RESEARCH ARTICLE

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The Role of Stromal Tumour Infiltrating Lymphocytes (sTIL) Intensity and Programmed Death Ligand 1 () Expression in Breast Cancer Response to Neoadjuvant Therapy in Cipto Mangunkusumo Hospital (CMH), Indonesia

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Abstract

Background: Pathological responses to neoadjuvant therapy were still relatively poor, especially in CMH. Studies had been done to search for predictors of response such as sTIL intensity and expression, which is known to block sTIL action in killing cancer cells. This research assessed sTIL intensity and expression as predictors of response to neoadjuvant therapy in breast cancer. The preliminary data might be used to better tailored breast cancer patient therapy, considering the availability of anti-PD-1/ PD- L1 immunotherapy nowadays. **Objective:** To assess TIL intensity, expressions, and their roles as pathological predictors of breast cancer response to neoadjuvant therapy in Cipto Mangunkusumo Hospital (CMH). **Method:** This was an observational analytic retrospective cohort study on breast cancer patients undergoing biopsy/review of biopsy specimens, receiving neoadjuvant therapy and mastectomy in CMH from January 2014 to December 2021. Sixty cases fulfilled the inclusion and exclusion criteria. Total sampling was done. expression (immunohistochemistry, clone 22C3) and sTIL intensity (histopathology) was examined in the biopsy specimen. Linear regression analysis was done to determine the independent predictors of neoadjuvant therapy response (evaluated in the mastectomy specimen with residual cancer burden/ RCB score). **Results:** There were 60 female patients, median age 46 years old. 91,7% had invasive carcinoma of no special type. Median sTIL intensity was 10% (1%-70%). 58,3% patients had low sTIL intensity (≤10%). 28,3% patients had positive expression (CPS ≥1). Only 8,3% patients had pCR, while 90% patients had RCB class II-III. Every 1% increase in sTIL intensity, no lymphovascular invasion, and taxane chemotherapy were predicted to lower RCB score by 0,058, 0,781, dan 0,594, respectively. expression associated with pCR-RCB class I (p=0,048), but CPS score was not a predictor of RCB score in linear regression analysis. **Conslusion:** sTIL intensity was an independent predictor of breast cancer response to neoadjuvant therapy in RSCM. expression associated with pCR-RCB class I, but CPS score was not a predictor of RCB score.

Keywords: - programmed death ligand 1- sTIL- stromal tumour infiltrating lymphocyte- breast cancer

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Introduction

Breast cancer is still the leading cause of cancer death worldwide (GLOBOCAN, 2020, 2021). In Indonesia, more than 80% of breast cancer were diagnosed in an advanced stage (Youlden et al., 2014; Ministry of Health, 2019; Narisuari and Manuaba, 2020). In Cipto Mangunkusumo Hospital (CMH), most cases (42,7% cases) came with locally advanced stages (stage IIB-III) (Yang and Yulian, 2022). Neoadjuvant treatment is needed before definitive surgery for locally advanced cases to lower the stage, shrink the tumor size, and lower distant recurrence risk (Sikov et al., , 2022). The preferred method to assess pathological response to neoadjuvant therapy is by calculating the residual cancer burden (RCB) index, which can then be categorized to pathological complete response (pCR), RCB class I, II, and III. The RCB index is proved to be correlated with breast cancer prognosis (Symmans et al., 2017; Yau et al., 2022; Sikov et al., 2022).

Responses to neoadjuvant therapy is still relatively poor in many parts of the world, with pathological complete response (pCR) rate around 19% (Haque et al., 2018; Spring et al., 2020) In Dharmais Cancer Center Hospital, pCR rate was 3,3% (Karsono et al., 2019). In CMH pCR rate was 4,76%, partial tumor reduction 40,48%, and minimal reduction 19,05%, and no response 35,71% (Shintia et al., 2016).

The cause of poor neoadjuvant therapy response in

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Indonesia is still unknown. The tumor microenvironment, such as the stromal tumour infiltrating lymphocytes (sTIL) is thought to affect neoadjuvant therapy response. sTIL are able to kill tumour cells via the T-cell-mediated cytotoxicity. Programmed death ligand 1 () expression in cancer cells and its microenvironment can also affect sTIL and contribute to chemoresistance. It can hinder T cell action in killing tumour cells, which is hypothesized to worsen neoadjuvant therapy response (Bertucci and Gonçalves, 2017; Santos et al., 2018; Swoboda and Nanda, 2018; Xu et al., 2020). However, expression was found to correlate with high sTIL intensity, both of which associated with higher pCR rate. In contrary to its action in weakening cytotoxic T cell action, expression might also mean a robust and effective immune response to invasive tumour (Sabatier et al., 2015; Bertucci and Gonçalves, 2017; Burugu et al., 2017).

expression, sTIL intensity, and its role in predicting breast cancer neoadjuvant therapy response have not been well-studied in Indonesia. The availability of the data is expected for better and more personalized therapy plan, especially in this era of immunotherapy of anti-PD-1/ (Loi et al., 2019; Cortes et al., 2020; Nanda et al., 2020). In this paper, expression and sTIL intensity are discussed as predictors of pathological response to neoadjuvant therapy in CMH.

Materials and Methods

This is an observational analytical retrospective cohort study, using secondary data from the archive of Anatomical Department Faculty of Medicine Universitas Indonesia dan health record of CMH. The target population is locally advanced invasive carcinoma of the breast patients who received either anthracycline- or taxane-based neoadjuvant therapy. The accessible population were all cases with a biopsy specimen before neoadjuvant therapy and mastectomy specimen after neoadjuvant therapy from January 2014 to December 2021 in CMH. The rule of thumb was used to estimate the sample size in regression analysis (10 samples for every independent variable/ predictors of response). There were 6 predictor variables: age, molecular type, lymphovascular invasion, chemotherapy type, sTIL intensity, and expression.

The inclusion criteria were locally advanced breast cancer cases undergoing biopsy/ review of the biopsy specimen in CMH before neoadjuvant therapy, having neoadjuvant therapy and undergoing mastectomy in CMH, as well as having a complete clinicopathological data. The exclusion criteria were cases either without or insufficient paraffin block, cases with double primary tumours, cases with less than 100 invasive tumour cells in the biopsy specimen. Sixty cases fulfilled the inclusion and exclusion criteria. Total sampling was done.

The independent variables were expression and sTIL intensity in the biopsy specimen before neoadjuvant therapy. The dependent variables was RCB score. The confounder variables were age, lymphovascular invasion, molecular type, cytotoxic chemotherapy regimen.

First, breast cancer cases undergoing biopsy/review of a biopsy specimen, neoadjuvant chemotherapy, and mastectomy in CMH from 1 January 2014 to 31 December 2021 were collected. The biopsy, immunohistochemical examinations, and type of neoadjuvant therapy were then traced. Samples' clinicopathological data were collected such as age, gender, histological type, histological grade, lymphovascular invasion, molecular type, neoadjuvant therapy regimen, the completeness of the chemotherapy cycles, sTIL intensity in the biopsy specimen, and RCB score calculation in the mastectomy specimen. staining was then performed in the biopsy specimen . combined positive score (CPS) was blindly calculated. Then, we collected these variables from the patient's files: Age, gender, histological grade, lymphovascular invasion, molecular type of breast cancer, neoadjuvant chemotherapy regimen, the completeness of the chemotherapy cycles. We collected the histopathological slides and the paraffin blocks of the biopsy specimen that had been obtained before the patients underwent neoadjuvant chemotherapy. We measured the sTIL intensity in the histopathological slide of the biopsy specimen. We perform the staining in the paraffin blocks of the biopsy specimen, and determine the expression. Then, we calculated the RCB score as the dependent variable from the mastectomy specimen that was done after the neoadjuvant chemotherapy. Stromal TIL intensity, combined positive score (CPS), and the RCB score was blindly calculated.

expression in tumour cells is considered positive if it is staining the cell membrane of the invasive cancer cells, either weakly or strongly, and either completely or incompletely. It is negative if it only stains the cytoplasm. expression in lymphocytes and macrophages is positive if it stains either the cytoplasm or the cell membrane. CPS is calculated as follows: The number of cells expressing (tumour cells, lymphocytes, macrophages) divided by the number of viable tumour cells, and then multiplied by 100. expression is positive if the CPS≥1 (Erber and Hartmann, 2020; Núñez et al., 2022).

The data were processed by SPSS 26.0 using multivariate linear regression analysis to determine the independent predictor of pathological response. The result is significantly significant if the p value is less than 0.05.

Results

There were 60 patients, all female, with median age of 46 (28-74) years old. The histological type of invasive carcinoma in more than 90% of the sample was invasive carcinoma of no special type (NST), and the rest was lobular invasive carcinoma. Most (83.3%) were invasive carcinoma with intermediate-high Nottingham grade. More than half of the sample (46.7%) had lymphovascular invasion. Estrogen receptor was positive in most of the sample (76.6%). Fifteen percent was triple negative breast cancer (TNBC), the rest was almost equally divided into luminal-HER2 and HER2+. Most of the sample (7.17%) had high proliferation index. Almost half of the sample (43.3%) had taxane-based chemotherapy. The median sTIL intensity was 10% (1%-70%). Most samples (58.3%) had low sTIL intensity. expression was positive (CPS ≥ 1) in almost one third of the samples (28.3%). Only 8.3% achieved pCR. Most patients (90%) fell into RCB class

II-III (Table 1).

Med-high sTIL intensity was more often found in HER2+ type (p=0.026). In the post hoc analysis of statistically significant different of sTIL intensity between RCB classes (p=0.010), was higher in the pCR-RCB class I group compared to RCB class III group (p=0.045), and it was also higher in RCB class II group compared to RCB class III group (p=0.023). No difference in sTIL intensity was found between RCB class II and pCR-RCB class I (p=0.659). No association was found between sTIL intensity and expression (p=0.594), or other clinicopathological variables (Table 2).

was more often expressed in tumours with medium-high Nottingham grade (p=0.049) and TNBC (p=0.049). expression was also associated with better chemotherapy response/ pCR-RCB class I (p=0.048). No association was found between expression and other clinicopathological variables (Table 3).

Multivariate multiple linear regression analysis of the various clinicopathological variables was done to determine the independent predictor of RCB score (Table 4). The independent predictors of lower RCB score were no lymphovascular invasion, higher sTIL intensity, and taxane-based chemotherapy. Every 1% increase in sTIL intensity , no lymphovascular invasion, and taxane-based chemotherapy were predicted to lower RCB score by 0.058, 0.781, and 0.594, respectively.

Table 1. Clinicopathological Data

Parameter	N (%)	Median (Min-Max)
Age (years)		46 (28-74)
Gender		
Female	60 (100.0)	
Male	0 (0.0)	
Histological type		
No special type (NST)	55 (91.7)	
Special type (lobular)	5 (8.3)	
Nottingham tumour grade		
1	10 (16.7)	
2	35 (58.3)	
3	15 (25)	
Lymphovascular invasion		
Present	28 (46.7)	
Absent	32 (53.3)	
Molecular type		
Luminal-HER2-	27 (45.0)	
HER2+	24 (40.0)	
TNBC	9 (15)	
Chemotherapy regimen		
Taxane-based	26 (43.3)	
Non-taxane	34 (56.7)	
Chemotherapy cycles		
Complete	31 (51.7)	
Incomplete	29 (48.3)	
sTIL (%)		10 (1-70)
sTIL category		
Low	35 (58.3)	
Med-High	25 (41.7)	
CPS		
Positive (≥1)	17 (28.3)	
Negative (<1)	43 (71.1)	
RCB score		3.359 (0-5.328)
RCB class		
pCR	5 (8.3)	
I/ minimal burden	1 (1.7)	
II/ moderate burden	23 (38.3)	
III/ extensive burden	31 (51.7)	

Discussion

Med-high sTIL intensity was more often found in HER2+ compared to luminal-HER2 (62,5% vs 25,9%,

Table 2. Clinicopathological Characteristics of the Samples based on sTIL Intensity

Parameter	sTIL intensity (mean ± SD or n (%))		p value
	Med-High	Low	
Age (years)	46 (28-74)	46 (29-67)	0.722
Histological type			0.309
NST	24 (43.6%)	31 (56.4%)	
Lobular	1 (20.0%)	4 (80.0%)	
Nottingham grade			0.385
1	5 (50.0%)	5 (50.0%)	
2	12 (34.3%)	23 (65.7%)	
3	8 (53.3%)	7 (46.7%)	
Lymphovascular invasion			0.726
Present	11 (39.3%)	17 (60.7%)	
Absent	14 (43.8%)	18 (56.3%)	
Ki67 index			0.961
High	18 (41.9%)	25 (58.1%)	
Low	7 (41.2%)	10 (58.8%)	
Molecular type			0.026*
Luminal-HER2- º	7 (25.9%)	20 (74.1%)	
HER2+ º	15 (62.5%)	9 (37.5%)	
TNBC	3 (33.3%)	6 (66.7%)	
ER status			0.47
Positive	18 (39.1%)	28 (60.9%)	
Negative	7 (50%)	7 (50.0%)	
PD-L1 expression			0.594
Positive	8 (47.1%)	9 (52.9%)	
Negative	17 (39.5%)	26 (60.5%)	
RCB class			0.010*
pCR-RCB class I	4 (66.7%)	2 (33.3%)	
RCB class II	13 (56.5%)	10 (43.5%)	
RCB class III·	8 (25%)	23 (74.2%)	

*p<0.05 = significant; º, In the post-hoc analysis, there was significant difference between luminal-HER2- and HER2+ (p=0,008). No significant difference was found between luminal-HER2- group and TNBC (p=0,686), as well as between HER2+ and TNBC (p=0,239); ·, In the post hoc analysis, significant difference was found between RCB class III and RCB class II (p=0,023), as well as between RCB class III and PCR-RCB class I (p=0,045). No difference was found between RCB class II and pCR-RCB class I (p=0,659).

Table 3. Clinicopathological Characteristics According to *PD-L1* Expression

Parameter	*PD-L1* expression (mean ± SD atau n (%))		p value
	Positive	Negative	
Age (years)	49.82 ± 14.36	47.88 ± 10.39	0.617
Histological type			0.309
NST	17 (30.9%)	38 (69.1%)	
Non-NST	0 (0.0%)	5 (100%)	
Tumour Notttingham grade			0.049*
Med-High	17 (34.0%)	33 (66.0%)	
Low	0 (0%)	10 (100%)	
Lymphovascular invasion			0.078
Absent	6 (18.8%)	26 (81.3%)	
Present	11 (39.3%)	17 (60.7%)	
Ki67 index			0.907
High	12 (27.9%)	31 (72.1%)	
Low	5 (29.4%)	12 (70.6%)	
Molecular type			0.049*
TNBC	5 (55.6%)	4 (44.4%)	
Non-TNBC	12 (23.5%)	39 (76.5%)	
ER status			0.19
Positive	11 (23.9%)	35 (76.1%)	
Negative	6 (42.9%)	8 (57.1%)	
sTIL	10 (5-70)	10 (1-45)	0.749
sTIL intensity			0.594
Med-High	8 (32.0%)	17 (68.0%)	
Low	9 (25.7%)	26 (74.3%)	
RCB class			0.048*
pCR-RCB class I	4 (66.7%)	2 (33.3%)	
RCB class II-III	13 (24.1%)	41 (75.9%)	

*p<0.05, significant

p=0,008), which went along with meta-analysis by Denkert (2018), He et al., 2020, and Stanton et al., (2016). This study found no difference in sTIL intensity between TNBC and luminal-HER2- (33,3% vs 25,9%, p=0,686), as well as between TNBC and HER2+ (33,3% vs 62,5%, p=0,239), in contrast with various meta-analysis which found higher sTIL intensity in TNBC and HER2+ (Denkert et al., 2018; He et al., 2020; Li et al., 2021) It was presumably caused by the limited sample size in TNBC (9 samples). The more unstable genome of TNBC and HER2+ breast cancer is hypothesized to cause higher sTIL intensity. The higher mutation rate and novel protein formed are strong stimulants of the immune response (immunogenic), which cause higher sTIL intensity in both types of breast cancer (Li et al., 2021)

was expressed more often in TNBC (55,6% vs 23,5%, p=0,049), in accordance with meta.-analysis by Wang (2017) and Zhang (2017). TNBC is the most immunogenic breast cancer (Cancer Genome Atlas Network, 2012; Disis and Stanton, 2018; Narang et al., 2019). expression is one of the defense mechanism of such tumour to evade the immune system and proliferate (Wang et al., 2017). The activated tumor infiltrating lymphocytes (TIL) around the tumor, which were higher in number in TNBC, will secrete interferon- γ (IFN- γ), which induces expression in tumour cells via the IFNGRs/ JAK/ STAT signaling pathway (Schalper, 2014) In addition, TNBC is also the most often type of breast cancer with PTEN loss of function (Lazaridis et al., 2019) which will then induce expression in tumor cells (Schalper, 2014;,Mittendorf et al., 2014).

was also expressed more often in tumors with medium-high Nottingham grade (34% vs 0%, p=0,049), in accordance to meta-analysis by Wang (2017) and Zhang (2017). Higher histological grade tumors, hence more immunogenic tumour in other organs such as lung adenocarcinoma, glial tumour, prostatic adenocarcinoma, and urothelial cancer also express more often, as it associate with higher rate of PTEN loss of function Lebok et al., 2015; Li et al., 2017; Takahashi et al., 2020).

There were 3 independent predictor variables to determine lower RCB score (better chemotherapy response) in multivariate multiple linear regression analysis: no lymphovascular invasion, higher sTIL intensity, and taxane-based chemotherapy regimen.

Every 1% increase in sTIL intensity was predicted to lower RCB score by 0.058 (0,039-0,078). Median sTIL was higher in pCR-RCB class I compared to RCB class III, in accordance to studies by Denkert (2018) and He (2020). The cytotoxic effect of chemotherapy seems to be augmented in tumour with higher sTIL intensity. In addition to the lethal damage effect to the cancer cells, chemotherapy also induces immunogenic cell death (ICD) by cytotoxic T lymphocytes. The injured cancer cells will release damage associated molecular patterns (DAMPs), which will then activate dendritic cells, the most potent antigen presenting cells (APCs). The activated APCs will present the tumour antigen to the cytotoxic T lymphocytes which will subsequently attack the tumour cells. Taxane, anthracycline, and anti-HER2 monoclonal antibody also have immunostimulatory effect, activating dendritic cells that will in turn activate the TILs around the tumour. Those immune response mechanism seem to be augmented in tumours with higher sTIL intensity at baseline/ before chemotherapy was given (Li et al., 2021).

No lymphovascular invasion was predicted to lower RCB score by 0.781 (0.241-1.321). Uematsu (2011) and Vasudevan (2015) also found that no lymphovascular invasion was a predictor of pCR. The cancer cells in the vessels are more resistant to chemotherapy compared with those in the stroma (Sahoo and Lester, 2009; Park et al., 2016).

Taxane-based chemotherapy regimen was also predicted to lower RCB by 0.594 (0.037-1.152). It was also proven in previous studies to associate with better pCR rate compared to anthracycline-based chemotherapy (20.93% vs 4.34%, p=0.0237) (Diéras et al., 2004; Sanker et al., 2016). Anthracycline intercalates cell DNA and inhibit topoisomerase involved in DNA proliferation. Anthracycline-based regimen uses drugs that interfere with DNA function such as cyclophosphamide (Voelcker, 2020) and 5-fluoroucaril (Longley et al., 2003) Taxane-based regimen combines drugs interfering with DNA function, as well as taxane which inhibits microtubule function in a cell. The cell cycle then stops at G2 and M phase, and result in cell death. The addition of taxane

Table 4. Multiple Linear Regression to predict RCB score

Independent Variable	Full Model. Adjusted R2 0.412		Final Model. Adjusted R2 0.448	
	Adjusted β regression coefficient	(CI 95%)	Adjusted β regression coefficient	(CI 95%)
Lymphovascular invasion				
Absent	-0.657	(-1.282-(-0.032))*	-0.781	(-1.321-(-0.241))*
Present	1			
sTIL	-0.058	(-0.080-(-0.035))*	-0.058	(-0.078-(-0.039))*
Chemotherapy regimen				
Taxane	-0.753	(-1.436-(0.071))*	-0.594	(-1.152-(-0.037))*
Non-taxane	1			
Molecular type				
Luminal-HER2-	1			
HER2+	0.4	(-0.283-1.083)	0.323	(-0.237-0.882)
TNBC	0.268	(-0.686-1.221)		
Age	0.01	(-0.018-0.037)		
Histologic type				
Lobular	-0.342	(-1.543-0.858)		
NST	1			
Nottingham tumour grade				
Grade 1	1			
Grade 2	-0.463	(-1.307-0.380)		
Grade 3	-0.665	(-1.628-0.297)		
CPS	-0.006	(-0.022-0.010)		
Chemotherapy cycle				
Complete	1			
Incomplete	-0.357	(-1.088-0.374)		

*p<0,05; CI, Confidence interval.

potentiates the antitumour efficacy of chemotherapy (Gradishar, 2012).

CPS was not found to be a predictor of RCB score, presumably because the relatively low CPS scores in the samples. 71.1% had CPS score 0, only 6.7% had CPS ≥10, while the rest (22.2%) had CPS score <10. The prediction of RCB scores seemed to be impossible using the relatively homogenous low CPS scores.

In Table 4.3, expression is higher in pCR-RCB class I compared with RCB class II-III (p=0.048), in accordance with Hoffmann (2021). They found expression in 80% of the pCR group, but only 53.9% of the RCB class I-III (p=0.02). In contradiction with action in repressing the immune system, expression was found to be associated with better neoadjuvant chemotherapy pathological response. It seems to signify a robust and effective immune response in the tumour microenvironment (TME) as the immune checkpoint inhibitor such as will be expressed physiologically after T cell activation by tumour. expression acts as a negative feedback mechanism to prevent excessive normal tissue destruction. Thus, it not only acts as an immune repressor, but also depicts an effective activation of cytotoxic T cell ; Bertucci and Gonçalves, 2017).

There were a few limitations in this study. The International Association for the Study of Lung Cancer does not recommend the use of paraffin blocks that are more than 3 years old for immunohistochemistry as it may cause underexpression of (Gagné et al., 2019) Despite the fact that the oldest paraffin block age in this study was 8 years, expression was still found in every age of the paraffin block. There were few samples in TNBC. The limited samples in TNBC hindered generalization of the TNBC result to the population. The RCB score calculation in this study was done retrospectively. The tumor bed area was determined from the macroscopic data in the histopathologic report.

In conclusion, was expressed (CPS ≥1) in 28.3% of breast cancer in CMH. We found 41,7% of breast cancer had medium-high sTIL intensity. Only 10% of samples had better chemotherapy response (pCR-RCB class I). There were several predictors of better pathological response to neoadjuvant chemotherapy/ lower RCB score: higher sTIL intensity, no lymphovascular invasion, and taxane-based chemotherapy. Every 1% increase in sTIL intensity, no lymphovascular invasion, and taxane-based chemotherapy were predicted to lower RCB score by 0.058, 0.658, and 0.754, respectively. expression in the biopsy specimen before neoadjuvant therapy is associated with better pathological response/ pCR-RCB class I (p=0.048).

Author Contribution Statement

All authors contributed equally in this study.

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