CD8+ Serum as a predictor of Neoadjuvant Chemotherapy Response in Locally Advanced Breast Cancer

Nur Qodir1*, Mulawan Umar1, Nopriansyah Darwin, Theodorus2

1 General Surgery Department of Dr Mohammad Hoesin Hospital Palembang, Indonesia
2 Departement of Pharmacology Faculty of Medicine Universitas Sriwijaya, Indonesia
E-mail: dokterhakim@yahoo.com

Abstract. CD8+ Cytotoxic T-cell Lymphocytes (CTLs) had a major role in tumour apoptosis. Meanwhile, chemotherapy could induce tumour cell apoptosis and enhance CTL response. Therefore, pre-treatment immune status might predict the ability of chemotherapy to eliminate cancer cells. The aim of this study was to evaluate the efficacy of neoadjuvant chemotherapy to CD8+ serum in locally advanced breast cancer patient. A randomized clinical trial has been conducted in Dr Mohammad Hoesin Palembang from October 2017 until January 2018. There were 30 samples who fulfil the inclusion criteria. All samples were taken neoadjuvant chemotherapy and analysis data was using SPSS ver.21. The range of age patient with locally advanced breast cancer in this study 30 - 66 y.o with mean 45±10.526 y.o. After neoadjuvant chemotherapy, the CD8+ serum was significantly decreased (p=0.000). CD8+ serum before chemotherapy has sensitivity 42.86% and specificity 43.48% to predict neoadjuvant chemotherapy response with cut off point 660.7 cell/mm3. Neoadjuvant chemotherapy decreases CD8+ significantly in locally advanced breast cancer patient.

1. Introduction
Breast cancer is the most common malignancy in women. International Agency for Research on Cancer (IARC) said that in 2012 breast cancer was the first number disease in the world as the new case (43.3%) and the most common cause of death (12.9%). More than 80% of breast cancer cases are found at an advanced stage [1]. Most of them (50-60%) are locally Advanced Breast Cancer (LABC) [2]. And the treatment of LABC is neoadjuvant chemotherapy. It aims to minimize tumour mass, allowing surgery and adjuvant therapy [3]. First line option is anthracycline-based chemotherapy [4,5]. Neoadjuvant chemotherapy reduces primary tumour mass, reduces lymphatic metastases in axilla and eradication of micrometastases, resulting in operability breast cancer can changes [6,7].

A neoadjuvant chemo-therapy response can be assessed by comparing the size of the mass before and after chemotherapy. The clinical response criteria used were RECIST [8]. Adaptive and innate response immunity plays an important role in immune surveillance of tumours and may limit the development and growth of neoplasms. Chemotherapy may precipitate an immune response that contributes to the treatment response [9-11]. In its development, tumour infiltrative lymphocytes (TILs) are an important predictive factor of chemotherapy response in breast cancer patients [12].

Cells from innate (neutrophils, monocytes, macrophages, and APC hosts) and adaptive immune systems (B and T lymphocytes) work together to respond the various pathogens, including tumour antigens. Innate immune cells are required by B and T cells to identify immuno-genetic proteins and allow adaptive immunity to form memory cells (lymphocytes that remain in the lymph nodes). Self-proteins in breast cancer that can stimulate T cells and induce immune responses. T-cell infiltration includes T helper (CD4+) and cytotoxic (CD8+) in which CD4+ T-helper facilitates antigen
presentation through cytokine secretion and activation of antigen presenting cells (APC), while CD8+ cytotoxic T-cells contribute to tumour destruction [12-14].

In breast cancer, cytotoxic CD8+ T-cell infiltration is closely related to patient long-term survival and good response to chemotherapy. Mao et al reported that CD8+ lymphocytes were the effective major cells in immune responses, which showed better disease-free survival in breast cancer patients [15].

Al Saleh et al. suggested that high CD8+ expression could predict significantly the pathologically complete response after neoadjuvant chemotherapy and represent an independent prognostic factor against Overall Survival (OS) [16]. According to Seo et al, CD8+ cytotoxic T lymphocytes are an important component of TILs which is associated with a chemotherapy response and can be used as a predictor of response to anthracycline or anthracycline/taxane-based on breast cancer [17]. According to Cabioglu et al, it was reported that the percentage of CD8+ cytotoxic suppressor T-cells increased following chemotherapy. While the percentage of CD4+/CD8+ ratios decreased with chemotherapy. Murta et al found that patients who received FEC neoadjuvant chemotherapy had an increase in CD8+ [18].

Considering all these aspects, investigators wanted to investigate the effects of neoadjuvant chemotherapy on the level of CD8+ plasma in LABC patients at Dr Mohammad Hoesin General Hospital in Palembang.

2. Methods

Indonesia more than 80% of cases are found at an advanced stage 1 Locally Advanced Breast Cancer (LABC) is still the largest (50-60%) of cancer patients coming to the clinic or hospital in Indonesia. It is a non-comparable clinical trial by looking at serum CD8+ levels in patients with locally advanced breast cancer before and after neoadjuvant chemotherapy administration at Dr Mohammad Hoesin General Hospital in Palembang. There were 30 samples who fulfil the inclusion criteria.

Sampling is done by consecutive sampling, all subjects who come and meet the criteria of sample selection are included in the study until the required number of subjects is met. All patients were given informed consent prior to participating as a sample of the study. CD8+ examined with flow cytometry BD FACSCanto.

Characteristics of breast cancer patients divided by age, birth control history, family history, and clinicopathology are described descriptively and data are presented in tabular form and analysed using paired T-test to determine effectiveness with SPSS program version 21.

3. Results

General Characteristics Based on age, the mean age of patients with advanced breast cancer is 45.97 ± 10.526 years old with age range 30-66 years, 15 (50%) people with ≥ 40 years old and 15 (50%) people with <40 years old. The majority of patients with locally advanced stage breast cancer patients (96.7%) have given birth and only 1 person (3.3%) have never given birth.

Locally advanced breast cancer patients with a hormonal contraception history were 17 (56.7%) and 43.3% had no hormonal contraception. 13 people (43.3%) with advanced breast cancer have a history of the same disease in the family.

In this study, there is positive ER in 20 people (66.7%), positive PR in 25 people (83.3%) and Her2/Neu positive in 9 people where +3 Her2/Neu in 4 (13.3%) people, +2 Her2/Neu in 3 people and 2 people (6.7%) have + Her2. Ki67 levels ≥ 20% were 15 patients (50%) and <20% were 9 patients (30%). 30 patients with locally advanced stage breast cancer, 23 patients have a good chemotherapy response (76.7%) and 7 patients with the poor response (23.3%).

From the statistical analysis, there was a difference between serum CD8+ levels before and after chemotherapy (p = 0.000) in which serum CD8+ levels after chemotherapy decreased significantly compared before chemotherapy. The value of serum CD8+ levels before chemotherapy had a sensitivity of 42.86% and a specificity of 43.48% with a cut of point 660.7 cells/mm³. The value of serum CD8+ after chemotherapy has a sensitivity of 71.43% and specificity of 69.57% with a cut of point 280.2 cells/mm³.
4. Discussion
In this study, the mean age of breast cancer patients was 45.97 ± 10.526 years with age range 30-66 years, where patients were found to be ≥ 40 is equal with <40 years (50%). The average age of Arzu et al study in 2017 is higher than the current study (51.1 ± 6.7 years old). FZ Lamiri et al study in 2015 found that the average age of breast cancer patients is 45.83 ± 11.05 where patients with age ≥ 45 years old (58%) are more than patients <44 years old (42%) [19].

![Figure 1. General Characteristic](image)

**Table 1. Clinicopathology characteristic**

| Characteristic | N  | %   |
|----------------|----|-----|
| ER Positive    | 20 | 66.7|
| ER Negative    | 10 | 33.3|
| PR Positive    | 25 | 83.3|
| PR Negative    | 5  | 16.7|
| HER2 Positive1(+) | 2 | 6.7 |
| HER2 Positive2(+++) | 3 | 10.0 |
| HER2 Positive3(++++) | 4 | 13.3 |
| HER2 Negative  | 21 | 70.0|
| Ki67 ≥ 20%     | 15 | 50.0|
| Ki67 < 20%     | 9  | 30.0|
| Ki67 Negative  | 6  | 20.0|
| Chemotherapy Response Poor | 7  | 23.3|
| Chemotherapy Response Good | 23 | 76.7|

**Table 2. Effectiveness of Neoadjuvant Chemotherapy to CD8⁺**

| Variable   | Before Therapy | After Therapy | Changes | P*   |
|------------|----------------|---------------|---------|------|
| CD8⁺      | 658.26 ± 370.15 | 462.73 ± 309.79 | 195.5 ± 151.15 | 0.000|

"Wilcoxon, p= 0.05"

**Table 3. Sensitivity and Specificity of CD8⁺ value to Chemotherapy response**

| Variable       | Cut Off      | Sensitivity | Specificity |
|----------------|--------------|-------------|-------------|
| CD8⁺ Before Therapy | 660.7 cells/mm³ | 42.86% | 43.48% |
| CD8⁺ After Therapy  | 280.2 cells/mm³ | 71.43% | 69.57% |

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The history of hormonal contraception in this study (56.7%) is lower than the FZ Lamiri study in 2015 (74.5%) [20]. In this study patients with a family history of breast cancer were 43.3%. However, a study conducted by F.Z Lamiri et al in 2015 showed only 14.3% of patients with a family history of breast cancer.

According to MD Anderson, a family history of breast cancer increases the risk of breast cancer. Risks generally depend on the number of families suffering from breast cancer, age at diagnosis and unilateral or bilateral breast cancer [21].

![Figure 2. Cut off sensitivity and specificity, CD8+ value prior to chemotherapy of locally advanced breast cancer patients](image)

Immunohistochemistry characteristics of breast cancer patients in this study showed that positive ER of 66.7%, PR of 83.3% and Her2/Neu +3, +2 and +1 of 13.3%, 10% and 6.7% respectively. Ki67 levels ≥ 20% in breast cancer patients in this study were similar to KI67 ≤20%. Immunohistochemistry is used not only for the prognosis but also determines the therapy for breast cancer patients [22].

By statistical analysis using the ROC curve, cut off point of CD8+ serum before neoadjuvant chemotherapy of 660.7 cells/mm$^2$ while the serum CD8+ serum levels after chemotherapy of 280.2 cells/mm$^2$.

Chemotherapy facilitates the antitumor immune response by reducing tumour burden and modifying the tumour microenvironment to enable a more effective immune response. Neoadjuvant chemotherapy has been shown to reduce the size of primary tumours and kill micrometastases lymph nodes. It is expected that tumour resectability is better [23]. In this study, patients with poor chemotherapy response were 23.3%.

In this study, the assessed immunity was serum CD8+ level, whereas serum CD8+ levels after neoadjuvant chemotherapy significantly decreased than before. From the statistical analysis showed that there was a difference between serum CD8+ levels before and after chemotherapy (p = 0.000). This is in contrast to Cabioglu et al, where there is an increase in serum CD8+ after chemotherapy. According to Murta et al, there is also an increase in CD8+ in patients who have received neoadjuvant FEC chemotherapy. This may be due to immune factors in breast cancer such as nutritional status, immuno-modulatory and other factors. It needs a comprehensive assessment of breast cancer patients so that the immune system after chemotherapy is still good.

Previously, chemotherapy has the concept of inducing bone marrow suppression that causes myelosuppression and leucopenia. So that the immune system to destroy cancer cells especially CD8+ also decreases. However, in a recent concept, suggested that the cell death (apoptosis) triggers an immune system response and chemotherapy can improve the cytotoxic response of lymphocytes and provide permanent anti-tumour immunity. Thus, chemotherapy causes cell death and releases antigen tumours that are processed by APC and activates specific tumours of CD8+ T cells. Therefore,
chemotherapy establishes an immunotherapy, where the immune status before therapy can predict the ability of chemotherapy to destroy cancer cells [24].

According to Seo A. N et al, high CD8+ in tumour tissue can be a prognostic factor in the clinical outcome of breast cancer. Although it is unclear why CD8+ is a major component of TILs associated with the chemotherapy response but this study shows that high CD8+ is a predictive factor for PCR in breast cancer patients treated with anthracycline/taxane-based. It is contrary to this study in which blood CD8+ did not show improvement post-chemotherapy. In this study, serum levels of CD8+ before chemotherapy had a sensitivity of 42.86% and specificity of 43.48%.

5. Conclusion
This study is representing that the ability of CD8+ serum levels before therapy to detect a good treatment response of 42.86% while the serum CD8+ serum levels before therapy to detect a poor treatment response of 43.48%. Therefore, CD8+ serum levels cannot be used as a predictor factor to determine the chemotherapy response in breast cancer like CD8+ in tumour tissue.

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