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Relationship of Expression Ki67 Pre Neoadjuvant Chemoterapy with Prognosis in Stadium IIIB Breast Cancer Patients that Performed Mastectomy Surgery at Dr. Mohammad Hoesin Hospital Palembang

Mulawan Umar[1*], Aulia Muhardi[1], Erial Bahar[2]

[1] General Surgery Department, Dr. Mohammad Hoesin Hospital, Palembang, Indonesia
[2] Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

A B S T R A C T

Background: Breast cancer is 42.1 per 100,000 population with an average of 17 deaths per 100,000 population. Breast cancer management includes surgery, radiotherapy, chemotherapy and hormonal therapy. Neoadjuvant therapy has a several goals in breast cancer: reducing the volume of initially inoperable tumors, evaluating in vivo chemosensitivity and analyzing micrometastasis management. Cell proliferation is often measured through Ki67 resulting in independent prognostic markers and as predictive markers of responsiveness or resistance to chemotherapy or endocrine therapy. In breast cancer with high risk has a higher expression of Ki67, so it will have a worse prognosis. This study was conducted to find out the relationship of Ki67 pre chemotherapy neoadjuvant levels with prognosis in patients with stage IIIB breast cancer who performed mastectomy surgery at Moehammad Hoesin Palembang General Hospital. **Methods:** Cohort Retrospective research on 40 samples Breast cancer patients in the Medical Record Room and Anatomical Pathology Room of Dr. Moehammad Hoesin Palembang in July-September 2021. **Results:** There were 14 out of 40 samples that had high Ki67 levels (>30%), chi square and fisher exact analysis showed Ki67 levels >30% were significantly associated with mortality (p<0.05, OR 13,8(2,7-70,2)). At cut off points Ki67 >20% is also significantly associated with mortality (p<0.05, OR 7,1 (0,8-63,1)) with a sensitivity value of 91,6% and specificity of 39,2%. **Conclusion:** Ki67 >20% can be a worse prognosis in predicting death and overall survival.

1. Introduction

Breast cancer is the most common malignancy and the leading cause of death in women worldwide. According to World Health Organization (WHO), 8-9% of women will develop breast cancer. The chance of cancer growing rapidly depends on age, the older the age the faster the cancer develops.[1] Various factors are suspected and related to the incidence of breast cancer including gender, age, family history, genetic disorders, race, hormones, radiation, obesity and dietary factors.

Breast cancer management includes surgery, radiotherapy, chemotherapy and hormonal therapy.

Chemotherapy is the treatment using a combination of drugs that aims to destroy or slow the growth of cancer cells.[5] Neoadjuvant chemotherapy has become the standard in the treatment of locally advanced breast cancer and is the treatment of choice for operable early stage breast cancer. The advantages in breast cancer treatment is to reduce tumor volume that were initially inoperable, evaluate chemosensitivity in vivo and evaluate micrometastases.

Ki67, a nuclear protein that is expressed exclusively during the active phase of the cell cycle,

holds great promise as an independent prognostic marker and as a predictive marker of responsiveness or resistance to chemotherapy or endocrine therapy.

This study aims to determine the association between Ki67 levels pre neoadjuvant chemotherapy and prognosis in stage IIIB breast cancer patients who underwent mastectomy surgery at Dr Mohammad Hoesin Hospital Palembang.

2. Methods

This study was an observational analysis with a retrospective cohort design using secondary data. The collection of samples used consecutive sampling where every patient with stage III B breast cancer who underwent neoadjuvant chemotherapy and underwent surgery after chemotherapy was included in the study for a certain period of time until the sample was met.

This research was conducted in the medical record room and anatomical pathology room, Dr. Mohammad Hoesin Hospital Palembang. Research data collection was carried out on July-September 2021.

All patients with stage III B breast cancer who underwent neoadjuvant chemotherapy and underwent surgery after chemotherapy would be included as inclusion criteria. Patients who did not have a history of Ki67 examination before surgery and whose medical record data were incomplete or loss of follow-up were excluded from this study.

This study was analyzed using the method chi-square or Fisher's exact test and ROC analysis to obtain the AUC, sensitivity and specificity values. This research data will be presented in the form of tables and flowcharts which will be analyzed univariate, bivariate and multivariate using SPSS 25.

3. Results
Demographic characteristics

In 40 research samples, 16 (40%) of them are 50 years old with a mean age of 53.5 (34-69) years. The majority of samples have a normal body mass index (BMI) of 28 (70%) with a median of 22.03 (16.02 to 38.79). (Table 1)

Table 1. Demographic characteristics

	n	%
Age, Mean	53,5 (34-69)	
Age Classification		
≤50 years	16	40
>50 years	24	60
BMI, Mean	22,03 (16,02-38,79)	
BMI		
Normal	28	70
Under	5	12,5
Over	7	17,5
Histopatology grading		
Grade 3	24	60
Grade 2	16	40
Lymph Nodes Status		
N3	14	35
N2	16	40
N1	7	17,5
N0	3	12,5
Cancer Subtypes		
Luminal A	5	12,5
Luminal b	26	65
Triple negative	5	12,5
Her2neu	4	10
Mortality		
Alive	28	70
Died	12	30

Histopathological grading was dominated by grade 3 of 24 (60%) while grade 2 of 16 (40%). There were 16(40%) samples of lymph node status with N2 of 16(40%), N3 of 14(35%), N1 of 7(17.5%) and N0 of 3(12.5%) samples. In this study, breast cancer subtypes were dominated by luminal B by 26(65%), luminal A and triple negative respectively 5(12.5%) and Her2neu 4(10%). After undergoing adjuvant therapy, 18 (45%) samples were alive and 22 (55%) samples died. The mean of Overall Survival (OS) was a median of 28 months with the lowest OS being 5 months and the longest being 38 months. (Table 1)

Pre-Neoadjuvant chemotherapy Ki67 levels in patients with stage III B breast carcinoma

In Table 2, the data was seen in samples with Ki67 levels <14% by 12 (30.0%), samples with Ki67 levels 14-30% by 14 (35%) and samples with Ki67 levels >30% by 14(35%).

Table 2. Pre-Neoadjuvant chemotherapy Ki67 levels in 3 years

	n	%
Ki67 Levels		
Low (<14%)	12	30
Intermediate (14-30%)	14	35
High (>30%)	14	35

The association between Ki67 and mortality can be seen in Table 3. where the majority of samples with Ki67 levels >30% had died by 9 (64.3%). The results of the analysis using Chi square showed that there was a significant association between Ki67 levels and mortality.

Table 3. The association between Ki67 and mortality in 3 years

Ki67 Levels	Outcome n (%)		p
	Died	Alive	0,002
Low (<14%)	1(8,3%)	11(39,2%)	
Intermediate (14-30%)	2(16,7%)	12(42,9%)	
High (>30%)	9(75%)	5(17,9%)	

Chi square test, p<0,05

The outcome (Mortality) of neoadjuvant chemotherapy response in stage III B breast cancer patients

Table 4 shows the association between pre neoadjuvant chemotherapy Ki67 levels and the incidence of mortality in stage III B breast cancer patients. It can be seen that 9 (64.3%) breast cancer samples had Ki67 ≥30% levels and had died. Fisher exact test results show that there is a significant association between Ki67 levels before chemotherapy and mortality with a risk factor of 13.8 times with a sensitivity of 75% and a specificity of 82.1%. So it can be concluded that Ki67 levels ≥30% are specific but not sensitive as a prognosis for overall mortality rate in 3 years in patients with stage III B breast cancer.

Table 4. The association between Ki67 and mortality in 3 years

Ki67	Outcome (n(%))		p	OR (Lower-Higher)
	Died (n=12)	Alive (n=28)		
High (≥30%)	9(64,3%)	5(35,7%)	0,001	13,8(2,7-70,12)
Low (<30%)	3(11,5%)	23(88,5%)		
				Sensitivity : 75% Specificity : 82,1% NR + : 64,2 NR - : 88,5 Youden's index : 57,1

Fisher exact test, P<0,05

The cut off point of Ki67 levels pre-chemotherapy that can be used to determine the relative risk in Stage III B breast cancer patients

Table 5 shows the cut off point of Ki67 levels in sample of stage III B breast cancer patients is ≤20% with a sensitivity of 72.7% and a specificity of 72.1%.

Table 5. Cut off point of Ki67 on mortality of stage III B breast cancer patients

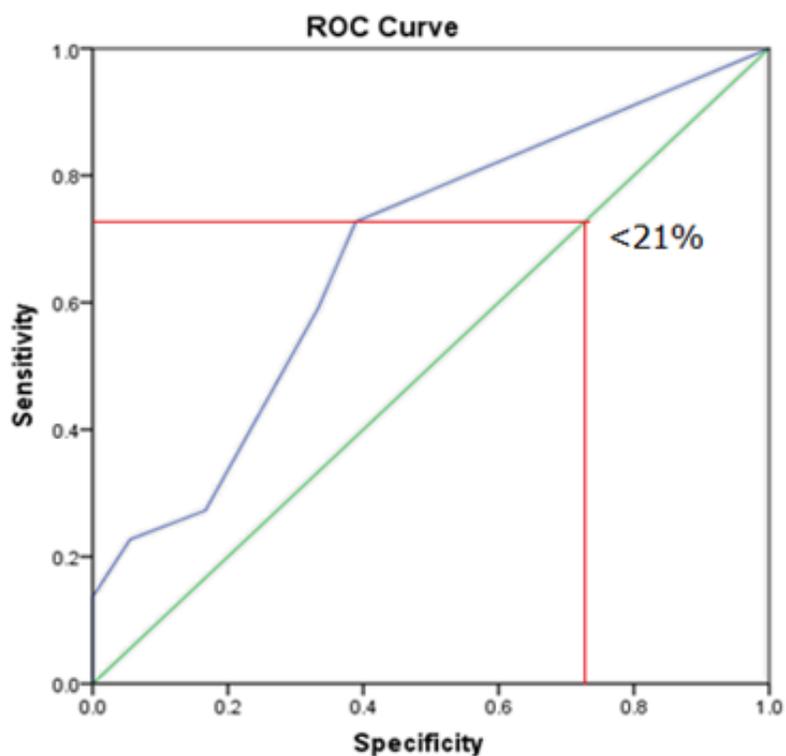| Ki67 *Cut Off Point*				
Ki67	Sensitivity	Specificity	AUC	p
≤20%	72,7%	72,1%	0,674	0,041

Figure 1. The ROC curve shows the cut off point of Ki67 is <20%.

Table 6. shows that at the Cut Off Point ≥20% there is a significant association with the outcome. It can be concluded that patients with pre-chemotherapy stage III B breast cancer with a Ki67 levels ≥20% had a risk factor of 7.1 times experiencing death with a sensitivity of 91.6% and a specificity of 39.28%. So that it is concluded that Ki67 levels ≥20% are sensitive but not specific as a prognosis for overall mortality rate in 3 years in patients with stage III B breast cancer.

Table 6. The association between Ki67 ≥20% and mortality in 3 years

Ki67	Outcome (n(%))		p	OR(Lower-Higher)
	Died (n=12)	Alive (n=28)		
≥20%	11(39,3%)	17(60,7%)	0,042	7,1(0,8-63,1)
<20%	1(8,3%)	11(91,7%)		
				Sensitivity : 91,6% Specificity : 39,2% NR + : 39,2 NR - : 91,6 Youden's index : 30,8

Fisher Exact Test, p<0,05

Table 7. shows that if the cut off point ≥25%, there is a significant association with the outcome. It can be concluded that pre-chemotherapeutic patients for stage III B breast cancer with Ki67 levels ≥25% had a risk factor of 9 times experiencing death with a sensitivity of 83.3% and specificity of 64.2%, so it was concluded that Ki67 levels ≥25% were sensitive but not specific as overall mortality rate prognosis in 3 years in stage III B breast cancer patients.

Table 7. The association between Ki67 ≥25% and mortality in 3 years

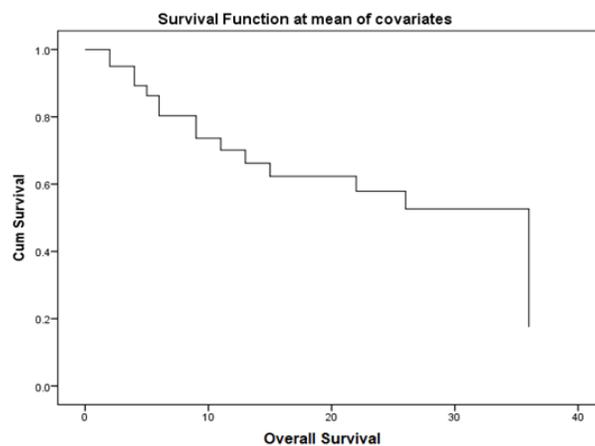| Ki67	Outcome (n(%))		p	OR(Lower-Higher)
	Died (n=12)	Alive(n=28)		
≥25%	10(50%)	10(50%)	0,007	9(1,6-49,4)
<25%	2(10%)	18(90%)		
				Sensitivity : 83,3% Specificity : 64.2% NR + : 83,3 NR - : 64,2 Youden's index : 47,5

Fisher Exact Test, p<0,05

Figure 2. Graph of overall survival on mortality

4. Discussion

The levels of Ki67 neoadjuvant pre-chemotherapy in patients with stage III B breast cancer

In this study, it was shown that there was a significant association between Ki67 levels with mortality and overall survival. Korde et al (2021) stated in the consensus results of The International Ki67 in Breast Cancer Working Group (IKWG) that the average breast cancer sample with stage IIIB showed that the Ki67 levels which had a good Free Disease Survival (FDS) and OS prognosis was >5% and <30%.[6] Ding et al (2020) found that most samples of stage IIIB breast cancer had Ki67 levels>20%. The higher the Ki67 levels, the more aggressive the tumor proliferation will be.[32]

The outcome (mortality) of neoadjuvant chemotherapy response in patients with stage III B breast cancer

The researchers of this study determined the outcome of neoadjuvant chemotherapy response mortality in breast cancer patients with a cut off point >30% referred based on the IKWG (2019).[6] 9 (64.3%) breast cancer samples with Ki67≥30% levels had died.

The consensus carried out by the IKWG in 2019 found samples with Ki67 levels> 30% had a worse prognosis for mortality, FDS and OS.[6] Likewise, study of Tao et al (2017) showed that Ki67 levels pre-neoadjuvant chemotherapy >30% had a worse prognostic value for mortality, FDS and OS.[39] Ragab et al (2018) stated that there was no decrease in Ki67 levels after neoadjuvant chemotherapy and a high Ki67 value in neoadjuvant prechemotherapy illustrates a worse prognosis for mortality. In a study conducted by Ragab et al (2018), 7716 breast cancer samples showed that at a cut off point >40% post-neoadjuvant had very high accuracy in predicting death.

The analytical validity of the Immunohistochemistry (IHC) Ki67 can be achieved by careful attention to preanalytic problems and a calibrated standard visual assessment. Presently, the clinical utility of IHC Ki67 in the treatment of breast cancer remains limited to prognostic assessment in stage I or II breast cancer.[3]

The cut off point of Ki67 pre-chemotherapy levels that can be used to determine relative risk in III B breast cancer patients

The results of the Area Under Curve (AUC) analysis showed that the cut off point of Ki67 in mortality prognosis was Ki67 20% with a sensitivity of 72.7% and a specificity of 72.1%. Patients with pre-neoadjuvant chemotherapy stage III B breast cancer with Ki67 levels ≥20% had a risk factor of 7.1 times experiencing death with a sensitivity of 91.6% and a specificity of 39.28%. Patients with pre chemotherapy stage III B breast cancer with Ki67 levels ≥25% had 9 times risk of death with a sensitivity of 83.3% and a specificity of 64.2%.

Cabrera et al (2018) concluded that breast cancer patients who had underwent neoadjuvant chemotherapy with non-decreasing Ki67 levels with a cut off point >20% would have a worse prognosis.[35] Ding et al (2020) also stated a cut-off point of Ki67 by 20% and concluded that stage IIIB breast cancer patients whose Ki67 levels did not decrease <20% after neoadjuvant chemotherapy had a worse prognosis for OS and FDS.[38]

Research conducted at Sichuan University by Wu et al (2019) concluded that high Ki-67 expression is a worse` prognostic factor in triple negative breast cancer (TNBC). A Ki67 truncation of 40% is associated with a greater risk of recurrence and death compared with lower expression levels, although the Ki67 threshold with the greatest prognostic significance is unknown.[34] Rim et al stated that the biomarker Ki67 nuclear proliferation has a potential prognostic, predictive and monitoring role in breast cancer. Unacceptable variability between laboratories has limited its clinical value. The International Ki67 in the Breast Cancer Working Group investigated whether the IHC of Ki67 could be analytically validated and standardized across laboratories using automated machine-based assessment. Assessment of Ki67 levels > 20% automatically can also describe the prognosis of OS and FDS in breast cancer patients.[36]

5. Conclusions

1. Ki67 levels <14% were 12(30%), samples with Ki67 levels of 14-30% were 14(35%) and samples with Ki67

levels >30% were 14(35%)

2. Ki67 levels ≥30% pre-neoadjuvant chemotherapy was significantly associated with mortality but was only specific and not sensitive as a prognosis for overall mortality rate >3 years.

3. The cut-off point of Ki67 for worse prognosis was ≥20% pre-neoadjuvant chemotherapy was significantly associated with mortality but was sensitive and non-specific, and the cut-off point of Ki67 ≥25% was sensitive but not specific for overall mortality rate >3 years

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