 39 (52.0%) had high TIL values. We also performed a ROC analysis on the data to determine this study's ideal TIL cut-off value. We discovered that 15% was this study's ideal TIL cut-off number. Using this cut-off value, we found that 36 patients (48.0%) had a low TIL value and 39 patients (52.0%) had a high TIL value. We noticed that using two different cut-off values; we had the same number of patients classified into high TIL and low TIL groups. The sensitivity, specificity, PPV, NPV, and accuracy of TIL to predict the 24-month DFS was 80.5%, 82.4%, 84.6%, 77.8%, and 81.3%, respectively. In addition, we also examined the association between the sample's proportion of TIL and DFS. We found a significant association between TIL value and DFS (p=0.001). High TIL was also a protective factor against recurrence in the first two years based on the univariate analysis with an OR of 0.052.

To evaluate TIL's potential to estimate patients' DFS, this study also used regression analysis. According to the regression analysis, every 10% increase in TIL percentage could raise the DFS by 5.45 months (p=0.001). Based on the Kaplan-Meier curve, as shown in Figure 2, we found that patients with low and high TIL values had different disease-free survival periods. Patients with high TIL value (red line in Figure 2) had higher survival than those with low TIL value (blue line in Figure 2).

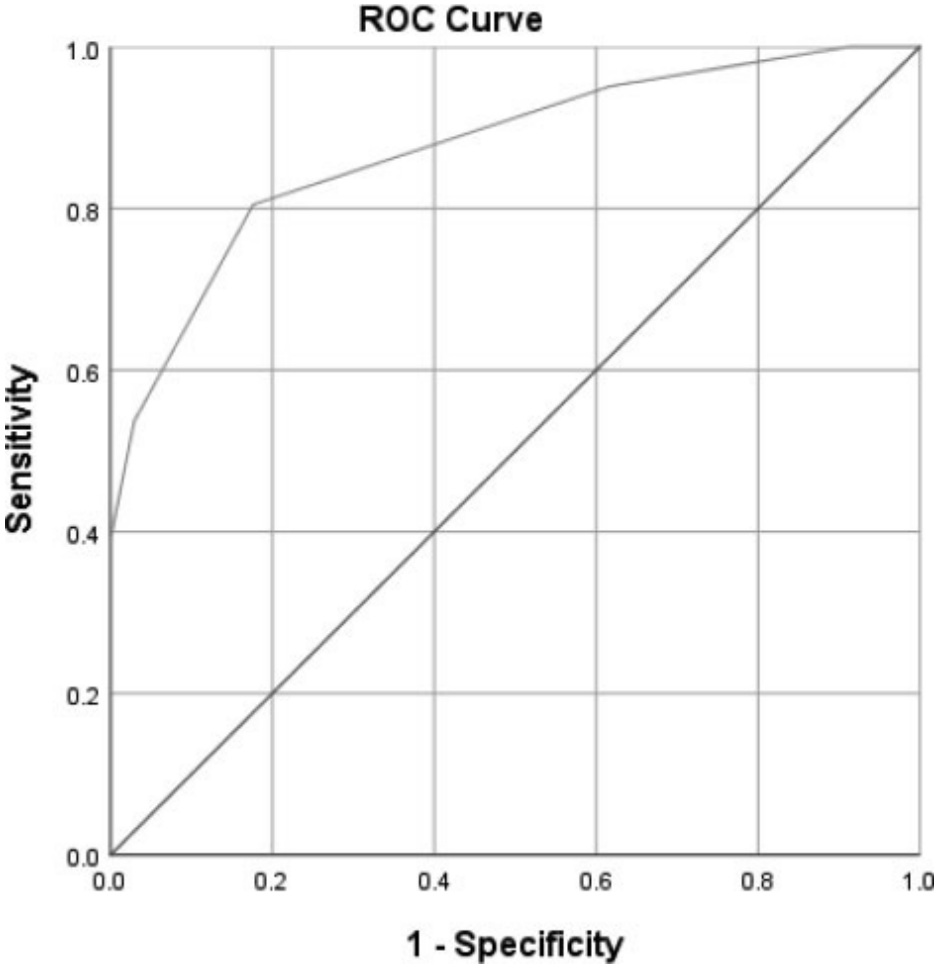

Figure 1. The ROC analysis to determine the optimal cut-off value in this study.

DISCUSSION

Breast cancer recurrence, whether local, regional, or distant, is the main cause of death and tends to have an unfavorable prognosis. It is crucial when choosing additional therapy for breast cancer patients after mastectomy. The prognosis of breast cancer recurrence is affected by the patient's age, tumor size, lymph node status, histopathological grade, hormonal state, and cancer's biomolecular characteristics. The immune system also plays a crucial part in preserving tissue homeostasis by conducting ongoing immunosurveillance and eliciting inflammatory responses that activate innate and adaptive immune cells. Recent data suggest that tumor immunogenicity affects breast cancer biology and treatment response. Immune cells known as tumor-infiltrating lymphocytes (TIL) have been investigated in several solid tumors, including breast cancer. TIL can

Table 3. The cut-off value of TIL and DFS.

TIL	DFS		Total	p-value	OR	95% CI
	< 24 months	≥ 24 months				
Cut-off 20%*						
High	6 (15.4%)	33 (84.6%)	39	0.001	0.052	0.016-0.168
Low	28 (77.8%)	8 (22.2%)	36		1	
Cut-off 15%**						
High	6 (15.4%)	33 (84.6%)	39	0.001	0.052	0.016-0.168
Low	28 (77.8%)	8 (22.2%)	36		1	

* The determination of high and low TIL was using the cut-off 20% reported in the previous study.[13,14]
** The determination of high and low TIL was using the cut-off 15% found in this study using ROC analysis

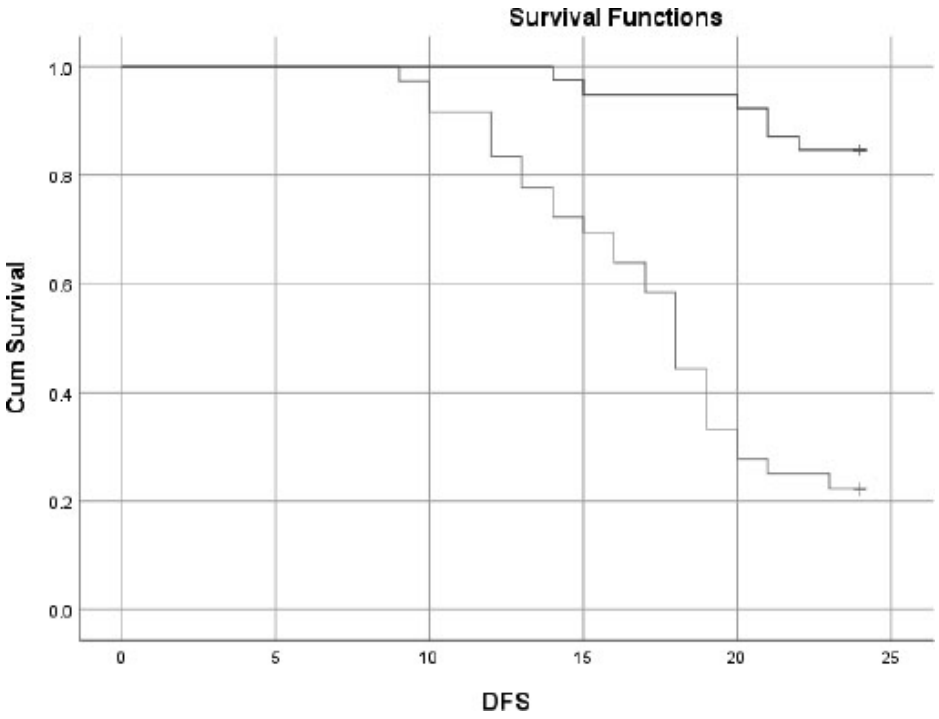

Figure 2. The survival analysis using the Kaplan-Meier curve.

be semi-quantitatively measured using hematoxylin and eosin (H&E) staining and comprises cytotoxic T cells, helper T cells, B cells, macrophages, NK cells, and dendritic cells.[15]

Lymphocytes known as stromal TIL are scattered throughout the stroma between cancer cells but are not in direct touch with them. The development of more advanced examination methods that reveal more details about lymphocyte subpopulations, such as flow cytometry, immunohistochemical staining, gene expression microarrays, and PCR, has also been made possible. However, these methods are considered impractical, expensive, and require tissue processing.[16]

The majority of the samples are older than 40 years old. The analysis demonstrated that the sample age and DFS had no statistically significant association. However, a previous study revealed a substantial connection between breast cancer patients' DFS and advancing age.[17] Only 18.7% of our sample's population is under 40 years old, which may be the cause of this difference.

The majority of the samples in this study had tumors that were larger than 2 cm. The analysis demonstrated no statistically significant correlation between tumor size and DFS. However, the previous study does not support this. The prognosis and DFS of breast cancer patients were found to be significantly correlated with tumor size in a study by Kasangian AA et al., particularly in individuals with the Luminal A subtype.[18] The unequal distribution of tumor sizes in our study sample may be the main reason for this different finding, where only 12% of the sample in our study had tumors larger than 2 cm.

Stages IIIA, IIIB, and IIIC were used to categorize tumor stages in this study. This study's analysis showed no significant correlation between tumor stage and DFS. This contradicts previous research that found a correlation between advancing tumor stage and a worsening of patient outcomes. Breast cancer patients' overall survival (OS) and disease-free survival (DFS) decreased as tumor stage increased.[19]

Ductal and lobular carcinoma were distinguished based on their histological characteristics. Invasive lobular carcinoma (ILC) and invasive ductal carcinoma are two major histological types of breast cancer. ILCs accounted for 10-15% of all breast cancers, and compared to IDC, ILCs are difficult to detect with standard imaging modalities such as mammography. In general, ILC is commonly detected in older advanced-stage patients. In addition, ILC patients have relatively late relapses and poor long-term survival. In this study, we found that the histology type of the tumor and DFS are not significantly correlated. Our result is consistent with the previous study, which revealed that patients with lobular and ductal histological characteristics had identical DFS rates.[20]

This study divided the metastatic lymph node status into N0, N1, N2, and N3. We found no statistically significant correlation between improved lymph node status and sample DFS. In previous studies, there were contradictory results where lymph node metastasis was a strong prognostic factor in breast cancer patients. In breast cancer patients, the number of positive lymph nodes is linked to a reduction in DFS.[21] The existence of other factors that affect recurrence may be the cause of these different results. Most of the study samples had tumors of grades II and III. Our findings indicated no statistically significant correlation between tumor grade and DFS. A different study also discovered that tumor grade did not significantly affect DFS or OS.[19] Tumor subtypes and DFS were found to be statistically significantly correlated. Our result was in line with a study conducted in China which revealed a substantial association between various tumor subtypes and DFS.[22]

Researchers and clinicians have not yet agreed on a standard for the proportion of stromal TIL in breast cancer. In this study, anatomical pathologists performed the stromal TIL examination using the recommendations of the International TIL Working Group for Breast Cancer.[16] According to studies by Kashiwagi S et al., the values of high and low TIL are as follows: low <10%, moderate 10-50%, and high >50%.[13] Another study by Nasrudin AH et al. used a cut-off 20% to differentiate high TIL and low TIL levels.[14] The optimal cut-off point we found based on the ROC analysis was 15%. Based on the median value of the TIL data, the cut-off value was 20%. The performance of TIL to predict the 24-month DFS by using the cut-off of 15% and 20% was identical.

In this study, we obtained data on the percentage of stromal TIL and its relation

to disease-free survival. Our results showed that many patients with low TIL had a higher prevalence of recurrence within 2 years compared to patients with high TIL. Patients with a high stromal TIL experienced the most recurrence after 2 years compared to patients with a low stromal TIL presentation. This is inconsistent with earlier studies by Loi S et al., who found a strong positive linear correlation between rising stromal TIL and a longer DFS. TIL is one of the body's adaptive defenses that helps the body to get rid of cells that have turned cancerous. The ability of CD8+ and CD4+ T-cells to cytolyze tumor cells induces CTL to produce granule exocytosis for cell apoptosis. Both CD8+ and CD4+ have potent anticancer properties to suppress carcinogenesis. Therefore, it is reasonable to conclude that TIL value could be used as an immunity parameter to predict the patients' outcome, especially the recurrence of LABC.[23]

Even though the rate has decreased, the recurrence in patients with high TIL value might still happen after more than 2 years. This is supported by several theories which state that cancer cells might evade the body's immune system by concealing their antigens and decreasing antigen presentation so that T cells are unaware of their presence. Additionally, cancer cells can protect themselves against T lymphocytes by using the FasL/Fas (PDL-1/PD1) pathway. By expressing the Fas receptor (PD-1), activated CD8+ T-cells in the tumor microenvironment also cause counter-immune reactions. The idea is that cancer cells can prevent these lymphocytes from entering tumors by overexpressing FasL (PDL-1) on their surface.[22,23]

Additionally, it is thought that the production of FasL results in "exhausted" lymphocytes and is linked to the apoptosis of tumor-infiltrating lymphocytes in vivo. There is currently no information about the onset of PDL production by cancer cells. Therefore, the stromal TIL presentation may be used as a determinant of when cancer cells will produce PDL.[24]

In this study, our data showed that every 10% rise in TIL could extend disease-free survival by 5.45 months, with the TNBC subtype, in particular, having a better prognostic value than other breast cancer subtypes. According to another study, patients with a high TIL percentage had a better outcome even if they did not achieve a complete pathological response after neoadjuvant chemotherapy. In contrast, those with a low TIL percentage had a poor disease-free interval, especially in the first three years (67%).[16] According to Dieci MV et al. studies, TIL in residual tumors has a strong predictive value for metastases-free survival and 5-year overall survival, especially in the TNBC subtype. The human epidermal growth factor receptor-2 (HER2) and the hormone receptor are not expressed in triple-negative breast cancer (TNBC), which has a worse prognosis than other subtypes and is more likely to return in the first three years.[25]

By obtaining a significant relationship between the percentage of stromal TIL and recurrence, pre-therapy stromal TIL can be used as a prognostic biomarker and has advantages in the implications of developing therapeutic modalities, such as immunotherapy, especially in TNBC and HER2-overexpression subtypes which have a worse prognosis and are more aggressive. Immune checkpoints such as the CD28/CTLA4 and PD-1/PD-L1 pathways have been the focus of recent developments in immunotherapy. The most effective immunotherapy that can broadly engage the immune system to focus antigen-specific T-cell-mediated and anticancer immune responses is immune checkpoint inhibitor therapy, such as anti-PD1/PD-L1, anti-CTLA-4.[26,27]

The need to assess stromal TIL presentation during neoadjuvant chemotherapy in LABC and if it has a better predictive value than pre-therapy TIL is another point of discussion. It is well established that most chemotherapy's antitumor effects come from immune system regulation. The assessment of stromal TIL in residual tumors following neoadjuvant chemotherapy is distinct from pre-therapy TIL because it can represent the immune microenvironment response to chemotherapy.[28]

This study has several limitations. First, this study was carried out in a single general academic hospital. As a result, the conclusions of this study may not be sufficient to characterize the situation in Indonesia. However, the findings in this study might be important to provide preliminary data regarding TIL in breast cancer patients in Indonesia. Second, the number of participants in this study is relatively small. Therefore, further research with larger study participants or a nationwide multi-center study may be required to provide more detailed results.

CONCLUSION

There was a significant correlation between the percentage of stromal TIL and the recurrence rate in patients with LABC. Therefore, the pre-therapy stromal TIL percentage can be employed as a biomarker to predict breast cancer recurrence, particularly in the first two years.

CONFLICTS OF INTEREST

No competing interests were declared.

ETHICAL CLEARANCE

This study was reviewed and approved by the Medical Ethical Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (Ref. No.: 0890/LOE/301.4.2/IV/2022), following the guidelines of the Declaration of Helsinki.

AUTHOR CONTRIBUTION

Conceived the study: DN. Designed the study: DN, DHS, and DGAS. Analyzed the data: DN, DHS, DF, PAW, and DGAS. Wrote the manuscript: DN and DGAS. Review the manuscript: DHS and DGAS.

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