Clinicopathology in Early Triple-Negative Breast Cancer in H. Adam Malik Hospital Medan

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Abstract

Introduction: Triple-negative breast cancer is defined as the absence of estrogen, progesterone, and HER-2 receptors obtained by immunohistochemistry, with an incidence of 24% of newly diagnosed breast cancers. Several breast tumours have substantial lymphocytic infiltration, and tumour-infiltrating lymphocytes (TILs) are the potential surrogate markers of adaptive immune response and are positively associated with better survival in TNBC. This study examines the relationship between tumour infiltrating lymphocytes and clinicopathology in early-stage triple-negative breast cancer at HAM Hospital.

Methods: This research is an observational analytic study with a case series design where data collection was taken from secondary data using medical records. Inclusion criteria.

Results: The most common patient age group was between 31-40 years (52.7%), the most common tumour size was in the T2 category (63%), the most common nodule category was in the N1 category (35.6%), and Grade III (58.9%). In patients with early-stage triple-negative breast cancer, tumour-infiltrating lymphocytes in the moderate infiltration category (30-60%) were most commonly found (43.2%). A significant association between age, grade, and nodules for TIL was found with $p < 0.001$.

Conclusion: A significant association was found between tumour infiltrating lymphocytes and age, grade and lymph node nodules in early-stage triple-negative breast cancer.

Keywords: tumour-infiltrating lymphocytes, triple-negative breast cancer, early stage.

I. BACKGROUND

Triple-negative breast cancer lacks estrogen, progesterone, and HER-2 receptors obtained from immunohistochemistry. This type is often considered a subtype with aggressive tumour biology with a high recurrence rate and death rate, representing 24% of newly diagnosed breast cancer, and a steady increase has been reported in its incidence. In 2018 around 2,088,849 cases of TNBC were reported, making it common cancer in women. TNBC data in Indonesia is rarely available, but it is said to occur in 25% of all cases in clinicopathological correlation studies of breast cancer subtypes and mostly occurs in medium to high-grade tumours. TNBC in Indonesia is also reported to be associated with a relatively high risk of death. TNBC survival study in the Indonesian population reported on locally advanced TNBC with disease-free survival at five years of 11.4%(1,2).

A previous study conducted in Medan by Hermansyah et al. in 2021 showed that out of 79 patients, the average age at clinical presentation was the 41-50 year group with 35 samples (44.3%). The largest tumour size was in T2 in 51 patients; 100% were invasive ductal carcinoma, of which 83.6% were grade 3. Angioinvasion was found in 60.8%, 60.8% were lymphatic invasion, and lymph node metastases were found in 55.7 % of cases (3).

Some breast tumours have substantial lymphocytic infiltration, and tumour-infiltrating lymphocytes (TILs) have recently been proposed as a surrogate marker of the adaptive immune response. Immune system interactions with tumour cells in breast cancer appear to be associated with triple-negative breast cancer and HER2-positive breast cancer. They are thought to be more immunogenic than luminal A carcinomas (4). Recent studies have revealed tumour infiltrating lymphocytes (TILs) to be a promising predictive biomarker for response to therapy, especially in TNBC. Tumour infiltrating lymphocytes are cytotoxic lymphocytes that infiltrate the tumour and stromal areas in response to the host's immune response. Greater lymphocyte infiltration rates, particularly in the tumour stroma, enhance

the anti-tumour effect of the therapy(5).

TIL has been reported to be positively associated with better survival, particularly in TNBC. Previous studies have shown that 30% to 50% of all breast cancers have increased programmed cell death ligand one receptor (PD-L1) on the surface of tumour cells. Previous research by Hermansyah et al. in 2021 showed that TIL had a moderate and high risk of 73.4%. TIL increased tumour proliferation associated with high histological grade, high Ki67, larger tumour size, and lymph node metastases. Some investigators found TIL to be at high TNBC risk(3).

Another study found that 69.41% of TNBC patients with low TIL scores (<10%). About 20% of TNBC express strong TIL, which means more than 50% lymphocytic infiltrate. Several studies have shown that a greater amount of TIL in the tumour stroma is associated with a higher chance of cure in early-stage TNBC and a better chance of pCR after receiving neoadjuvant chemotherapy (NACT). Moreover, a high TIL in residual disease after NACT appears to be associated with a better outcome than expected(6). It is important to identify prognostic factors and markers to select high and low-risk subsets of TNBC patients for a better therapeutic approach because TNBC subtypes may respond differently to certain agents. Based on the above background, researchers wanted to see the profile of tumour infiltrating lymphocytes in triple-negative breast cancer.

II. METHOD

This type of research is observational analytic with a case series design. Data collection is taken from secondary data using medical records to see the correlation between tumour infiltrating lymphocytes and clinicopathology in early-stage triple-negative breast cancer at H. Adam Malik General Hospital. This research was conducted with the approval of the USU FK Health Ethics Commission. Research and data collection will be carried out starting from March 2023, followed by processing and analysis of the data that has been collected.

The population in this study were all TNBC patients at HAM Hospital who received treatment between 1 January 2018 – 1 March 2023. The sample for this study was part of the population that met the inclusion and exclusion criteria. The planned sampling method was non-probability sampling, namely consecutive sampling, where patients were taken sequentially according to the inclusion and exclusion criteria until the sample size was met. However, because all available samples met the inclusion and exclusion criteria, in the end, this study used a total sampling technique. The inclusion criteria of this study were all patients diagnosed with early-stage triple-negative breast cancer, which had been confirmed histopathologically and immunohistochemically and had complete research data in the medical record.

The calculation results show that the minimum sample size required is 134 people. However, the number of patients who meet the criteria in this study in the time interval 1 January 2018-1 March 2023 is 146 people, so all of them are included and analyzed. All data will be analyzed descriptively using statistical software, presented in percentage form, and displayed in a frequency distribution table. The data will be tested for normality using the Kolmogorov-Smirnof test and interpreted as normal data if $p>0.05$. Bivariate analysis with a numerical-categorical variable measuring scale will use the One-way Anova test and the Kruskal-Wallis test as an alternative if the data is not normally distributed.

III. RESULTS

This research is an observational analytic study with a case series design. Data collection is taken from secondary data using medical records to seek the association between tumour infiltrating lymphocytes and clinicopathology in early-stage triple-negative breast cancer at HAM General Hospital. Research and data were collected from TNBC patients at HAM Hospital who received treatment between 1 January 2018 – 1 March 2023; as many as 146 samples met the inclusion criteria.

Table 1. Frequency Distribution of Research Respondents (n=146)

Characteristics		
Age (n, %)		
<30 years	20	13.7%
31-40 years	77	52.7%
41-50 years	34	23.3%
51-60 years	15	10.3%
>60 years	0	0%
Gender (n, %)		
Woman	146	100%
Man	0	0%
Tumour size (n, %)		
T1	7	4.8%
T2	92	63.0%
T3	47	32.2%
Nodules (n, %)		
N0	49	33.6%

N1	52	35.6%
N2	45	30.8%
Grades (n, %)		
Grade I	19	13.0%
Grade II	41	28.1%
Grade III	86	58.9%

Table 1 shows the age group of patients most commonly found between 31-40 years with 77 patients (52.7%), then 41-50 years with 34 patients (23.3%), under 30 years with 20 patients (13.7%) and 51-60 years as many as 15 patients (10.3%). A total of 146 samples were female (100%). The most common tumour size was in the T2 category in 92 patients (63%), T3 in 47 patients (32.2%) and T1 was found in 7 patients (4.8%), and there were no patients with T4. The most common nodule category was the N1 category in 52 patients (35.6%), then N0 in 49 patients (33.6%), and N2 in 45 patients (30.8%). Grade III was the most common in 86 patients (58.9%), then grade II in 41 patients (28.1%) and grade I in 19 patients (13%).

Table 2. Frequency Distribution of Infiltrating Lymphocytes Tumors in Early Stage Triple Negative Breast Cancer (n=146)

***Tumour-infiltrating lymphocytes*(TIL)**	**Frequency**	
	n	**Percentage (%)**
Mild infiltration (<30%)	31	21.2%
Moderate infiltration (30 - 60%)	63	43.2%
Severe infiltration (>60%)	52	35.6%

Table 2 shows that in patients with triple-negative breast cancer, Tumor Infiltrating Lymphocytes in the moderate infiltration category (30-60%) were most often found in 63 patients (43.2%). The category of severe infiltration (> 60%) was found in 52 patients (35.6%), and mild infiltration (<30%) was seen in 31 patients (21.2%).

Table 3. Frequency Distribution of Infiltrating Lymphocytes Tumors with Clinicopathology in Early Stage Triple Negative Breast Cancer (n=146)

Variable	**Tumour Infiltrating Lymphocytes (TIL)**			**p-value**
	Mild infiltration (<30%) (n, %)	**Moderate infiltration (30-60%) (n, %)**	**Severe infiltration (>60%) (n, %)**	
Age				<0.001*
<30 years	2 (6.5%)	6 (9.5%)	12 (23.1%)	
31-40 years	8 (25.8%)	41 (65.1%)	28 (53.8%)	
41-50 years	9 (29%)	13 (20.6%)	12 (23.1%)	
51-60 years	12 (38.7%)	3 (4.8%)	0 (0%)	
Grade				<0.001*
Grade I	10 (32.3%)	4 (6.3%)	5 (9.6%)	
Grade II	13 (41.9%)	4 (6.3%)	24 (46.2%)	

Grade III	8 (25.8%)	55 (87.3%)	23 (44.2%)	
Tumour size				0.194
T1	2 (6.5%)	1 (1.6%)	4 (7.7%)	
T2	23 (74.2%)	40 (63.5%)	29 (55.8%)	
T3	6 (19.4%)	22 (34.9%)	19 (36.5%)	
T4	0 (0%)	0 (0%)	0 (0%)	
Nodules				<0.001*
N0	10 (32.3%)	13 (20.6%)	26 (50%)	
N1	21 (67.7%)	19 (30.2%)	12 (23.1%)	
N2	0 (0%)	31 (49.2%)	14 (26.9%)	

Regarding the frequency distribution of Tumor Infiltrating Lymphocytes with clinicopathology in early-stage triple-negative breast cancer shown in Table 3, a significant relationship was found between age, grade, and kgb nodules to TIL with a p-value <0.001. Meanwhile, tumour infiltrating lymphocytes did not significantly correlate with tumour size.

IV. Discussion

Triple-negative breast cancer is a highly heterogeneous disease with varied clinical, pathological and molecular features. This type of breast cancer is characterized by size only over many years, with significant management limitations. In this study, the most common patient age group was between 31-40 years, 77 patients (52.7%), then 41-50 years, 34 patients (23.3%), under 30 years, 20 patients (13.7%) and 51-60. years as many as 15 patients (10.3%). In this study, all 146 samples were female (100%). Pathology records of the 4715 samples studied. TNBC was found in 815 patients. The revealed frequency is significantly closer to the upper margin of the worldwide quoted range. The majority of patients are <50 years of age at presentation. The mean age at diagnosis of TNBC was 46.26 ± 12.22 years, significantly younger than quoted worldwide.

A study conducted at Women's College Hospital and University, Toronto, Canada, revealed a frequency of 11.2% with an average age of presentation of 53 years. The results of this study differ from previous studies conducted in Belgium in 2014, which found that the average age of patients with triple-negative breast cancer was 50.9 years, with a range of 25-66 years. The average age of presentation for breast cancer in India is less than 50 years, which is lower than in developed countries. Eighty per cent of patients are under 65 years of age. Patients are often diagnosed late, with approximately 70% being in an advanced clinical stage at presentation (7–9).

Triple-negative breast cancer is a unique type clinically because it is more common in young women <50 years, African-American women, oral contraceptive use > one year, BRCA-I mutation carriers and women in low socioeconomic groups. Histologically aggressive triple negative breast cancer with poor prognosis, high mitotic rate, large tumour size, more aggressive expression profile with low bcl-2 but high p53 and ki67 expression causing overall survival (OS), breast cancer-specific survival (BCSS) and worse relapse-free survival (RFS) (9,10).

The most common tumour size was found in the T2 category in 92 patients (63%), T3 in 47 patients (32.2%) and T1 in 7 patients (4.8%). Previous research also found that 51 out of 79 patients were 20–50 mm (T2) in size. Previous research on tumour size and lymph node metastases found that TNBC tumour sizes > 2 cm were 54.5% in patients without lymph node metastases and 75.9% with lymph node metastases which were statistically significant.

The most common category of KGB nodules was the N1 category in 52 patients (35.6%), then N0 in 49 patients (33.6%), and N2 in 45 patients (30.8%). In this study, Grade III was the most common in 86 patients (58.9%), then Grade II in 41 patients (28.1%) and Grade I in 19 patients (13%). Previous studies found all patients with the histopathological type of invasive ductal carcinoma. In another study, 76.7% of triple-negative breast cancers were found with invasive ductal carcinoma histopathology (3).

A study on TNBC and lymph node metastases found that the histopathological types of patients with and without axillary lymph node metastases were mostly related to the histopathological types of invasive ductal carcinoma, 85.7% and 89.2%, respectively. Previous studies also obtained triple negative histological grade results of breast cancer with and without lymph node metastases, mostly grade III (89.3% and 82.1%). Another study on 30 samples found histological grade III only 66.7%, but for tumour size in that study, there were more tumours with a size > 50 mm(11–13).

TNBC has many clinicopathological characteristics distinct from other subtypes, including a young age of onset and large tumour size. Histological features include proliferative activity and high grade, no infiltrative margins, focal necrosis, lack of glandular formation, central scarring/fibrotic foci, and a predominant lymphoplasmacytic infiltrate. Because of this, most of these features are nonspecific and are found in other high levels of hormone receptor-positive breast cancer. While TNBCs comprise 25-30% of grade 3 tumours, approximately 77-90% of TNBCs are grade 3. Most (80-93%) are poorly differentiated ductal carcinomas of no special type. The second most common type is invasive lobular carcinoma which accounts for 1-2% of the total TNBC. Almost all cases of typical medullary carcinoma have a triple-negative phenotype, consisting of ~2% TNBC. Atypical medullary breast cancer and cancers arising in young age cohorts carrying the BRCA1 mutation almost exclusively exhibit the TNBC phenotype(14–16).

Previous research showed that lymphovascular invasion was found in 60.8%. A cross-sectional study with a sample size of 2017 patients found that only 22.7% of TNBC patients out of 88 had a lymphovascular invasion. Peritumoral lymphovascular invasion affects prognosis and influences survival and recurrence of TNBC. TNBC, according to the literature, occurs in 10-20% with characteristics of

early metastases, chemotherapy resistance, and poor survival. However, there is a paradoxical term for TNBC which shows a good response to neoadjuvant chemotherapy but has the worst average survival compared to other types of molecular subtypes (2,3).

TNBC mostly express proteins that are either characteristic of breast basal epithelial cells or that are associated with rapid multiplication and poor prognosis. Various studies reported 50-80% expression of basal cytokeratins (CK5/6, CK14 & CK17), P-cadherin, vimentin and EGFR. Other expressions include nestin, osteonectin, c-KIT, caveolins 1 and 2, laminin and aB crystallin. Mutations in the TP53 gene were seen in a high proportion of TNBC, as were changes in pRB and cell cycle checkpoints at p16 G1/S. A small proportion of TNBCs have anatomy (15).

In this study, in patients with triple-negative breast cancer, tumour infiltrating lymphocytes in the moderate infiltration category (30-60%) were most commonly found in 63 patients (43.2%), then the category of severe infiltration (> 60%) was found in 52 patients (35.6%)). Mild infiltration (<30%) was found in 31 patients (21.2%). This study's results align with previous studies, which found that TIL was found with a medium and high risk of 73.4%. TIL increased tumour proliferation associated with high histological grade, high Ki67, larger tumour size, and lymph node metastases. Some investigators found TIL to be at high TNBC risk. On the other hand, another study found 69.41% of TNBC patients with low TIL scores (<10%)(2,3).

In this study regarding the frequency distribution of tumour infiltrating lymphocytes with clinicopathology in early-stage triple-negative breast cancer, a significant association was found between age, grade, nodule, and LVI to TIL with a p-value <0.001. Meanwhile, tumour infiltrating lymphocytes did not significantly correlate with tumour size. A prospective, retrospective study reported that TIL was significantly associated with prognosis and therapeutic efficacy in triple-negative and HER2+ subtypes (2).

Previous studies demonstrated that increased TIL was associated with better outcomes for patients with TNBC treated with anthracycline-based adjuvant chemotherapy, with similar hazard ratio estimates to those reported previously. The results of this study are clinically relevant because the TIL can be useful as a stratification or adjustment factor in future clinical studies, as well as providing a rationale for evaluating immunotherapeutic approaches (4)

V. CONCLUSION

The most common patient age group was between 31-40 years (52.7%), the most common tumour size was in the T2 category (63%), the most common nodule category was in the N1 category (35.6%), and Grade III (58.9%). In patients with early-stage triple-negative breast cancer, tumour-infiltrating lymphocytes in the moderate infiltration category (30-60%) were most commonly found (43.2%). A significant relationship was found between tumour infiltrating lymphocytes and age, grade and KGB nodules in early-stage triple-negative breast cancer.

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