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Association between Ki67 After Neoadjuvant Chemotherapy and Disease-Free Survival in Breast Cancer

Aan Setiawan[1], Mulawan Umar[2*] Erial Bahar[3]

[1]Department of Surgery, Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia

[2]Oncology Unit, Surgery Department of Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia

[3]Department of Anatomy Faculty of Medicine Sriwijaya University, Palembang, Indonesia

*Corresponding Author Email: mulawanumar@yahoo.com

Abstract

Background: Post Chemotherapy Ki67 in recent year has been investigated as a predictive and prognostic factor in locally advanced breast cancer patient undergoing neoadjuvant chemotherapy.

Objectives: To describe the relationship between post-chemotherapy Ki67 and disease-free survival after neoadjuvant chemotherapy in patients with locally advanced breast cancer at Dr. Hospital. Mohammad Hoesin Palembang.

Methods: This study is an analytical observational study with prognostic test design conducted in 30 stage III B breast cancer patient. A retrospective search of a prospectively maintained clinical database was performed to identify patient treated with neoadjuvant chemotherapy at the Mohammad Hoesin Hospital. The expression of Ki67 was assessed using immunohisto-chemistry in post therapy surgical excision specimen.

Results: From 30 patients, there was a significant relationship between Post Chemotherapy Ki67 and disease- free survival in patients with locally advanced breast cancer (r = -0,742, p = 0.000). The cut off point value of Ki67 was 27,5% with an area under curve (AUC) of 0,716. The results of the post-chemotherapy Ki67 prognosis test included sensitivity 64%, specificity 60%, Positive Predictive Value 88,9%, Negative Predictive Value 25%, accuracy 63,3%.

Conclusion: There is a significant relationship between Post Chemotherapy Ki67 to disease free survival and Post Chemotherapy Ki67 can be used as a prognostic biomarker in breast carcinoma patients undergoing neoadjuvant chemotherapy.

Keywords: Post Chemotherapy Ki67, disease free survival, neoadjuvant chemotherapy, breast cancer

1. Introduction

Cancer is one of the leading causes of death worldwide. In 2012, approximately 8.2 million deaths in Indonesia were caused by cancer. Breast cancer is one of the most common types of cancer in Indonesia. Based on Pathological Based Registration in Indonesia, breast cancer ranks first with a relative frequency of 18.6%. It is estimated that the incidence rate in Indonesia is 12 / 100.000 women[1,2].

According to the Indonesian Cancer Foundation, currently breast cancer is the leading cause of death with a mortality rate of 198.000 people per year. Among them there are 882.9 cases (per 100.000) originating from developing countries. In Indonesia, more than 80% of breast cancer cases are found to be at an advanced stage. Currently, locally advanced breast cancer is still the largest proportion (50-60%) of cancer patients who come to polyclinics or hospitals in Indonesia. In RSUP Mohammad Hoesin Palembang in 2014, the highest distribution of clinical stages was stage III, namely stage III A 26.53% and stage III B 48.98% [3-6].

Breast cancer management includes surgery, radiotherapy, chemotherapy and hormonal therapy. Chemotherapy is a treatment using a combination of drugs that aims to destroy or slow down the growth of cancer cells. Currently chemotherapy is a very important component in the treatment of breast cancer[7]. Neoadjuvant chemotherapy has become the standard in the management of locally advanced breast cancer and is the therapy of choice in operable early-stage breast cancer. Neoadjuvant chemotherapy has many advantages in the management of breast cancer, including providing a better surgical option and being able to assess chemotherapy response [7-8].

Neoadjuvant therapy in stage III B breast carcinoma patients aims to increase disease free survival, reduce the risk of relapse and reduce mortality. The advancement of neoadjuvant therapy causes the mortality rate to decrease sharply, but the incidence of relapse is still quite common[9].

Research conducted by Gunter et al in Germany stated that the recurrence rate was 39.5% and the mortality rate was 23.6% of the 667 patients observed for 5 years10. Relapse incidence is often associated with an increased risk of death regardless of type of treatment[11].

The 2009 St Gallen Consensus stated that Ki67 could be a potential biomarker for prognosis and chemotherapy indicators in advanced breast cancer.[12] The St Gallen Consensus in 2011 and 2013 concluded that research in the last 32 years of Ki67 has been widely evaluated as a prognostic and / or predictive marker. breast cancer and other types of tumors. Ki67 has been suggested to be a biomarker for the definition of luminal A and luminal B in tumors.[13-15] However, cancer biomarkers are still not completely integrated in clinical decision making and the International Ki67 in Breast Cancer Working Group in 2011 reported that no cut of point was found ideal in clinical practice for the Ki67 value as a breast cancer Biomarker[2].

Ki67 is an antigen that is closely related to the cell cycle and mitosis, so the percentage of Ki67 represents the proliferative fraction of cancer.[11] Where Ki67 is expressed in all phases of the cell cycle except G0 and at the peak of phase M, so it is very appropriate to be used as a tumor proliferation biomarker.[11] In breast cancer those with high risk have higher Ki67 expression, so they will have a worse prognosis when compared to tumors that give a lower image of Ki67 expression.16 Breast cancer also describes high Ki67 with low interpretation for Ki67 levels <14%; intermediate for levels of Ki67 14% -30% and high for levels> 30% .[17]

A systematic study conducted by Luporsi et al[18] concluded that the results of a prognostic test performed by ki67 can be used to determine which follow-up therapy will be selected clinically if it is presented in a journal that is complete and clearly reports according to guidelines, such as REMARK criteria[19]. Simon et al obtained results from a systematic review that determines the level of evidence (LoE) on various variations of clinical data in breast cancer trial studies, the role of Ki67 can be used as a prognostic marker[20]. Two different settings to differing performance of Ki67 for predicting chemotherapy response, Ki67 was significantly associated with clinical or pathophysiological response in 7 of 9 studies[21-22].

A systematic review conducted by Luporsi et al reported that Ki67 for LoE at III B was associated with neoadjuvant response[18]. In the second setting, the study was about predictive evaluation of survival in response to neoadjuvant therapy. In this case, the role of Ki67 as a chemotherapy response versus no chemotherapy has been observed and there is a significant relationship with a strong correlation between the role of Ki67 as a biomarker and survival evaluation

in response to neoadjuvant and adjuvant therapy[18-19]. Therefore, in contrast to the neoadjuvant situation, the predictive role for Ki67 may not necessarily be established in adjuvant chemotherapy trials.[20]

Fasching et al in their study showed that it is impossible to determine the best cut off point for Ki67, because the cut off point for each breast cancer subtype is different. In his research, Fasching used a cut off point of Ki67 > 13%, obtained Ki67 as an independent predictor for pathological complete response (pCR) (OR 3.5; 95% CI, 1,4, 10.1) and for overall survival (HR 8.1; 95%). CI, 3.3 to 20.4) and distant disease-free survival (HR 3.2; 95% CI, 1.8 to 5.9) [21].

2. Methods

This study is an observational analysis study with a prognostic test design. The prognostic test is a study that aims to make the best model that can estimate the output value or the likelihood of output or the possibility of a subject with certain characteristics in the future. In this study a prognostic test was carried out to determine the relationship between Ki67 levels and the prognosis of stage III B breast cancer patients underwent neoadjuvant chemotherapy at Dr. Hospital. Mohammad Hoesin Palembang

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 stage III B breast cancer who have undergone surgery following neoadjuvant chemotherapy. The exclusion criteria were patients without available ki67 staining in the surgical specimen after neoadjuvant chemotherapy.

There were 30 subjects in this study. The independent variables were age and Ki-67 levels post neoadjuvant chemotherapy. The dependent variable was disease free survival. The confounding variables were ER, PR, Her2 receptors, lymph nodes status, histo-pathological grading, adjuvant therapy and obesity

The data were analyzed using the Kendall Tau, Wilcoxon, Spearman rho's, Kaplan Meier and Cox Regression methods as well as the ROC analysis to obtain the area under curve (AUC), sensitivity and specificity. The data were presented in tables and flowcharts which will be analyzed univariate, bivariate and multivariate using SPSS 23.

3. Results

Characteristics distribution

There were 30 patients with stage III B breast cancer and met the inclusion criteria. Examination of Ki67 patients was performed before and after neoadjuvant chemotherapy. Ki67 is divided into low (<14%), medium (14-30%) and high (> 30%). In this study, 10 samples were obtained with Ki67 post chemotherapy neoadjuvant low, medium and high respectively 10 samples (33.3%).

In this study, with the Kendall tau-c and Kendall tau-b tests it was found that there was no relationship between age (r = - 0.267; p = 0.203), grade (r = 0.178; p = 0.330), lymph node status (r = - 0.178; p = 0.318), adjuvant therapy (r = 0.158; p = 0.406), body mass index (r = 0.000; p = 1.000) and cancer subtype (r = - 0.053; p = 0.728) with Ki67 level post neoadjuvant chemotherapy. The results of the study are presented in table 1.

Table 1. Ki67 level post neoadjuvant chemotherapy based on sample characteristics

Characteristics	Ki67 level post neoadjuvant chemotherapy			r	p
	Low (< 14%) (n = 10) n (%)	Medium (14-30%) (n = 10) n (%)	High (> 30%) (n = 10) n (%)		
Age					
≤ 50 years old	3 (30)	0 (0)	6 (60)	-0.267	0.203[a]
> 50 years old	7 (70)	10 (100)	4 (40)		
Grading					
2	4 (40)	5 (50)	2 (20)	0.178	0.330[a]
3	6 (60)	5 (50)	8 (80)		
Lymph node status					
Positive	6 (60)	7 (70)	8 (80)	-0.178	0.318[a]
Negative	4 (40)	3 (30)	2 (20)		
Adjuvant therapy					
None	2 (20)	0 (0)	1 (10)	0.158	0.406[b]
Hormonal	7 (70)	9 (90)	7 (70)		
Radiotherapy	1 (10)	1 (10)	2 (20)		
Body mass index					
Underweight	1 (10)	2 (20)	1 (10)		
Normalweight	8 (80)	6 (60)	8 (80)	0.000	1.000[a]
Overweight	0 (0)	2 (20)	0 (0)		
Obese	1 (10)	0 (0)	1 (10)		
Cancer Subtype					
Luminal A	1 (10)	2 (20)	1 (10)		
Luminal B	6 (60)	8 (80)	7 (70)	-0.053	0.728[a]
Her2Neu	2 (20)	0 (0)	1 (10)		
Triple negative	1 (10)	0 (0)	1 (10)		

[a]*Kendall tau-c*, p = 0,05

[b]*Kendall tau-b*, p = 0,05

The effectiveness of neoadjuvant chemotherapy against Ki67 level on stage III b breast cancer patients

The mean of pre-neoadjuvant chemotherapy Ki67 level was 44.30 ± 24.41 with a range of 0 - 90%, then post-neoadjuvant chemotherapy the mean of Ki67 level decreased to 25.77 ± 17.26 with a range of 1-60%. It was found that 20 of the 30 samples showed a decrease in Ki67, 8 samples showed an increase and 2 samples did not show a change in Ki67. With the Wilcoxon statistical analysis, it was found that there was a significant change in Ki67 before and after neoadjuvant chemotherapy (p = 0.004). The results of the study are presented in table 2.

Table 2. The effectiveness of neoadjuvant chemotherapy to Ki67 level

Characteristics	Time		p
	Pre-chemotherapy	Post- chemotherapy	
Ki67 (%)			
• Mean ± SD	44.30 ± 24.41	25.77 ± 17.26	0.004
• Median	40	30	
• Min – Max	0 - 90	1 - 60	

The correlation between Ki67 post neoadjuvant chemotherapy and disease-free survival (DFS) in stage III b breast cancer patients.

The mean of Disease-Free Survival (DFS) was 16.50 ± 11.98 with a range of 2 - 36 months. With the Spearman rho test, it was found that there was a significant negative correlation between Ki67 post neoadjuvant chemotherapy and DFS (r = - 0.742; p = 0.000). The higher Ki67 post neoadjuvant chemotherapy, the shorter the Disease-Free Survival / DFS, and vice versa. The results are presented in table 3.

Table 3. The correlation between ki67 post neoadjuvant chemotherapy and disease-free survival (DFS)

Characteristics	Ki67 post chemotherapy	Disease Free Survival	r	p
• Mean ± SD	25.77 ± 17.26	16.50 ± 11.98		
• Median	30	12.5	-0.742	0.000
• Min – Max	1 - 60	2 - 36		

The survival analysis of stage III B breast cancer patients.

The results showed that there was no difference in the DFS of breast cancer patients based on age (p = 0.142). Patients ≤ 50 years old were 0.539 times more likely to be relapse-free for up to 3 years than patients > 50 years old age but not significant (HR = 0.539; p = 0.154). However, based on the grade of disease, there was a difference in the DFS of breast cancer patients (p = 0.006). Patients with grade 2 were 3.531 times more likely to be relapse-free for up to 3 years than patients with grade 3 (HR = 3.531; p = 0.010).

In addition, there was no difference in the DFS of breast cancer patients based on lymph node status (p = 0.084). Patients with positive lymph node status were 0.433 times more likely to be relapse free for up to 3 years than patients with negative lymph node status but not significant (HR = 0.443; p = 0.096). Likewise, based on adjuvant therapy, it was found that there was no difference in the DFS of breast cancer patients between the three adjuvant therapies (p = 0.413). Patients without therapy had a 1.761 time more likely to be relapse-free for up to 3 years compared to patients with hormonal therapy but not significant (HR = 1.761; p = 0.453) and patients without therapy had 2.996 times more likely to be relapse free for up to 3 years compared to patients with radiotherapy therapy but not significant (HR = 2.996; p = 0.216)

In this study, the results also showed that there was no difference in the DFS of breast cancer patients based on body mass index (p = 0.785). Patients with normal BMI were 1,051 times more likely to be relapse free for up to 3 years than underweight patients but not significant (HR = 1.051; p = 0.957) and overweight patients were 1.207 times more likely to be relapse free for up to 3 years than underweight patients but not significant (HR = 1.207; p = 0.801) while obese patients had a 0.451 chance of being relapse free for up to 3 years compared to underweight patients but not significant (HR = 0.451; p = 0.516).

Based on the cancer subtypes, there was no difference in the DFS between the four cancer subtypes (p = 0.772). Patients with luminal A subtype were 1.176 times more likely to be relapse free for up to 3 years than patients with luminal B subtype but not significant (HR = 1.176; p = 0.795) and patients with luminal A subtype had 0.626 times more likely to be relapse free for up to 3 years than patients with Her2Neu subtype but not significant (HR = 0.626; p = 0.611), while patients with luminal A subtype had 1.686 times the chance to be relapse free for up to 3 years compared to patients with triple negative but not significant (HR = 1.686; p = 0.571).

Based on Ki67 post chemotherapy, there was a difference in DFS between the three levels of Ki67 (p = 0.000). Patients with low Ki67 levels had 1.342 times more likely to be relapse free for up to 3 years compared to patients with medium but not significant (HR = 1.342; p = 0.573) while patients with low Ki67 levels were 13.856 times more likely to be relapse free for up to 3 years compared patients with a high Ki67 level (HR = 13.856; p = 0.000)

Table 4. Survival analysis of stage III B breast cancer patients

Characteristics	Disease Free Survival (months)	p*	3 years relaps free	p**
Age				
• ≤ 50 years old	11.778 ± 3.937	0.142	0.539	0.154
• > 50 years old	18.524 ± 2.436			
Grade				
• 2	23.00 ± 3.965	0.006	3.531	0.010
• 3	12.74 ± 2.111			
Lymph node status				
• Positive	14.09 ± 2.121	0.084	0.443	0.096
• Negative	22.11 ± 4.676			
Adjuvant therapy				
• None	22.67 ± 8.555	0.413		
• Hormonal therapy	16.35 ± 2.342		1.761	0.453
• Radiotherapy	12.75 ± 5.977		2.996	0.216
Body mass index				
• Underweight	15.75 ± 5.888	0.785		
• Normoweight	15.64 ± 2.516		1.051	0.957
• Overweight	24.50 ± 8.132		1.207	0.801
• Obese	19.50 ± 7.500		0.451	0.516
Cancer Subtype				
• Luminal A	16.25 ± 6.097	0.772		
• Luminal B	15.95 ± 2.382		0.593	0.571
• Her2Neu	22.67 ± 8.555		0.698	0.633
• Triple Negative	13.50 ± 11.50		0.372	0.329
Ki67				
• Low	25.20 ± 2.403	0.000		
• Medium	19.00 ± 3.758		1.342	0.573
• High	5.300 ± 1.033		13.856	0.000

Table 5. Prognostic value of Ki67 post neoadjuvant chemotherapy against 3 years disease free survival

Diagnostic Value	
• Sensitivity	64 %
• Specificity	60 %
• Positive Predictive Value	0.889
• Negative Predictive Value	0.250
• Positive Likelihood Ratio	1.600
• Negative Likelihood Ratio	0.600
• Accuracy	0.633
• AUC	0.716 (CI95% 0.501 – 0.931)

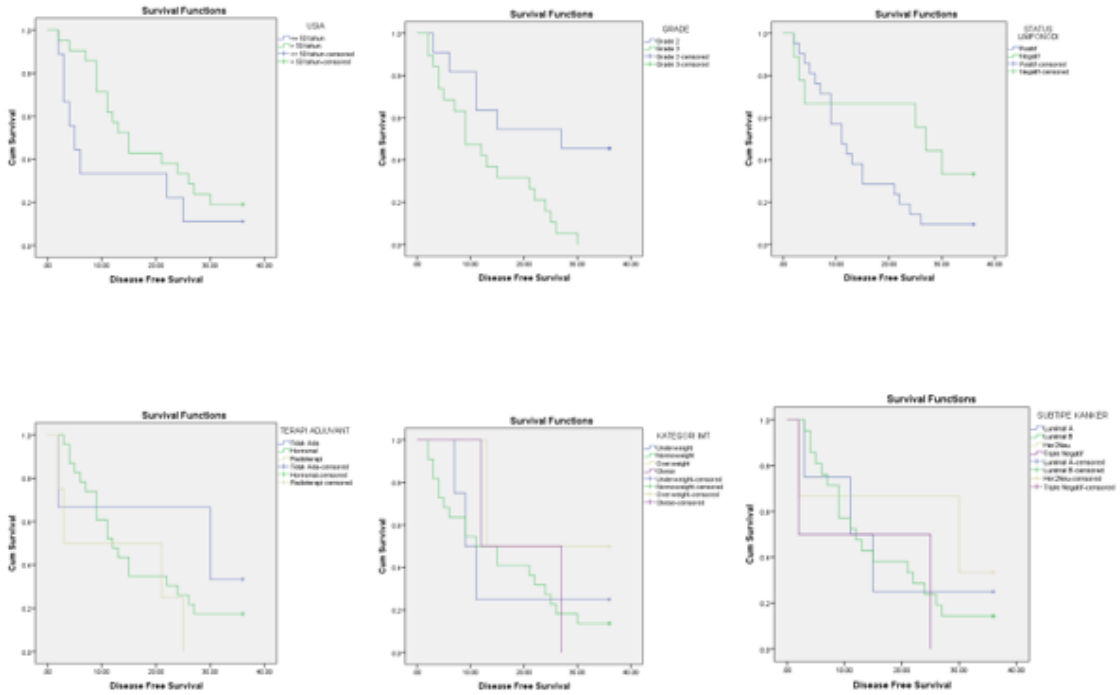

Figure 1. Kaplan-Meier curves with 3 years disease free survival rates according to age. grading, lymph node status, adjuvant therapy, body mass index, and breast cancer subtype

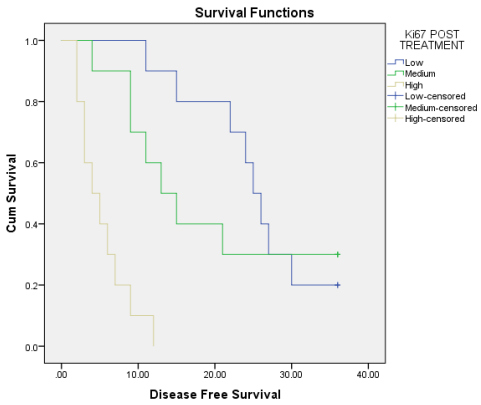

Figure 2. Kaplan-Meier curves with 3 years disease free survival rates according to Ki67 level post neoadjuvant chemotherapy

Prognostic value of Ki67 post neoadjuvant chemotherapy against 3 years disease free survival

Analyzes were performed with the receiver operating curve (ROC) curve to find the Ki67 cut off point to obtain prognostic sensitivity and specificity values. The value of Ki67 which has the best sensitivity and specificity is obtained at a value of 27.5%.

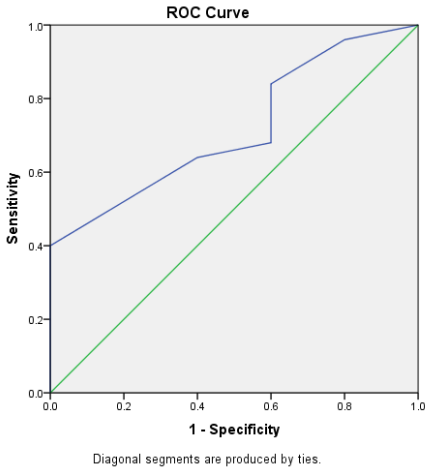

Figure 3. ROC curve for the prognostic value of Ki67.

4. Discussion

In this study, it was found that the majority of breast cancer patients who had Ki67 low levels were > 50 years old, patients with medium Ki67 levels were > 50 years old, while majority patients with Ki67 high levels were ≤ 50 years old, but statistically there was no relationship between age and the

level of Ki67 post chemotherapy. Research conducted by Tan et al, in 2014 also reported no relationship between age and Ki67 level (p = 0.358), all Ki67 categories, both low, medium and high, were majority aged ≤ 50 years.[17] Another study conducted by Hertati et al. In 2014, the results also showed that there was no significant relationship between Ki-67 expression and the age group.[56]

Grade 3 breast cancer was found more frequently at the high Ki67 level, while at the low and medium grade 2 and grade 3, Ki67 levels were found almost the same. However, statistically there was no significant relationship between grade and Ki67 level in this study. The results of this study are in line with the study by Tanei et al., In 2011 which reported no relationship between tumor grade and Ki67 level (p = 0.100).[57] However, Tan et al's study gave different results, grade 3 was more common at high Ki67 level, while at low and medium Ki67 level, the majority of grades 1 and 2 were found, besides that there was a significant difference in grade between the Ki67 levels (p = 0.026).

The majority of patients had positive lymph node status both at low, medium and high Ki67 levels, the higher the Ki67 level the greater the percentage of positive lymph nodes status, but statistically there was no significant relationship between lymph node status and Ki67 level. These results are supported by the research of Von Minckwitz et al., In 2013 which stated that the majority of patients had positive lymph node status both at low, medium and high Ki67 levels, the higher the Ki67 level the greater the percentage of positive lymph node status, but not significant (p. = 0.089) .[58] Based on the type of adjuvant therapy and body mass index, there was also no significant relationship between the two variables with the Ki67 level. Likewise with the cancer subtype, there was no relationship between the cancer subtype and the Ki67 level, the majority of all Ki67 levels had luminal B cancer subtype.

With statistical analysis in this study, the results showed that there was no relationship between age, grade, lymph node status, adjuvant therapy, body mass index and cancer subtype, so it can be concluded that the Ki67 level of post-neoadjuvant chemotherapy was not influenced by age, grade, lymph node status, adjuvant therapy, body mass index and cancer subtypes.

By statistical analysis, it was found that there was a significant relationship between Ki67 and DFS. The relationship between these two variables is negative, which means that if the Ki67 level got high, the DFS got short.

With survival analysis, it was found that there were differences in DFS based on tumor grade and Ki67 level. Patients with tumor grade 2 had a longer DFS and had 3.531 times a chance of being relapse-free for up to 3 years than patients with tumor grade 3. In addition, it was found that patients with high Ki67 levels had the lowest DFS. Patients with a low Ki67 level had 1.342 times a chance to be relapse-

free for up to 3 years compared to patients with a medium Ki67 level and 13.856 times a chance to be relapse free for up to 3 years compared to patients with a high Ki67 level. In line with this study, the study by Chen et al. Reported that patients with tumor grade 1-2 had a longer DFS and 3.422 times a chance of relapse-free up to 5 years than patients with tumor grade 3.

Some literature suggests that high levels of Ki-67 expression are associated with a poor prognosis. Several studies have examined the prognostic significance of Ki-67 in breast cancer. Studies have shown that Ki-67 overexpression correlates with disease free survival and overall survival. In contrast, patients with tumors with high proliferation rates have a better response to chemotherapy. [43.51-52].

In this study, the Ki67 cut-off point was 27.5%. The prognostic test show that Ki67 have a sensitivity of 64% and specificity of 60% in predicting DFS. This means that as many as 64% of stage IIIB breast cancer patients with a Ki67 level ≥ 27.5% relapse within 3 years and 60% of patients with a level < 27.5% do not experience relapse until 3 years. Positive predictive value value (PPV) 88.9% means that the possibility of stage III B breast cancer patients experiencing a relapse is 88.9% if post-adjuvant chemotherapy Ki67 level ≥ 27.5% and negative predictive value (NPV) 25% means the possibility of stage III breast cancer patients. III B did not experience a relapse, only 25% if the post-adjuvant chemotherapy Ki67 level was <27.5%. The accuracy of the Ki67 level in predicting the incidence of relapse in stage III B breast cancer patients is good, this means that the Ki67 level can be used to predict the incidence of relapse 3 years after chemotherapy.

The research by Chen et al. Found that the Ki67 cutoff point was smaller, namely 25%. The Ki67 level has a sensitivity of 65.6% and a specificity of 68.3% in predicting the incidence of relapse after neoadjuvant chemotherapy in stage III breast cancer patients. Kilickap et al. Study, in 2014 found the cut point of Ki67 was 20%, the Ki67 level in the study it has a sensitivity of 93% and specificity of 66% in predicting the incidence of relapse within 5 years after neoadjuvant chemotherapy in stage III breast cancer patients B.59

5. Conclusions

There was a significant correlation between Ki67 post neoadjuvant chemotherapy and disease-free survival on stage III B breast cancer patients. The higher the Ki67 level, the shorter the Disease-Free Survival / DFS. The post neoadjuvant chemotherapy Ki67 could be used as a prognostic marker on stage III B breast cancer patients.

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