

Accuracy of Neutrophil/Lymphocyte Ratio as Predictor of Neoadjuvant Chemotherapy Response in Locally Advanced Breast Cancer at Dr. Mohammad Hoesin General Hospital Palembang

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ABSTRACT

Introduction: Breast cancer is one of the most common neoplasm occurs in women. Neoadjuvant Chemotherapy (NACT) is widely used in Locally Advanced Breast Cancer (LABC) and Inflammatory Breast Cancer (IBC), to reduce tumor size (down-sizing), and further allowing breast-conserving surgery to be performed. Accurate markers are needed to prevent impractical chemotherapy that allows patients to receive definitive surgery in a timely manner. Previous study showed neutrophil/lymphocyte ratio (NLR) was superior in predicting long-term outcomes over PLR. This study aims to evaluate the relationship between the NLR value and neoadjuvant chemotherapy response in patients with locally advanced breast cancer at Dr. Mohammad Hoesin General Hospital Palembang.

Method: This study is a prognostic test of the NLR accuracy to predict the response of neoadjuvant chemotherapy in patients with locally advanced breast cancer. The study included 113 patients as study subjects.

Results: The obtained NLR cut off point value was ≤ 2.92 with an area under the ROC curve (AUC) of 92.2%. The diagnostic measures of the NLR prognostic test had sensitivity of 93%, specificity of 93%, Positive Predictive Value of 97%, Negative Predictive Value of 82%, and accuracy of 93%. There is a significant relationship between NLR and chemotherapy response in patients with locally advanced breast cancer ($p = 0.000$).

Conclusion: There is a significant relationship between NLTR and chemotherapy response. NLR can be used as a predictor of neoadjuvant chemotherapy response with a good degree of accuracy.

Keywords: neutrophil/lymphocyte ratio, neoadjuvant chemotherapy, breast cancer

Introduction

Breast cancer is the most common neoplasm in women and is the second leading cause of cancer death in women in industrialized countries.¹ Neoadjuvant Chemotherapy (NACT) is widely used in Locally Advanced Breast Cancer (LABC) and Inflammatory Breast Cancer (IBC) to reduce the size of the tumor (down-sizing), and further allowing breast-conserving surgery to be performed. It has been suggested that the pathologic complete response (pCR) after NACT is thought to replace disease free survival (DFS) and overall survival (OS) markers, especially in patients with more aggressive subtypes, such as triple-negative or HER2-positive breast cancer.¹ Locoregional control (LRC), recurrence free survival (RFS), and overall survival (OS) rate were 99, 98, and 100% at the first year and 89, 69 and 77% at the fifth year, respectively. Response to NACT was not correlated with LRC ($p > 0.05$) but was correlated with RFS and OS ($p < 0.05$).²

Apart from molecular subtypes, there are no other biomarkers that have been validated as predictive factor for pCR or prognostic factor for DFS and/or OS after NACT, including Ki67 and Residual Cancer Burden. Recently, the immune system has been shown to play an important role in breast cancer response to chemotherapy. Although breast cancer does not produce high neoantigen, breast cancer cells are often

infiltrated by lymphocytes (tumor-infiltrating lymphocytes, TIL), which may vary widely across molecular subtypes. The presence of TIL in breast cancer is highly correlated with pCR after NACT was performed. Different tumor infiltration cell subtypes, such as T-regulatory lymphocytes (Treg) and myeloid derived-suppressor cells (MDSC), lead to immune system suppression, and have been shown to reduce the success of NACT in patients with breast cancer. Consistently, breast cancer tends to respond to immune checkpoint inhibitors (ICI), although the success rate is lower compared to other neoplasms such as melanoma, kidney and lung cancers.¹

Some studies suggest that the use of markers, such as gene mutations, associated with response to neoadjuvant chemotherapy treatment is still difficult to assess, and no clinical biomarkers have been shown to generally predict cancer response after NACT to date.^{1,3} Therefore, accurate markers are needed to prevent impractical chemotherapy that enables patients to receive definitive surgery in a timely manner.

The chronic systemic inflammatory response is continuously studied in terms of breast cancer progression and prognosis. Parameters using peripheral blood test have been investigated as factors that reflect the immune response. Circulating white blood cells, which result in changes in the proportion of neutrophils, lymphocytes, and monocytes, are associated with systemic inflammatory response. The relationship between cancer prognosis and absolute monocyte count (AMC), absolute lymphocyte count (ALC), lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) has been studied in various types of cancer.⁴

In patients with breast cancer, Ni et al. reported that increased LMR was a beneficial prognostic factor after neoadjuvant chemotherapy (NACT).⁵ In addition, Azab et al. reported that NLR was superior in predicting long-term outcomes over PLR.⁶ In this study, we aimed to evaluate whether the neutrophil lymphocyte ratio (NLR) could predict the outcome of chemotherapy response in breast cancer patients with peripheral blood parameters. Additionally, we aimed to estimate the value of immune-related predictor markers in response to NACT.

Methods

This study is a prognostic test of the accuracy of the NLR to predict the response of neo-adjuvant chemotherapy in patients with locally advanced breast cancer.

The subjects of this study were patients with locally advanced breast cancer who received neo-adjuvant chemotherapy at Dr. Muhammad Hoesin Palembang General Hospital who met the inclusion criteria.

The inclusion criteria were patients with locally advanced breast cancer who were diagnosed based on the criteria of the American Joint Committee of Cancer and received adjuvant chemotherapy with Karnofsky index ≥ 50 . The exclusion criteria were patients who had undergone previous chemotherapy and were immunocompromised.

There were 113 subjects in this study. The independent variables were the immunohistochemistry subtypes and the neutrophil/lymphocyte ratio. The dependent variable was the adjuvant chemotherapy response. The sociodemographic characteristics were age, age at menarche, history of hormonal contraception usage, age at first pregnancy, family history of breast cancer.

The data are presented in distribution and narrative tables. The receiver operating characteristics (ROC) curve statistical test was performed using the MedCalc 2000 software to determine the NLR cut off point value in predicting chemotherapy response. Chi square test was used to evaluate the sociodemographic relationship to the NLR value, and to evaluate the relationship between the NLR value and the chemotherapy response.

Results

Sociodemographic characteristics distribution

There were 113 patients who participated in the study. The average age was 47 years old, with the youngest was 27 years old and the oldest was 68 years old. There were 89 patients aged above 40 years old (78.8%). There were 84 patients (74.3%) with age at menarche at under 15 years old. There were 55 patients (48.7%) who had their first pregnancy at under 30 years old. As many as 51 patients (45.1%) had menopause at the age above 45 years old. There were 102 patients (90.3%) with no family history of cancer. There were 86 patients (76.1%) with history of hormonal contraceptive usage. There were 90 patients (79.6%) with childbirth history of more than one time. As many as 78 patients (69%) had Luminal B subtype breast cancer. The results of the study are presented in table 1.

Table 1. Sociodemographic characteristics distribution

Sociodemographic characteristics	Total (n)	Percentage (%)	P value
Age			
≤ 40 years old	24	21.2	0.475
> 40 years old	89	78.8	
Age at Menarche			
≤ 15 years old	84	74.3	0.733
> 15 years old	29	25.7	
Age at first pregnancy			
Never been pregnant	22	19.5	0.223
> 30 years old	36	31.9	
≤ 30 years old	55	48.7	
Age at menopause			
Not yet menopause	33	29.2	0.333
≤ 45 years old	29	25.7	
> 45 years old	51	45.1	
Family history of breast cancer			
Yes	11	9.7	0.438
No	102	90.3	
History of hormonal contraception usage			
Yes	86	76.1	0.113
No	27	23.9	
History of childbirth			
0	23	20.4	0.100
≥ 1 time	90	79.6	
Subtype			
Luminal A	22	19.5	0.405
Luminal B	78	69.0	
Triple negative	10	8.8	
HER2 over	3	2.7	

Distribution of chemotherapy response in locally advanced stage breast cancer patients

The distribution analysis results showed that 83 of the 113 patients (73.5%) had good chemotherapy response, while 30 patients (26.5%) had poor response. The research results are presented in table 2.

Table 2. Chemotherapy response distribution

Chemotherapy Response	N	Percentage (%)
Good response	83	73.5
Poor response	30	26.5

Relationship of sociodemographic characteristics and chemotherapy response in locally advanced stage breast cancer patients

The relationship between sociodemographic characteristics and chemotherapy response of patients with locally advanced breast cancer were analyzed using the chi square statistical test. It is found that there was no significant relationship between sociodemographic characteristics (age, age at menarche, age at first pregnancy, age at menopause, family history, contraception, parity and subtype) to chemotherapy response ($p > 0.05$).

Distribution of the Neutrophil/Lymphocyte Ratio (NLR)

The distribution of the NLR based on chemotherapy response showed the NLR value on good chemotherapy responses had a mean value of 1.90 ± 0.95 , while the NLR value on poor chemotherapy responses had a mean value of 5.77 ± 4.26 . The results of the study are presented in table 3.

Table 3. Neutrophil/lymphocyte ratio (NLR) distribution

Chemotherapy Response	N	Mean \pm SD	Median (Min-Max)
Good response	83	1.90 ± 0.95	1.77 (0.11 – 6)
Poor response	30	5.77 ± 4.26	4.70 (0.93 – 23)

Neutrophil/Lymphocyte Ratio (NLR) Cut Off Point Value Based on Chemotherapy Response in Locally Advanced Stage Breast Cancer Patients

The ROC analysis result of the NLR value based on the chemotherapy response in patients with locally advanced breast cancer obtains a cutoff point value of ≤ 2.92 with an Area under the ROC curve (AUC) value of 92.2%. The results of the analysis are presented in table 4.

Table 4. Table of neutrophil/lymphocyte ratio (nlr) and chemotherapy response

Neutrophil/Lymphocyte Ratio (NLR)	Chemotherapy Response		Total N (%)	P value
	Good	Poor		
	N	N		
NLR ≤ 2.92	77 (a)	2 (b)	79 (a+b)	0.000
NLR > 2.92	6 (c)	28 (d)	34 (c+d)	
Total	83 (a+c)	30 (b+d)	113 (a+b+c+d)	

Based on the 2x2 table between the NLR value and the chemotherapy response, it is found that the diagnostic measures had a sensitivity value of 93%, specificity of 93%, Positive Predictive Value of 97%, Negative Predictive Value of 82%, accuracy value of 93% and AUC value of 92.2%. The results of the analysis are presented in table 5.

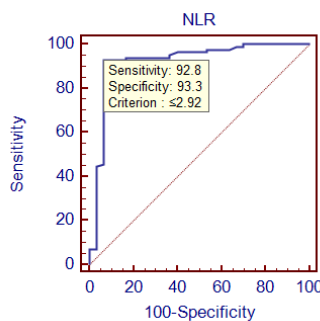


Table 5. Neutrophil/lymphocyte ratio (NLR) prognostic test on chemotherapy response in locally advanced stage breast cancer patients

Diagnostic measures	Percentage value	95% Confidence interval
Sensitivity	93%	84 – 97
Specificity	93%	76 – 99
Positive Predictive Value	97%	90 – 100
Negative Predictive Value	82%	65 – 93
Accuracy	93%	86 – 97
Area under the ROC curve (AUC)	92.2%	
Youden index J	0.8610	85.6 – 96.4

Relationship of neutrophil/lymphocyte ratio (NLR) and chemotherapy response in locally advanced stage breast cancer patients

The analysis of the relationship between NLR and chemotherapy response in patients with locally advanced breast cancer found that 97.5% cancer patients who had $NLR \leq 2.92$ had a good chemotherapy response, compared to 17.6% cancer patients who had $NLR > 2.92$. The results of statistical tests using the chi square test found that there was a significant relationship between NLR and chemotherapy response in patients with locally advanced breast cancer (p value = 0.000) with an alpha value of 0.05 ($p < \alpha$).

Discussion

The chronic systemic inflammatory response is continuously studied in terms of breast cancer progression and prognosis. Parameters using peripheral blood test have been investigated as factors that reflect the immune response. Circulating white blood cells, which result in changes in the proportion of neutrophils, lymphocytes, and monocytes, are associated with systemic inflammatory response. The relationship between cancer prognosis and absolute monocyte count (AMC), absolute lymphocyte count

(ALC), lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) has been studied in various types of cancer.⁴

There is a known association between the systemic inflammatory response and changes in circulating white blood cells, particularly the presence of neutrophilia and relative lymphocytopenia. Routine blood test in cancer patients is an objective parameter that is easily measured and is able to show the degree of systemic inflammatory response. Systemic inflammation in cancer patients is thought to reflect cytokines profile produced by tumors and as component of the host response to tumors. Increased level of systemic inflammation has been shown to correlate with poor survival outcomes and poor response to treatment in some patients with solid tumors. Patients who are able to maintain a low NLR during chemotherapy have the most favorable prognosis, whereas patients with increased NLR have the shortest survival rate.⁴

This study examined 113 patients with locally advanced breast cancer, in which 89 patients (78.8%) were above 40 years old with age range between 27-68 years old, and average age of 47 years old. Research conducted by Javier et al. in 2018 reported that out of a total of 288 patients, the average age was 51 years old with age range from 27 - 85 years old, with 58% of patients aged > 40 years old.¹ Another study conducted by Graziano et al. in 2019 reported that out of a total of 373 patients, the average age was 50 years old with with age range from 26 - 82 years old, the youngest 26 years old and the oldest 82 years old, with 192 (51.5%) patients aged < 50 years old.¹

Most of the breast cancer patients in this study had Luminal B subtype breast cancer (69%). In previous research conducted by Graziano et al. in 2019, reported Luminal A as the most common subtype with 132 (35.4%) patients out of 373 total patients.¹

Relationship of sociodemographic characteristics and *Neutrophil Lymphocyte Ratio (NLR)* in Locally Advanced Stage Breast Cancer Patients

The relationship of sociodemographic characteristics and Neutrophil/Lymphocyte Ratio (NLR) in patients with locally advanced breast cancer were analyzed using the chi square statistical test. It is found that there was no significant relationship between sociodemographic characteristics (age, age at menarche, age at first pregnancy, age at menopause, family history of breast cancer, history of hormonal contraception usage, history of childbirth and cancer subtype) and NLR ($p > 0.05$). This results are in line with research

conducted by Zhang M et al, Zhu Y et al, Asano et al which stated that there was no relationship between clinical pathology characteristics and NLR.⁷

Neutrophil/Lymphocyte Ratio (NLR) Distribution

The distribution of the NLR based on chemotherapy response showed the result of the NLR value analysis on good chemotherapy responses had a mean value of 1.90 ± 0.95 , while the NLR value on poor chemotherapy responses had a mean value of 5.77 ± 4.26 . These results are in line with research conducted by Graziano et al. who reported in their study that patients with low NLR values (≤ 2.42) had a good chemotherapy response compared to patients with high NLR value.¹

Neutrophil/Lymphocyte Ratio (NLR) Cut Off Point Value Based on Chemotherapy Response in Locally Advanced Stage Breast Cancer Patients

The results of the ROC analysis of the NLR value based on the chemotherapy response of patients with locally advanced breast cancer obtained a cut off point value of ≤ 2.92 with an Area under the ROC curve (AUC) value of 92.2%.

Based on the 2x2 table between the NLR value and the chemotherapy response, it is found that the diagnostic measures had a sensitivity value of 93%, specificity of 93%, Positive Predictive Value of 97%, Negative Predictive Value of 82%, accuracy value of 93% and AUC value of 92.2%. These results conclude that NLR can be used as a predictor of neo-adjuvant chemotherapy response in breast cancer patients and has a good degree of accuracy.

Relationship of Neutrophil/Lymphocyte Ratio (NLR) and Chemotherapy Response in Locally Advanced Stage Breast Cancer Patients

The analysis of the relationship between NLR and chemotherapy response in patients with locally advanced breast cancer found that 97.5% patients who had $NLR \leq 2.92$ had a good chemotherapy response, compared to 17.6% cancer patients who had $NLR > 2.92$. The results of statistical tests using the chi square test found that there was a significant relationship between NLR and chemotherapy response in patients with locally advanced breast cancer (p value = 0.000) with an alpha value of 0.05 ($p < \alpha$).

These results are in line with research conducted by Iimori et al. which reported that breast cancer patients with NLR value lower than the cut off value had a better pathological complete response (pCR) and better survival outcomes after NACT.⁸

Neutrophil is a type of granulocyte leukocyte that is produced in 7-14 days, and remains in circulation for 6 hours. The main function of neutrophil is phagocytosis (killing and digesting microorganisms). Acute bacterial infections and trauma trigger neutrophil production. This increase in neutrophil number is referred as "shift to the left", which indicates an acute bacterial infection.^{9,10} There is a known association between the systemic inflammatory response and changes in circulating white blood cells, particularly the presence of neutrophilia and relative lymphocytopenia. Routine blood test in cancer patients is an objective parameter that is easily measured and is able to show the degree of systemic inflammatory response. In 2017, a study by Lu et al. showed the components of the white blood cell count, particularly neutrophils and lymphocytes, have a role in predicting the life expectancy of cancer patients.¹¹

Immunohistochemically, lysis of tumor cells by chemotherapy agents can be associated with the release of tumor-related antigens. This can assist the immune response against cancer cells, which will be important in patients with immunity sensation to these tumor antigens before starting the chemotherapy regimen. This means, NLR can be a prognostic sign to predict the effectiveness of adjuvant chemotherapy after surgery or NACT.^{12,13}

A study by Azab et al. in 2011, stated that breast cancer patients with $NLR > 3.3$ had a higher mortality rate than patients with $NLR < 1.8$. Research shows that breast cancer patients with an NLR ratio of ≥ 2.5 had a lower life expectancy compared to those with an NLR ratio < 2.5 .¹⁴

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