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# Relationship between Tumor Infiltrating Lymphocytes and Neoadjuvant Chemoterapy Responsiveness: A Cross-Sectional Study on Breast Cancer Patients

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**ABSTRACT**

Breast cancer is still a significant health problem in the world, with chemotherapy as an essential component in its management. Limited research on tumor- infiltrating lymphocytes (TIL) as a biomarker for the effectiveness of breast cancer treatment was conducted and is still contradictory. This study aims to investigate the relationship between TILs and the clinical response to neoadjuvant chemotherapy in breast cancer. The research used a cross-sectional study on eligible breast cancer patients in Wahidin Sudirohusodo Hospital in Makassar. TIL levels were grouped based on the histopathological examination results of breast tissue samples into three categories: low (0% to 10%), medium 15% to 50% and high (55% to 100%). Responsiveness was assessed based on changes in tumor size after neoadjuvant chemotherapy. The Chi-Square test is the primary analysis in this study. Results of 40 participants, the sample had a median age of 40 (27-73 years), mainly in the clinical stage III (40.0%), had moderate TIL concentration (47.5%), and was responsive to the neoadjuvant chemotherapy (87.5%). There was no significant association between TIL concentration and chemotherapy responsiveness ($p > 0.05$). However, in post-chemotherapy conditions, the median value of tumor size in the group with the high TIL category was significantly lower than in the low and medium TIL groups ($p=0.034$). There was no association between TIL levels and the clinical response to neoadjuvant chemotherapy in breast cancer patients. Further research is needed to confirm these findings.

**KEYWORDS:** Breast Neoplasms, Cross-Sectional Studies, Neoadjuvant Therapy, Lymphocytes, Tumor-Infiltrating

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## INTRODUCTION

The ductus epithelium (85%) and lobules (15%) in the glandular tissue of the breast are the primary sites of origin for breast cancer (BC). Based on statistics from GLOBOCAN 2020, breast cancer is the most common cancer globally in all genders and in women. An estimated 2.3 million new instances (or 11.7% of all new cancer cases) are reported annually, with 685,000 patients dying from the disease (6.9%). BC accounts for 24.5% of all new instances of cancer in women and has a 15.5% mortality rate. 7.8 million Women were still alive at the end of 2020 and had had a BC diagnosis for longer than five years. The most prevalent type of cancer worldwide is BC (1). Breast cancer is the most frequent type of cancer in Indonesia, accounting for 16.6% of new cases across all genders and 38.6% of cases in women. There were 396,914 new cases in 2020. Before the age of 75, the female population has a 14.9% chance of acquiring breast cancer (2).

Chemotherapy is currently an integral part of the paradigm for treating BC.[3] The most common application of anthracycline-based chemotherapy is in the neoadjuvant

and adjuvant stages of treatment for breast cancer (4). The findings of a meta-analysis of many randomized trials, which demonstrated an increase in overall survival (OS) and disease-free survival (DFS) from the use of anthracycline chemotherapy compared with non-anthracycline, provide support to this. The combination of anthracycline with Taxane and Cyclophosphamide (TAC) is quite widely used, with efficacy reaching 78% (5). However, anthracycline-based chemotherapy also has limited effectiveness in BC due to drug resistance (6). Chemoresistance is a problem that hinders the effectiveness of chemotherapy (7).

For cancer patients, a positive prognosis is associated with tumor-infiltrating lymphocytes (TIL). All lymphocyte cell populations that have infiltrated tumor tissue are considered TILs. Several solid tumors, including BC, have been reported to include TILs. Cytotoxic (CD8+) and helper (CD4+) T cells make up the majority of TILs in BC, with a little amount of B- and NK cells. T follicular helper (Tfh) cells, which are essential for the development of lymphocytes, are also present in tertiary lymphoid structures, which are correlated with high TIL counts. TIL has been shown in earlier research to be a significant biomarker for predicting therapy efficacy and outcome (8). TIL clearly indicates the response to neoadjuvant treatment for slightly advanced local BC (9). Conversely, in patients with locally advanced BC undergoing neoadjuvant chemotherapy, TIL wasn't related to a pathological response (10).

A limited study has been done in BC to determine the association between TIL and theneoadjuvant chemotherapy response. With neoadjuvant chemotherapy, this study investigated the association between TIL and clinical response in BC.

## MATERIAL AND METHODS

This study used a cross-sectional design on all BC patients at Dr. Wahidin Sudirohusodo Hospitals from 1st April 2023 – 30th September 2023. Patient tissue samples that meet the criteria are examined using the Hematoxilin Eosin staining method in an accredited laboratory. All research designs have been reviewed and approved by the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University (No. 567/UN4.6.4.5.31/PP36/2023) on July 28, 2023.

The number of samples was calculated based on the cross-sectional study design calculation formula with the α 5%, β 80%, P1 0.6, and P2 0.25. The minimum samples required is 40. Patients with a diagnosis of BC based on histopathological examination results, willing to participate, and had treated with neoadjuvant chemotherapy were included in this study. Meanwhile, samples with other types of cancer and did not accomplish the chemotherapy requirements will be excluded from this study. In addition, data on medical records in the form of age, clinical stage, and tumor size of the patients were also collected.

BC in this study is defined as malignancy in breast tissue which can originate from the ductal epithelium or lobules.. The American Joint Committee on Cancer (AJCC) classified tumor size (T), the presence or absence of distant metastases (M), and regional lymph node metastases (N) in their 2010 classification, which was followed in this study's BC staging. The phases are divided into four groups: I, II, III, and IV. TIL are defined as mononuclear cells found in tumor tissue that show an immune response against tumor cells. TIL density was measured in the tumor mass's stromal compartment and expressed as a percentage of the stromal area, rounded to the closest five percent (11).

Neoadjuvant chemotherapy referred to in this study is any type of chemotherapy given before surgery. The response to chemotherapy is divided into two, namely non- responsive and responsive. Non-responsiveness was determined if the tumor size decreased <30% unidimensionally, the tumor size remained the same, the tumor size increased or a new tumor was found. The terms stable disease and progressive disease can be included in this category. Furthermore, responsiveness is determined if the tumor mass disappears, or at least there is a reduction in tumor size of > 30% unidimensionally and no new tumors are found. The terms partial response and complete response can be included in this category (12).

The patient's surgical/tissue biopsy material was taken in a sterile condition and then put into a bottle containing 10% formalin buffer solution to be sent to the Accredited Anatomical Pathology Laboratory. Next, paraffin blocks were made according to the standard method, followed by cutting the tissue with a thickness of 4 μm and placing it on a glass slide.

Hemactocillin Eosin staining averages throughout slides—not only hotspots—are used to calculate scores. The assessment was limited to TILs located within the invasive tumour margin, disregarding inflammation and dysplastic and in situ locations, including growth confined to the lamina propria. While other inflammatory cells, such as neutrophils and granulocytes, were eliminated, all mononuclear cells, such as lymphocytes and plasma cells, were included as TILs. TILs within nests of epithelial cells were removed from assessment, and only stromal TILs were evaluated, excluding areas of necrosis, including the central "gross necrosis" characteristic of BC. Three categories are used to group the percentage of TIL scores: low (0% to 10%), medium (15% to 50%), and high (55% to 100%).[11] Interpretation of TIL scores is carried out by a pathologist blindly without knowing the status of the patient's response to chemotherapy.

The SPSS version 20.0 software (IBM, USA) will be used to analyze the data in accordance with the measurement scale and research objectives. Numbers and percentages are used to express data on a categorical scale, while mean ± standard deviation or median (min–max) are used to convey data on a numerical scale. The Paired T, or Wilcoxon signed ranked

test, was used to compare the tumor size before and after chemotherapy. The Spearman rank correlation test and Chi-square test were used to examine the relationship between TIL and chemotherapeutic response. Furthermore, the Kruskal-Wallis test was used to examine the relationship between TIL and tumor growth both before and after chemotherapy. If the p-value is less than 0.05, the test findings are considered significant.

## RESULTS

Forty people with BC participated in this study. Table 1 provides a summary of the participants' clinical and demographic data. With the youngest age range being 27 years old and the oldest being 73 years old, the sample's median age is 40 years old. The. sample has a mean age of 50. The majority of samples were in stage III breast cancer. In general, TIL levels in patients are dominated by the medium category, followed by the high and low categories. The response to chemotherapy is generally in the responsive category. Table 2 displays the findings of the examination of variations in tumor size before and after chemotherapy. The median tumor size after chemotherapy is substantially smaller than the median tumor size prior to chemotherapy (p=0.000). This shows an improvement in tumor size after chemotherapy given to the patient. The analysis of the relationship between TIL levels and response to chemotherapy can be seen in Table 3. The majority of samples were in the category of responsiveness to chemotherapy, both in groups with low, medium and high TIL levels. This was confirmed by the statistical analysis carried out which found no significant relationship between TIL levels and chemotherapy response (p=0.596). Even though a positive correlation was found, the relationship between these two variables was not significant (r=0.156, p=0.596). The analysis of the relationship between TIL levels and tumor size can be seen in Table 4. In the pre-chemotherapy condition, the median value of tumor size in groups with low, medium and high TIL categories was comparable (p= 0.502). On the other hand, in the post-chemotherapy state, the high TIL group's median tumor size value was substantially smaller than the low and medium TIL groups' median tumor size values (p=0.034).

**Table 1. Characteristic Respondents**

| Characteristics | n (%) |
|---|---|
| Age (Median (Min – Max) | 49 (27-73) years |
| Clincal Stage | |
| Stage II | 4 (10.0) |
| Stage III | 36 (90.0) |
| TIL Level | |
| Low | 9 (22.5) |
| Moderate | 19 (47.5) |
| High | 12 (30.0) |
| Chemotherapy Responsiveness | |
| Non-responsive | 5 (12.5) |
| Responsive | 35 (87.5) |

**Table 2. Comparison of the tumor size between pre and post chemotherapy**

| Pre (cm) | Post (cm) | p-value |
|---|---|---|
| 11.9 (3-27) | 7.0 (1-24) | 0.000 |

## DISCUSSION

The present study found that the ages of BC patients varied quite widely, with the youngest being 27 years old and the oldest being 73 years old. This is not much different from data on people living with breast cancer who received treatment at Wahidin Sudirohusodo Hospital in Makasar in 2005-2009, with a peak frequency aged 40-49 years (13).

This study also found that the majority of samples were in the stage III category. This is in line with data at Cipto Mangunkusumo Hospital in 2014, which showed that the majority of BC sufferers sought treatment for the first time when they were in advanced and advanced local stages (stage III/IV), as much as 82.4% (14). Several factors contribute to delayed medical treatment. The patients still prioritizing alternative treatments tend to provide opportunities for cancer to grow and develop, which ultimately causes severe pain and even death in people living with cancer. Another cause is the continued development of myths and wrong education in society. These myths and wrong education make people with cancer afraid to seek medical treatment as the mainstay of treatment for their disease (15).

This study found that the majority of TIL levels were in the medium category. Solid tumors generally consist of a variety of immune cells, including T and B lymphocytes, NK cells, macrophages, and neutrophils. Adaptive immunity and immunosuppression always show different expression levels

in tumors, and many immune genes are related to inflammation. Interactions between immune cells determine the induction or inhibition of tumor development, angiogenesis, and metastasis. Low lymphocytes are associated with poor outcomes (16). The subsets of T lymphocytes necessary to mediate tumor rejection differ. CD8+ T cells are the subset with a significant role. Tcs that can induce tumor-killing are immediately recognized by peptide antigens, which are presented by MHC class I tumor molecules. The majority of tumors are positive for MHC class I but negative for MHC class II (17).

The majority of samples in this study had a positive response to chemotherapy. This is consistent with earlier studies conducted on patients with breast cancer at Dr. Mohammad Hoesin Hospital in Palembang, which yielded positive responses of 70.6% and negative responses of 29.4%. The group that had the highest success rate, with positive responses of 75% (9 of 12 respondents), used combination-based regimens (TAC), followed by Taxane-based regimens (TC) of 71.4% (10).It can be seen from this that most patients who had neoadjuvant chemotherapy responded by having their tumor size decrease.

There was a lack of association between TIL levels and treatment response in the current study. Previous research has not been able to demonstrate any significant relationship between TILs and pathological response in patients with locally advanced breast cancer after neoadjuvant chemotherapy. The appraisal of TIL can be influenced by a number of factors, including the smear collection procedure and the pathologist's assessment of the data. But histopathological grading, hormone receptors, HER-2 expression, and the histological kind of breast cancer also contribute to the difference bias (10). TIL that is high but does not respond to chemotherapy can occur because there is no sample uniformity. In contrast, in several studies, good responses occurred mostly in the HER-2 and TNBC subtypes. Apart from that, chemotherapy drugs can work in two mechanisms, namely cytotoxic and immunotherapeutic. Another factor that influences the immune system is known as immune surveillance, where immunity to a tumor can occur through the elimination, equilibrium, and escape phases. In this escape phase, the tumor avoids the immune system so that tumor cell growth continues because tumor cells are considered normal cells by the immune system (18).

This study also found that tumor size was relatively smaller in breast cancer patients who had undergone chemotherapy and had high TIL levels. Lymphocytes are associated with their cytotoxic function. A good response is if an abundance of lymphocytes infiltrate the tumor cells. A study on high peripheral serum CD8 measurements had better survival, and lymphocyte counting showed better outcomes for high lymphocytes (16). In research conducted by Zgura et al., it was said that TILs are predictive and potential prognostic markers in BC. When relatively locally advanced breast cancer is treated with neoadjuvant chemotherapy, any TIL is a clear predictor of the response (9). Meta-analysis conducted by Wang et al. also reported that high TIL can predict a better response to chemotherapy given to breast cancer patients (19).

Not carrying out a more complex multivariate analysis is the main limitation of this study. Several factors may contribute to the chemotherapy responsiveness of breast cancer, such as histological type, histopathological grading, hormone receptors, and HER-2 expression. Joint analysis with these variables can provide a more valid relationship between TIL levels and responsiveness to neoadjuvant chemotherapy.

## CONCLUSION

In summary, our investigation revealed no relationship between TIL levels and the clinical response of neoadjuvant chemotherapy in BC. However, elevated TIL levels following chemotherapy often result in smaller tumors. Future studies with more variables to be examined are required to understand TIL better and the response to neoadjuvant chemotherapy in breast cancer patients.

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## CONFLICT OF INTEREST

All author declare no conflict of interest.

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