SJS SRIWIJAYA JOURNAL OF SURGERY

Sriwijaya Journal of Surgery Volume 3, Issue 1, Page No: 44-54
Available online: www.sriwijayasurgery.com
SJS 3(1) : 44-54

Effects of Neo-Adjuvant Chemotherapy on CD8 + Serum Levels in Local Advanced Stage Breast Cancer Patients

Nopriansyah Darwin[1*], Nur Qodir[1], Mulawan Umar[1], Theodorus[2]

[1]Department of Surgery, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

[2]Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

* Correspondence Author Email: nopriansyahdarwin@gmail.com

Abstract

Introduction: CD8 + CTLs are important components of tumor specific cellular adaptive immunity in breast cancer. Chemotherapy can increase the response of cytotoxic lymphocytes and provide anti-tumor immunity. Therefore, chemotherapy can cause cell death and trigger tumor antigens that are processed by APC and activate CD8 + cells to destroy tumor cells. Evaluation of the immune system before treatment can predict chemotherapy response. The purpose of this study was to assess the effect of neo-adjuvant chemotherapy on serum CD8 + levels in patients with locally advanced breast cancer.

Methods: Non-comparative clinical trials were conducted at Dr. Mohammad Hoesin Hospital Palembang from October 2017 to June 2018.The sample of this study was 30 breast cancer patients who met the inclusion criteria. All samples underwent FAC neo-adjuvant chemotherapy and data were analyzed with SPSS version 21.

Results: The mean age of advanced breast cancer patients was 45.97 ± 10,526 years with an age range of 30-66 years. Serum CD8 + levels after chemotherapy decreased significantly compared to before chemotherapy (p = 0.000). The value of serum CD8 + before chemotherapy has a sensitivity of 42.86% and specificity of 43.48% with a cut of point of 660.7 cells / mm3.

Conclusion: Neo-adjuvant chemotherapy significantly decreases serum CD8 + levels in local advanced breast cancer patients.

SJS SRIWIJAYA JOURNAL OF SURGERY

Keyword: CD8 +, locally advanced breast cancer, immune system, neo-adjuvant chemotherapy

1. Introduction

Breast cancer is the most common malignancy in women. In 2012 according to the International Agency for Research on Cancer (IARC) breast cancer was still a disease that ranked number one in the world as a new case, which was 43.3% and most often caused death, amounting to 12.9%.[1] In 2010, the incidence rate breast cancer in Indonesia 12 per 100,000 women, while in the USA about 92 per 100,000 women.[2] In Indonesia, more than 80% of cases are found to be at an advanced stage.[2] Locally advanced breast cancer (LABC) is still the largest part (50-60%) of cancer patients who come to polyclinics or hospitals in Indonesia.[3] According to Yudistira (2014) the distribution of the most clinical stadiums at Mohammad Hoesin Hospital Palembang is stage III or LABC, namely stage III A 26.53% and stage IIIB 48.98%.[4]

Neo-adjuvant chemotherapy is a therapeutic choice in patients with locally advanced breast cancer. The use of this therapy is increasing in patients with inoperable breast cancer who are not candidates for breast conservation surgery or have been shown to have lymph node metastases. Patients who show a complete pathology response to neo-adjuvant chemotherapy have a long-termdisease-free survival.[5] This aims to reduce the tumor mass, so that surgery can be performed and continued adjuvant therapy.[6] Neo-adjuvant therapy is given in 3 cycles.[7.8] The first line used, namely anthracycline based chemotherapy.[9-10] The administration of neo-adjuvant chemotherapy reduces primary tumor mass, reduces lymphatic metastases in axilla and eradication of micro- metastases, so that the operability of breast cancer changes.[11-12] The response of neo-adjuvant chemotherapy can be assessed by comparing mass sizes before and after chemotherapy. The clinical response criteria used are RECIST, which is complete, partial, stable and progressive.[13.] The immune system plays an important role in the development of cancer, especially breast cancer.Response Adaptive and innate immunity plays an important role in tumor immune-surveillance and can limit the development and growth of neoplasms. Chemotherapy can trigger an immune response which contributes to the therapeutic response[14-16.] In its development, tumor infiltrative lymphocytes

(TILs) are important predictive factors for the chemotherapy response in breast cancer patients.[17]

Cells from innate immune systems (neutrophils, monocytes, macrophages, and host APCs) and adaptive (B and T lymphocytes) work together to respond to various pathogens, including tumor antigens. Innate immunity cells are needed by B and T cells to identify immunogenic proteins, then allow adaptive immunity to form memory cells (lymphocytes that remain in the lymph nodes). Most antigens in breast cancer are self-proteins that can stimulate T cells and induce the regulator's immune response. T cell infiltration includes T Helper (CD4 +) and cytotoxic (CD8 +) where CD4 + T-helper facilitates antigen presentation through cytokine secretion and activation of presenting cell antigens (APC), while CD8 + cytotoxic T-cells play a role in tumor destruction.[17-19] In breast cancer, CD8 + cytotoxic T cell infiltration is closely related to the patient's long-term survival and good response to chemotherapy. Mao et al reported that CD8 + lymphocytes are the main cells that are effective in the immune response, which shows better disease-free survival in breast cancer patients.[20] Al Saleh et al in his study reported that high CD8 + expression could significantly predict pathologic complete responses after neo-adjuvant chemotherapy and illustrate an independent prognostic factor for Overall Survival (OS).[21] According to Seo et al, CD8 + cytotoxic T lymphocytes are important components of TILs that are associated with the chemotherapy response and can be used as predictors of response to anthracycline or anthracycline / taxane based on breast cancer.[22]

2. Methods

This type of research is a clinical trial without comparison by looking at the level of CD8 + serum in patients with locally advanced breast cancer before and after neo-adjuvant chemotherapy. The research was carried out in the oncology surgery clinic and inpatient installation of the Dr. General Central Hospital Mohammad Hoesin Palembang. Research time starts from October 2017 to June 2018. The study population was local advanced-stage breast cancer patients who were going to get neo-adjuvant chemotherapy at the Central General Hospital Dr. Muhammad Hoesin Palembang. All local advanced breast cancer sufferers who meet the inclusion and exclusion criteria. The method of sampling is done by consecutive sampling, where all subjects who come and meet the sample selection criteria are included in

the study until the required number of subjects is met. All patients provided informed consent before participating in the study sample. The parameter of the success of this study was a significant increase in CD8 + levels after neo- adjuvant chemotherapy. Data in this study will be presented in tabular form and analyzed using paired T test to determine effectiveness with SPSS version 21.

3. Results

General characteristics of research subjects are shown in Table 5. Based on age, the average age of advanced breast cancer patients is 45.97 ± 10.526 years with an age range of 30-66 years, where there are 15 patients (50%) and ages (40%) < 40 years as many as 15 people (50%). The majority of patients with locally advanced breast cancer have given birth (96.7%) and only 1 (3.3%) has never been. Patients with locally advanced breast cancer with a history of family planning were found as many as 17 people (56.7%) and the rest (43.3%) did not have a family history of family planning. A total of 13 people (43.3%) patients with advanced breast cancer have a family history of the same disease.

Table 1. General Characteristics of Research Subjects

Characteristics		n	%
Age	≥ 40 years	15	50
	< 40 years	15	50
KB history	Yes	17	56,7
	No	13	43,3
Childbirth History	Ever	29	96.7
	Never Yet	1	3.3
Family History	Have	13	43,3
	Have not	17	56.7

In this study, there were 20 ER positive people (66.7%), positive PR 25 people (83.3%)

and positive Her2 / Neu 9 people where Her2 / Neu + 3 were 4 people (13.3%) , Her2 / Neu +2 as many as 3 people and 2 people (6.7%) have Her2 +. Good Ki67 levels ≥ 20 are 15 people (50%). Ki67 <20% as many as 9 people (30%) and Ki67 negative as much as 20%. From 30 local advanced breast cancer patients, there were 23 good chemotherapy responses (76.7%) and 7 poor patients (23.3%).

Table 2. Clinical Pathology Characteristics of Research Subjects

Characteristics		N	%
ER	Positive	20	66.7
	Negative	10	33,3
PR	Positive	25	83,3
	Negative	5	16,7
HER2	Positive 1(+)	2	6.7
	Positive 2(++)	3	10,0
	Positive 3(+++)	4	13.3
	Negative	21	70,0
Ki67	≥ 20%	15	50,0
	< 20%	9	30,0
	Negative	6	20,0
Chemotherapy Response	Bad	7	23,3
	Good	23	76,7

Before conducting a statistical analysis, the Saphiro Wilk normality test is first performed. Obtained variable probability value CD8 + before < 0.05 so that the statistical test used is the Wilcoxon test.

Table 3. Effectiveness of Neo-adjuvant Chemotherapy (NAC) on CD8 +

Variable	Before Therapy	After Therapy	Change	P*
CD8+	658,26 ± 370,15	462,73 ± 309,79	195,5 ± 151,5	0,000

**Wilcoxon, p= 0,05*

From the statistical analysis it was found that there were differences in serum CD8 + levels before and after chemotherapy (p = 0,000) where the value of serum CD8 + after chemotherapy dropped significantly compared to before chemotherapy.

AUC value of the ROC curve CD8 + value can be seen below. AUC value is getting closer to 1, the better.

Table 4. AUC CD8 + values for chemotherapy responses

Variable	**CD8+**	
	AUC	***p-value***
Before Therapy	0,516	0,902
After Therapy	0,702	0,111

ROC analysis, 95% CI

AUC CD8 + values before chemotherapy had moderate discrimination, whereas after NAC therapy had good discrimination.

Table 5. Sensitivity and Specificity of CD8 + Values for Chemotherapy Response

Variable	**Cut Off**	**Sensitivity**	**Specificity**
CD8+ Before Therapy	660,7 sel/mm^3	42,86%	43,48%
CD8+ After Therapy	280,2 sel/mm^3	71,43%	69,57%

The receiver operating curve (ROC) curve was analyzed to find the cut-off point to obtain the sensitivity and specificity of serum CD8 + levels before chemotherapy. Determination of the cut-off value for serum CD8 + levels before chemotherapy, is done by making a curve between sensitivity, specificity and the value of serum CD8 + levels before chemotherapy for locally advanced breast cancer patients.

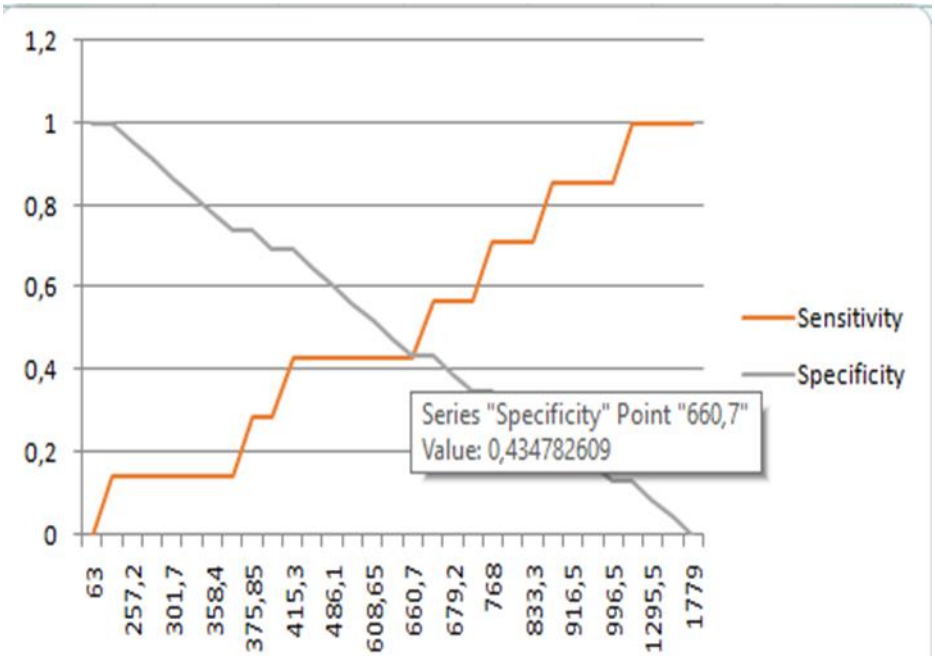

Figure 1. Curve intersection of sensitivity, specificity, CD8 + value before chemotherapy for locally advanced breast cancer patients

Figure 1. Is a cut point curve for CD8 + serum levels before chemotherapy for locally advanced breast cancer patients. From this figure, the best value for sensitivity and specificity is 660.7 cells / mm3.

Table 6. CD8 + After Chemotherapy as a Predictor of Therapeutic Response in Local Advanced Stage Breast Cancer Patients.

Cancer Breast		**Response**		**Total**
		Not good	**Good**	
CD8+	≤ cut off (660,7)	3^a	13^b	16
	> cut off (660,7)	4^c	10^d	14
Total		7	23	30

Based on table 6. The value of serum CD8 + before chemotherapy has a sensitivity of 42.86% and specificity of 43.48%.

The receiver operating curve (ROC) curve was analyzed to find the cut-off point to obtain the sensitivity and specificity of serum CD8 + levels after chemotherapy. Determination of the cut-off value for CD8 + serum levels after chemotherapy, is done by making a curve between

sensitivity, specificity and the value of CD8 + serum levels after chemotherapy for locally advanced breast cancer patients.

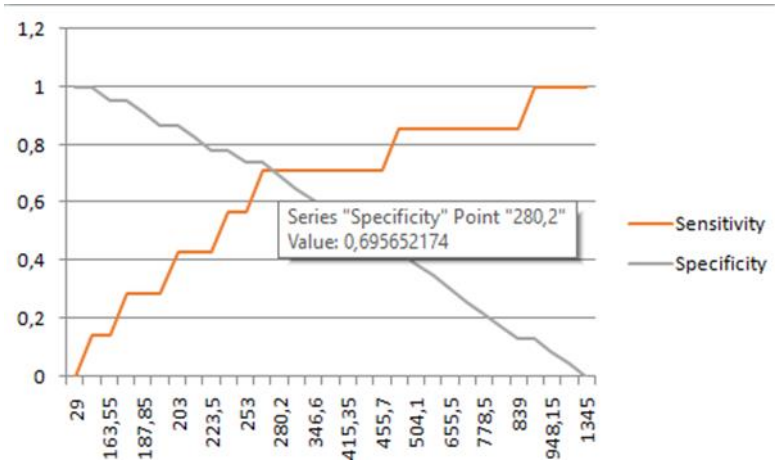

Figure 2. Curve intersection of sensitivity, specificity, CD8 + value after chemotherapy for locally advanced breast cancer patients

Figure 2. Is a cut point curve for CD8 + serum levels after treatment of locally advanced breast cancer patient. From this figure, the best value for sensitivity and specificity is 280.2 cell / mm3.

Table 7. CD8 + After Chemotherapy as a Predictor of Therapeutic Response in Local Advanced Stage Breast Cancer Patients.

Breast Cancer		**Response**		Total
		Bad	Good	
CD8+	≤ cut off (280,2)	5^a	7^b	12
	> cut off (280,2)	2^c	16^d	18
Total		7	23	30

Based on table 7. The value of serum CD8 + levels after chemotherapy has a sensitivity of 71.43% and specificity of 69.57%.

4. Discussion

The incidence of breast cancer increases with age, every ten years, the risk of cancer increases twice until the age of menopause. In this study, the average age of breast cancer patients was 45.97 ± 10,526 years with an age range of 30-66 years, which obtained patients

with age 40 patients and patients aged < 40 years as many as 15 patients (50%). According to the research of Arzu et al in 2017 found the average age of breast cancer patients is higher than this study which is 51.1 ± 6.7 years.[23-37] In addition, a study conducted by FZ Lamiri et al in 2015 showed the average age of breast cancer patients was 45.83 ± 11.05 where more patients aged ≥ 45 years (58%) were found than patients aged <44 years (42%).38 History of hormonal contraception in breast cancer patients in this study were 56.7%. This result is lower than the results of the study by F.Z Lamiri et al in 2015 where breast cancer patients with a history of hormonal contraception were obtained as much as 74.5%.[38] In this study patients who had a family history of breast cancer were 43.3%. However, a study conducted by F.Z Lamiri et al in 2015 showed that only 14.3% of patients had a family history of breast cancer.[38] According to MD Anderson, a family history of breast cancer increases the risk of developing breast cancer. The risk generally depends on the number of families suffering from breast cancer, the age at diagnosis and unilateral or bilateral breast cancer. For example, a 30-year-old woman who has a sister who has bilateral breast cancer before the age of 50 has a 55% probability of breast cancer at the age of 70 years. The probability of bias decreases by 8% if the affected breast is unilateral.[39]

Immunohistochemical characteristics of breast cancer patients in this study obtained 66.7% positive ER, 83.3% PR and Her2 / Neu +3, +2 and +1 respectively 13.3%, 10% and 6.7 %. The level of Ki67 ≥ 20% in breast cancer patients in this study was 50% and Ki67 <20% by 30%. Immunohistochemistry is used not only for prognosis but also determines the therapy given to breast cancer sufferers.40 Chemotherapy is a cancer treatment with anti-neoplasm cytotoxic drugs. Chemotherapy facilitates the antitumor immune response by reducing tumor burden and modifying the tumor microenvironment to enable a more effective immune response. Neo-adjuvant chemotherapy is chemotherapy that precedes primary therapy or primary therapy. Neo-adjuvant chemotherapy has been shown to reduce the size of the primary tumor and kill lymph node micro- metastases. With neo-adjuvant chemotherapy, tumor respectability is expected to be better.[24] In this study we found patients with poor chemotherapy response of 23.3%.

In this study immunity was assessed as a serum CD8 + level wherein the serum CD8 + level values obtained after neo-adjuvant chemotherapy dropped significantly compared to before chemotherapy. From the statistical analysis it was found that there were differences in serum CD8+ levels before and after chemotherapy (p = 0,000). This is different from Cabioglu

et al, where an increase in serum CD8 + after chemotherapy.36 According to Murta et al. There is also an increase in CD8 + in patients who have received FEC neo-adjuvant chemotherapy. This difference in results can be caused due to the immunity factors of breast cancer sufferers such as nutritional status, immune-modulators, infections and other factors. So it needs a comprehensive assessment in patients with breast cancer before chemotherapy and when undergoing chemotherapy so that the immune system after chemotherapy remains good. Previously chemotherapy had the concept of inducing bone marrow suppression which caused myelo-suppression and leucopenia.[36] So, the immune system to destroy cancer cells especially CD8 + also decreased. But in the latest concept, saying that cell death (apoptosis) triggers an immune system response and chemotherapy can increase lymphocyte cytotoxic responses and provide permanent anti-tumor immunity. Thus, chemotherapy causes cell death and releases tumor antigens that are processed by APC and activates specific tumor CD8 + T cells. Therefore chemotherapy forms an immunotherapy where immunity status before therapy can predict the ability of chemotherapy to destroy cancer cells.[41]

According to Seo A.N et al, a high CD8 + in tumor tissue can be a prognostic factor in the clinical outcome of breast cancer. Although it is still unclear why CD8 + is a major component of TILs associated with chemotherapy responses, this study shows that high CD8 + is a predictive factor for pCR in breast cancer patients treated with antracycline / taxane based. This is different from this study where CD8 + which was examined through blood did not show an increase after chemotherapy.[22] In this study it was found that serum CD8 + levels before chemotherapy had a sensitivity of 42.86% and specificity of 43.48%. This means that the ability of serum CD8 + levels before therapy to detect a good therapeutic response is 42.86% while the ability of serum CD8 + levels before therapy to detect poor response to therapy is 43.48%. Therefore, serum CD8 + levels cannot be used as predictors for determining chemotherapy responses in breast cancer such as CD8+ in tumor tissue.

5. Conclusion

There was a significant decrease in serum CD8 + levels after chemotherapy compared to before chemotherapy. The value of serum CD8 + before chemotherapy has a sensitivity of 42.86% and specificity of 43.48% with a cut point of 660.7 cells/mm^3. The value of serum CD8 + after chemotherapy has a sensitivity of 71.43% and a specificity of 69.57% with a cut point of 280.2 cells/mm^3.

6. References

1. Pusat Data dan Informasi Kemenkes RI. Situasi Penyakit Kanker dalam Buletin Jendela Data & Informasi Kesehatan. September 2015 ; hal 2-3
2. Komite Penanggulangan Kanker Nasional. Panduan Penatalaksanaan Kanker Payudara. Kementerian Kesehatan RI. 2015; hal 1
3. Purwanto H. Kanker Payudara Lanjut Lokal dan Kemoterapi Neoadjuvan sebagai Upaya Peningkatan Respon. Medicinus.2011.Vol 24;4-8
4. Ade, Y. Gambaran Penderita Kanker Payudara Usia Muda di RSUP Dr. Mohammad Hoesin Palembang. 2014
5. Khotimah S, Gondhowiardjo SA, Poetiray EC, Djoerban Z. Kemoradiasi Neoajuvan pada Kanker Payudara Lanjut Lokal. Radioterapi & Onkologi Indonesia. 2011; 2(1):1-4
6. Avril N, Sassen S, Roylance R. Response to therapy in breast cancer. JNuclMed.2009; 50: 55S-63
7. Kardinal CG, Cole JT, Perry MC. Breast Cancer. The Chemotherapy Source Book Ed. Philadelphia : Lippincott Williams & Wilkins; 2008.363-407
8. Wood WC dkk. Malignant Tumor of the breast In: De Vita, Helman S. Rosenberg SA Editor. Cancer Principles and practice of Oncology 8th Ed. Philadelphia: Lippincott; 2008:1145-77
9. Tiezzu DG, Andrade JM, Ribeiro-Silva A dkk. Her-2, p53, p21 and hormonal receptors protein expression as predictive factors of response and prognosis in locally advanced breast cancer treated with neoadjuvant docetaxel plus epirubicin combination. BMC Cancer. 2007; 7:1-10
10. Sahoo S, Lester SC. Pathology of Breast Carcinoma after Neoadjuvant chemotherapy. Arch Pathol Lab Med. 2009;133(4):633-42
11. Green MC, Giordano SH, Hortobagyi GN. Medical Therapy of Locally Advanced Breast Cancer in: Piccart M, Wood WC, Hung MC, Solin LJ et al editors. Breast Cancer