RESEARCH ARTICLE

Matrix Metalloproteinase-9 as Prognostic Factor for the Treatment of HER-2 Enriched Breast Cancer

Daan Khambri[1]*, Heldrian Dwinanda Suyuthie[2], Noza Hilbertina[3], Husna Yetti[4], Denni Joko Purwanto[5]

Abstract

Background: MMP-9 plays a significant role in invasion and migration of tumor cells and metastasis. A combination MMP-9 biomarker with HER2 and Ki-67 is expected to provide more specificity in prognosis for better breast cancer (BC) treatment options. **Methods:** A retrospective cohort of 34 patients with HER2 enriched breast cancer were studied from January 2016 until December 2020. Assessment MMP-9 by IHC using Monoclonal Mouse Anti-Human MMP-9 Antigen with semiquantitative immunoreactive scores methods was done. **Results:** The samples included patients aged 29 to 66 years. Patients' educational level was mostly high school graduation (n=17; 50%). Distant metastases occurred in 10 (29.4%) patients, histopathological Grade II was found in 29 (85.7%) patients, positive LVI was found in 18 (52.9%) patients, and high proliferation rate (Ki67 > 20%) was found in 32 (94.1%) patients. All the patients underwent MRM with a history of chemotherapy in 29 (85.3%) patients, radiotherapy in 14 (41.2%) patients, and targeted therapy in only seven (20.6%) patients. Most of the patients had locally advanced stage III (n=21; 61.8%). The MMP-9 High expression was found in 20 (58.8%) patients and 14 (41.2%) patients had a low expression. A significant relationship was found between MMP-9 expression and DFS (p=0.023); while a significant relationship was found with OS (p=0.093). The mean DFS for High expression MMP-9 was 37.3 months and 45.3 months for low expression. The mean OS was 37.6 months for High-intensity MMP-9 and 42.7 months for Low-intensity. **Conclusions:** The MMP-9 expression (p=0.037), age group<40 years old (p=0.024), and the history of radiotherapy (p=0.035) were significantly related to he occurrence of distant metastases. The MMP-9 High expression had a 7.5 times greater risk of distant metastases. **Impacts:** The MMP-9 can be used as a prognostic factor for distant metastasis in HER2 Enriched BC.

Keywords: MMP-9- biomarkers- metastasis- survival analysis

Introduction

Based on Globocan data in 2018, there were 2.1 million (11.6% of all cancers) new cases of breast cancer (BC) worldwide with a mortality rate of 625,000 (6.6% of the causes of death from all cancers). The BC is the second leading cause of cancer death after lung cancer. International Agency for Research on Cancer (IARC) in 2012, estimated new cases of BC equal to 43.1 per 100,000 population, with a death rate of 12.9 per 100,000 population (Badan Penelitian dan Pengembangan Kesehatan, 2018; Dyanti and Suariyani, 2016).

Prognosis in BC continues to improve with early detection and systemic therapy. Approximately, 75%-80% of BC patients in Indonesia come to the hospital at an advanced stage, which affects the prognosis and cure rate. The survival rate of BC patients decreases drastically with the increase in the stage of disease at diagnosis. If the tumour cells are still localized in the breast tissue, the five-year survival rate can be as high as 97%. However, this rate drops dramatically to 23% if the tumour cells metastasize to other organs. (Dyanti and Suariyani, 2016; Harahap, 2018; Panigoro et al., 2020).

Currently, the management treatment of BC emphasizes more on the molecular level. Good patient stratification and classification allows patients to get the right treatment and avoid unnecessary risks. The use of anti-hormone adjuvants, chemotherapy, and monoclonal antibody-based therapy (Trastuzumab) can increase the survival rate of high-risk BC patients as determined by several prognostic markers such as Estrogen/Progesterone hormone receptor gene expression, lymph node status, and HER2 gene amplification. Recently, it has been found that Matrix Metalloproteinase-9 (MMP-9) has prognostic

[1]Surgical Oncology at Dr. M. Djamil General Hospital/ Faculty of Medicine Universitas Andalas, Padang-Indonesia. [2]Trainee of Surgical Oncology at Dr. M. Djamil General Hospital/ Faculty of Medicine Universitas Andalas, Padang-Indonesia. [3]Patologist at Dr. M. Djamil General Hospital/ Faculty of Medicine Universitas Andalas, Padang-Indonesia. [4]Departement of Public Health and Community Medicine, Faculty of Medicine Universitas Andalas, Padang, Indonesia. [5]Surgical Oncology at Dharmais National Cancer Center Hospital, Jakarta, Indonesia. *For Correspondence: daankhambri567@gmail.com

and predictive value for BC (Yousef et al., 2014; Winer et al., 2018).

The HER2 has an important role in the proliferation of cancer cells. Dimerization between HER2 and the Epidermal Growth Factor ligand activate a cellular cascade pathway that promotes cancer cell invasion. Increased expression of HER2 is associated with increased expression of MMP-9 (Shan et al., 2015; Majumder et al., 2018).

The degradation of the ECM has an essential role in the metastatic process. Several proteolytic enzymes play a role in ECM degradation, including plasmin, cathepsin, and matrix metalloproteinases. The MMP-9 is a protease enzyme of the family of zinc-dependent endopeptidases. The MMP-9 has an essential role in tissue remodelling through cell differentiation, cell migration, proliferation, apoptosis and angiogenesis. (Soini et al., 2006; Winer et al., 2018; Yu and Stamenkovic, 2000).

Some biomarkers may lack sufficient sensitivity for clinical use when used as a single marker. The use of a combination of biomarkers is a strategy to increase the specificity of biomarkers. Combining the MMP-9 biomarker with other biomarkers such as HER2 and Ki-67 is expected to provide a high specificity for prognosis and better BC treatment (Duffy et al., 2017; Huang, 2018; Kos and Dabbs, 2016; Meng et al., 2004).

Materials and Methods

A cross-sectional study was conducted from April 2021 to October 2021 at the Department of Surgery and Anatomical Pathology Laboratory, Faculty of Medicine Universitas Andalas/Dr.M.Djamil General Hospital, Padang. The study population consisted of patients who had been diagnosed with BC recorded in the medical records at Dr. M. Djamil General Hospital Padang for the last five years, starting from January 2016 to December 2020. All the patients had an IHC examination (ER, PR, HER2, and Ki-67). The samples were paraffin block BC with Invasive Ductal Carcinoma of No Special Type (IDC of NST), negative hormonal receptor, and positive HER2. Based on the literature, it was found that the proportion of HER2 Enriched subtype in BC was 5-10% (Cobleigh et al., 2020; Vranić et al., 2021). The sample size was 34 patients with HER2 enriched subtype BC (Figure 1). The samples were taken based on existing data in medical records and selected based on predetermined inclusion criteria. Inclusion criteria were patients with available medical record data diagnosed with IDC of NST and with an IHC examination (ER, PR, HER2 and Ki-67) with the results of ER-/PR-/HER2(+) or HER2 Enriched Subtype.

The exclusion criteria were the paraffin block was cut off until no tumour cell was found, damaged or mouldy, and if the IHC examination was incomplete, damaged or missing data from the Medical Record Status. The paraffin block was then sent to the Anatomical Pathology Laboratory, Faculty of Medicine Universitas Andalas/ DR.M.Djamil General Hospital, Padang. The IHC staining of the MMP-9 was performed using Monoclonal Mouse Anti-Human MMP-9 Antigen, Genetex with a dilution of 1/400. The IHC MMP-9 staining was carried out automatically with Automatic IHC staining from the Leica Bond-Max brand. The IHC MMP-9 staining was also made for positive controls from lymphoid tissue.

The cells expressing MMP-9 appeared brown colored in malignant epithelial cells and stroma cytoplasm. Assessment of MMP-9 expression was made based on the Immunoreactive Score described in Table 1 (Fedchenko and Reifenrath, 2014).

Univariate descriptive analysis was conducted to describe the characteristics of the samples. Bivariate analysis was conducted to find the relationship between each variable. Multivariate analysis was used to find out the effect of the independent variable (MMP-9) on the incidence of BC metastases by taking into account confounding variables. The mathematical model used in this study is logistic regression analysis because the dependent variable is dichotomous. The significance test was set at $p < 0.05$. Data precision was determined by 95% Confident Interval (CI).

Kaplan-Meier method was used to check OS and DFS as prognostic factors for MMP-9. Kaplan Meier curves and data estimates provide a better way to analyze data in cohort studies. Kaplan Meier method is a non-parametric estimation method in survival function, which is generally used to describe the survival of a population or compare the survival of two populations (Pradika and Bambang, 2019).

Results

Thirty four paraffin embedded block and patients data collected from April to September 2021 of BC (IDC/NST) confirmed from histopathological examination and had an IHC examination; ER, PR, HER2 and Ki67.

The characteristics of the research samples obtained from medical record data of IDC of NST patients with Enriched HER2 (from January 2016 to December 2020) are listed in Table 2.

Out of 34 patients, 14(41.2%) patients were in age group 51-60, five (14.7%) were in age group younger than 40 and the rest were in age group (41-50) and age group

Table 1. Immunoreactive Score (IRS) for Assessment of MMP-9 Expression

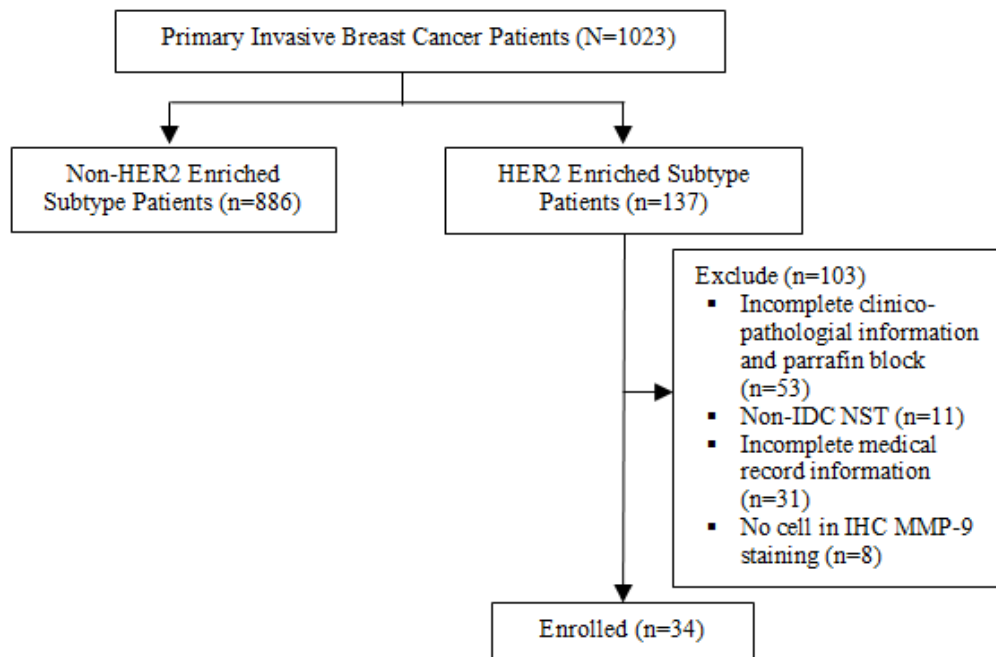| A (percentage of positive cells)	B (intensity of staining)	IRS score (multiplication of A and B)
0 = no positive cells	0 = no color reaction	0-1 = negative
1 = < 10% of positive cells	1 = mild reaction	2-3 = mild (+1)
2 = 10-50% positive cells	2 = moderate reaction	4-8 = moderate (+2)
3 = 51-80% positive cells	3 = intense reaction	9-12 = strongly positive (+3)
4 = >80% positive cells	Final IRS score (AxB): 0-12	

Figure 1. Patients Enrollment Chart. 1023 breast cancer patients were recorded from January 2016-December 2020. 137 patients with HER2 Enriched. 103 patients were excluded due to incomplete clinical-pathological information, non-IDC NST, incomplete medical record information, and No BC cell after IHC MMP-9 staining. 34 patients are enrolled in this research.

older than 60. In terms of education level, the majority of the patients had a high school diploma (n=17; 50.0%).

Most of the patients were diagnosed with BC in 2018 (n=14; 41.2%). Only three patients had a history of live births with nullipara (8.8%) and 31 patients had a history of multipara (91.2%). In addition, eight patients had a history of using hormonal therapy for birth control (23.5%).

Expression of MMP-9 in HER2 Enriched BC

The IHC examination was carried out at the Anatomical Pathology Laboratory Universitas Andalas Faculty of Medicine/Dr. M. Djamil Padang General Hospital. Two pathologists performed MMP-9 Expression Assessment
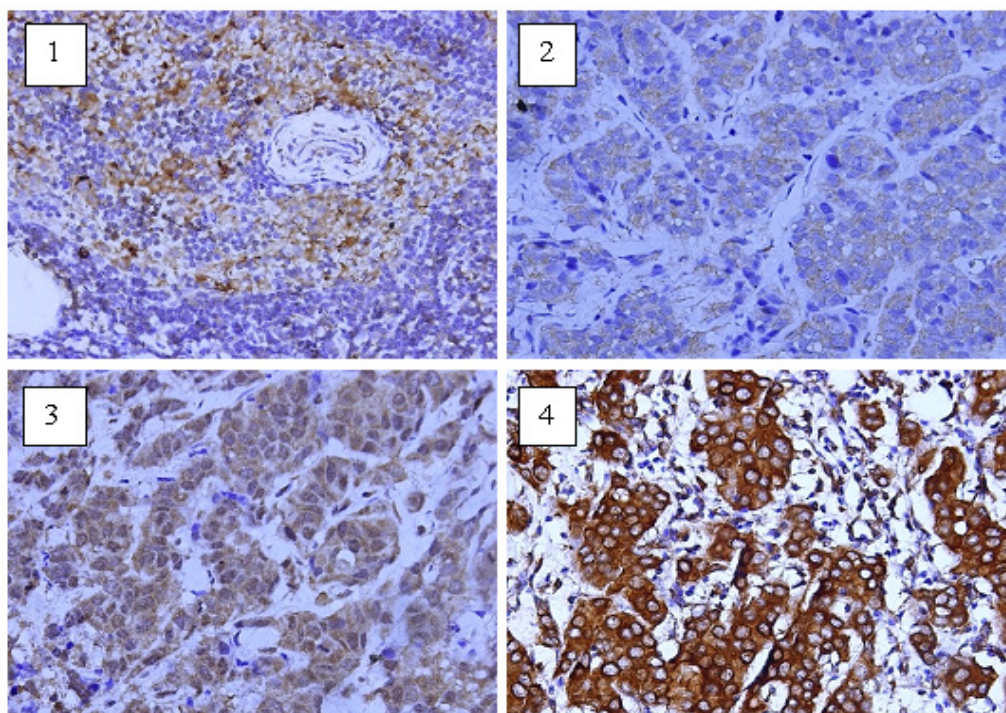

Figure 2. Original Magnification 400x; 1. Positive control, the cell was expressing MMP-9 appeared brown color in the cytoplasm of the malignant cell and stroma 2. Mild expression of MMP-9 (IRS: 2-3). 3. Moderate expression (IRS: 4-8), 4. High expression of MMP-9 (IRS 9-12).

(Figure 2). The difference in the assessment is carried out by taking the average value of the immunoreactive scores obtained and entering into the appropriate semi-quantitative criteria from the average results of the examination.

The MMP-9 expression, the history of radiotherapy (p=0.035), and the patient's age group (p=0.024) were significantly related to the incidence of distant metastatic in HER2 Enriched Subtype BC. The history of chemotherapy, history of targeting therapy, histopathological grading, LVI and Ki-67, and clinical stage of BC were not significantly related to the incidence of distant metastases

Table 2. The Demographic and Clinical Pathologic Characteristics Of Patient With HER2 Enriched Breast Cancer

Characteristics		Frequency	Percentage
Age group	< 40	5	14.7
	41-50	11	32.4
	51-60	14	41.2
	> 60	4	11.8
Educational Background	Elementary school	6	17.6
	High School	17	50.0
	Collage graduate	11	32.4
Year of diagnose	2016	4	11.8
	2017	2	5.9
	2018	14	41.2
	2019	11	32.4
	2020	3	8.8
Birth count	Nullipara	3	8.8
	Multipara	31	91.2
History of Hormonal therapy	Yes	8	23.5
	No	26	76.5
Distant Metastasis	Yes	24	70.6
	No	10	29.4
Histopatology Grading	Grade I	1	2.9
	Grade II	29	85.3
	Grade III	4	11.8
LVI	Positive	18	52.9
	Negative	14	41.2
	Missing	2	5.9
Ki-67	Low Proliferation	2	5.9
	High Proliferation	32	94.1
Chemotherapy	Yes	29	85.3
	No	5	14.7
Radiotherapy	Yes	14	41.2
	No	20	58.8
Targetting Theraphy	Yes	7	20.6
	No	27	79.4
Clinical Stage	Stage I	2	5.9
	Stage II	11	32.4
	Stage III	21	61.8
MMP-9 Expression	+1	4	11.8
	+2	10	29.4
	+3	20	58.8

HER2 Enriched Subtype BC (Table 3).

The multivariate analysis aims to determine whether there is an interaction between the primary variable (MMP-9 Expression) with confounder variables (age group, clinical stage, and history of radiotherapy, LVI) in the relationship between the primary variable and the dependent variables (Table 4).

After conducting multivariate analysis through testing the interaction between the main independent variables and confounding variables and its effect on the occurrence of distant metastases and confounding tests, the final model was obtained to explain the occurrence of distant metastases in overexpressed HER2 BC. As listed in Table 4, it is known that the MMP-9 Expression variable does not have a confounder variable. It can be concluded that HER2 overexpressed BC patients with MMP-9 expression (+3) have a 10.6 times greater possibility of metastases than BC with MMP-9 expression (+1 and +2) after taking into account confounding variables.

Disease Free Survival (DFS)

To assess Disease-Free Survival (DFS) from observations made for 60 months (January 2016-December 2020) on MMP-9 expression, the Kaplan Meier analysis test was carried out. The mean DFS for MMP-9 with High intensity (+3) was 37.3 months. The mean DFS for mild and moderate intensity (+1 and +2) was 45.3 months; and the p-Value of the Log Rank test was 0.023. Patients with high MMP-9 expression had 7.5 times greater risk of developing the metastatic disease than patients with low MMP-9 expression.

Overall Survival

The mean score of OS for patients expressing high expression of MMP-9 (+3) was 37.6 months, this figure for patients with Low expression of MMP-9 (+1 and +2) was 42.7 months. The patients with high expression of MMP-9 (+3) had a 3.5 times higher risk of death than patients with low expression of MMP-9 (+1 and +2); although it is not statistically significant.

Discussion

The IHC examination, ER, PR HER2, and Ki-67 are still not routine examinations and they were not available at Dr. M. Djamil Padang General Hospital until mid-2021. From January 2016 to December 2020, the IHC examination would be carried out only with the consent of patients who were willing to undergo the examination in another referral hospital and not covered by the National Health Insurance. The total number of patients with IHC examination data for five years was 1,023. Out of 1,023 patients, 137 had HER2 Enriched subtype. Out of 137, only 84 patients were able to regain paraffin block. And out of 84 paraffin blocks, only 47 paraffin blocks were in good condition and could be assessed for MMP-9 IHC. The fixation and storage of paraffin blocks are factors in a good paraffin block cutting results to be attached to the prepared coated slides. From 47 slides after the MMP-9 IHC examination, 34 patients were included in the study and met the inclusion criteria. The record of patient clinical

Table 3.Association Between MMP-9 Expression, Patient Characteristics and Distant Metastatis

Variable		Distant Metastasis		Total	Univariable OR (95% CI)
		No	Yes		
MMP-9 Expression	High Expression (+3)	11	9	20	10,636
		32.4%	26.5%	58.8%	(1.159 - 97.593)
	Low Expression (+1 and +2)	13	1	14	1.00 (reference)
		38.2%	2.9%	41.2%	
Age Group	≤ 40 years	1	4	5	15.333
		2.9%	11.8%	14.7%	(1,436 - 163,756)
	> 40 years	23	6	29	1.00 (reference)
		67.6%	17.6%	85.3%	
Clinical Stage	Locally Advance (Stage III)	12	9	21	9,000
		35.3%	26.5%	61.8%	(0.982 - 82,496)
	Local Stage (I dan II)	12	1	13	1.00 (reference)
		35.3%	2.9%	38.2%	
Chemotherapy	Absent Chemotherapy	4	1	5	1.8
		11.8%	2.9%	14.7%	(0,175 - 18.468)
	Receive Chemotherapy	20	9	29	1.00 (reference)
		58.8%	26.5%	85.3%	
Radiotherapy	No previous Radioterapi	17	3	20	5.667
		50.0%	8.8%	58.8%	(1,129 - 28,454)
	Receive Radioterapi	7	7	14	1.00 (reference)
		20.6%	20.6%	41.2%	
Targeting Therapy	No Targeting Therapy	19	8	27	1.053
		55.9%	23.5%	79.4%	(0,168 - 6.602)
	Receive Targeting Therapy	5	2	7	1.00 (reference)
		14.7%	5.9%	20.6%	
Grading Histopatology	High Grade Grade (III)	3	1	4	1,286
		8.8%	2.9%	11.8%	(0,117 - 14,089)
	Low and Intermediate Grade (I dan II)	21	9	30	1.00 (reference)
		8.6%	2.9%	11.4%	
LVI	Positive	11	7	18	3,818
		32.4%	20.6%	52.9%	(0,649 - 22,454)
	Negative	12	2	14	1.00 (reference)
		35.3%	5.9%	41.2%	
Ki-67	High Proliferation	23	9	32	2,556
		67.6%	26.5%	94.1%	(0,144 - 45,386
	Low Proliferation	1	1	2	1.00 (reference)
		2.9%	2.9%	5.9%	

data, risk factors, histopathological examination, IHC, the course of the disease, and treatment history were taken from the patient's medical record after obtaining Ethical Clearance from Health Research Ethics Committee Dr.

Table 4. Multivariate Analysis Model MMP-9 Expression, Clinical Stage, Age, Radiotherapy, LVI and Distant Metastasis

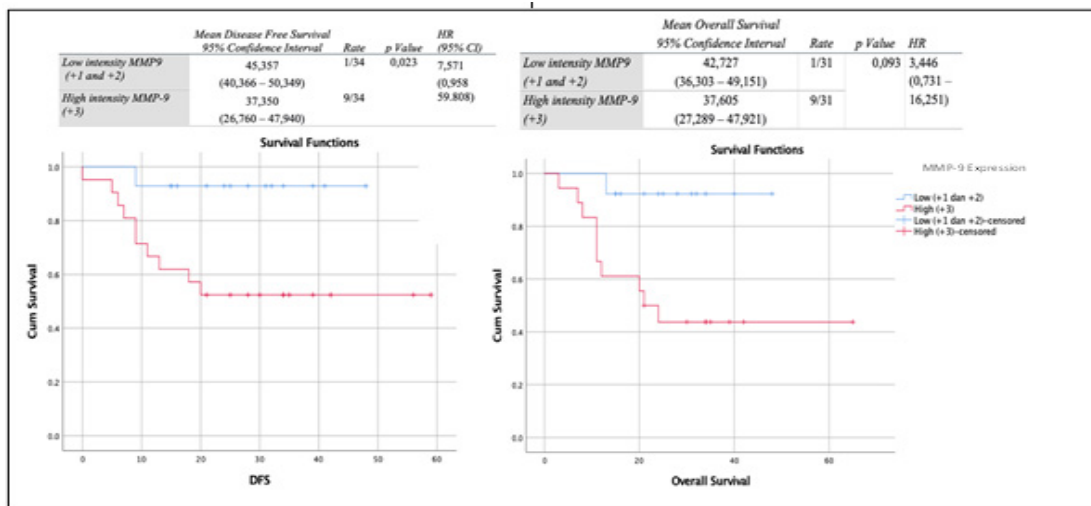| Confounding Variable (p<0,25)	OR (Before)	p-Value		P Value (Elimination)				OR (After)
			I	II	III	IV	V	
MMP-9 Expression	10,636	0.037	0.692	0.692	0.059	0.056	0.037	10,636
Clinical Stage	7,615	0.052	0.693	-	-	-	-	-
Age Group	9,857	0.024	0.692	0.693	-	-	-	-
Radiotherapy	4:05	0.035	0.324	0.102	0.072	0.073	-	-
LVI	3,818	0.096	0.203	0.082	0.081	-	-	-

Figure 3. Kaplan Meier Disease Free and Overall Survival Curves and Data. Mean DFS MMP-9 High expression (+3): 37.3 months. Mean DFS for Low expression of MMP-9 (+1 and +2): 45.3 months. p-Value: 0.023 HR: 7.5 Mean OS MMP-9 High expression (+3): 37.6 months. Mean OS for Low expression of MMP-9 (+1 and +2): 42.7 months. P Value 0.093, HR: 3.5

M. Djamil Padang General Hospital. Overall Survival Assessment was also carried out by contacting the patient via telephone to determine whether the patient is still on treatment or died at home and did not come for control to the hospital.

This study showed a relationship between age and the occurrence of distant metastases (p=0.024). It remains controversial whether young age at diagnosis is an adverse prognostic factor in primary breast cancer. Some studies have found that younger patients have worse clinical outcomes than older patients (Joseph et al., 2020). Others have reported that younger patients have a better prognosis or no relationship between outcome and age. Various explanations have been given for these conflicting results, including the study population, differences in patient age group selection criteria, and the age groups used in the analyses. In addition, it has been long debated whether breast cancer diagnosed at a young age is a clinically and etiologically different disease from breast cancer diagnosed at an older age. The occurrence of distant metastases in young people with BC has 15 times higher risk of developing distant metastases compared to the BC age group above 40 years (Han et al., 2016).

As the results showed, the highest educational degree was high school diploma in 17 patients (50%). The most patients were obtained from 2018 as many as 14 patients (41.2%). Most patients came with locally advanced stage (Stage III) (n= 21; 61.8%).

The high number of first visits at an advanced stage is still a problem in developing countries, one of which is Indonesia as a low-income and moderate-income country, with a per capita income below US$ 4,000. The unequal distribution of resources and the lack of early detection tools are some of the factors that cause the high number of

patient visits with locally advanced and metastatic stages (Prajoko et al., 2018). Dr. M. Djamil Padang General Hospital is one of the National Referral Center Hospitals so that cases of BC at this hospital are mostly referral cases with advanced local stage and metastases. According to the National Hospital Information System in Indonesia, cancer treatment faces various obstacles that cause nearly 70% of patients to be found in an advanced local stage. Based on medical records from the Adam Malik General Hospital in Medan, which is also one of the national referral hospitals, it was found that around 24 of 30 breast cancer patients came to the hospital for the first time in an advanced local stage (Deliana et al., 2019).

The LVI and histopathological grading have been predictors of poorer survival and prognostication. The LVI in BC is defined as the presence of tumor cells in the endothelial (lymphatic or vascular)-lined spaces in the breast surrounding the invasive carcinoma. This presence is associated with an increased risk of axillary lymph node involvement and metastasis (Resti Putri Firdaus et al., 2016; Simbolon and Pohan, 2021).

The histopathological grading showed that Grade II was the highest in 29 patients (85.3%), Grade III in four patients (11.4%) and Grade I only one patient (2.9%). In a systemic review study conducted by Yosef, it was found that Grade II constituted the majority of tumor grading of the entire study with a percentage reaching 63% followed by Grade III and Grade I which were 25% and 12%, respectively (Simbolon and Pohan, 2021).

The LVI in this study was positive in 18 patients (52.9%). This result is not much different from that obtained in a study conducted by Vashti in 2016, which found positive LVI in the HER2 subtype of 35.1%. Although in theory, lymphovascular infiltration has been

a predictor of poorer survival (Resti Putri Firdaus et al., 2016). This presence is associated with an increased risk of axillary lymph node involvement and metastasis (Simbolon and Pohan, 2021). In the bivariate analysis test, these two variables (Grading and LVI) did not show an association with MMP-9 expression or the incidence of distant metastases.

An examination of proliferation Ki-67 in this study found that 94.1% (n=32) of the patients had a Ki-67 value > 20% or high proliferation, while the rest two patients (5.9%) had a Ki-67 value <20% or low proliferation. There was no significant relationship between the proliferation of Ki-67 and the incidence of distant metastases. This result is also similar to a study conducted by Aswiyanti, who showed that there was also no correlation between Ki-67 expression and the degree of differentiation and lymphovascular invasion in triple-negative BC (Asri, Mayorita and Khambri, 2015).

Surgery still plays a significant role in BC treatment (Prajoko et al., 2018). In this study, all patients underwent MRM. The survival rate of BC patients undergoing surgical therapy (MRM) was doubled, with a 5-year survival rate of 40% in advanced stages, even without adjuvant therapy. According to a meta-analysis, patients with early stages can be treated with BCT, a procedure that combines BCS and whole breast radiation will result in long-term survival similar to mastectomy. In this study, no early-stage patients underwent BCT because of patients' preferences and limitations of radiotherapy facilities (Prajoko et al., 2018).

Administration of systemic therapy and targeted therapy in patients with HER2 Enriched can prolong DFS and OS by 33-52% and 34-41% (Perez et al., 2014). As shown by the results, out of the 34 patients who should have received targeted therapy with trastuzumab, only seven patients received targeted trastuzumab therapy and the remaining 27 patients (79.4%) did not receive the therapy because all of these patients had National Health Insurance (Jaminan Kesehatan Nasional/JKN). Targeted therapy was discontinued temporarily by JKN in 2018 and 2019 at Dr. M. Djamil Padang General Hospital.

The administration of neoadjuvant therapy and adjuvant chemotherapy in this study was not significantly associated with the incidence of distant metastases. Provision of locoregional radiotherapy in 19 patients (54.3%) gave significant results on the possibility of distant metastases. A meta-analysis involving 17 RCTs found that radiotherapy reduced the risk of locoregional and distant recurrence (35% without radiotherapy vs 19.3% with radiotherapy and Absolute Risk Reduction of 15.7%). Radiotherapy also reduced the risk of mortality due to BC from 25.2% to 21.4% (ARR 3.8%) (Darby et al., 2011). This study found a significant relationship between a history of radiotherapy and those who did not receive radiotherapy. Patients who did not receive radiotherapy had a 10-fold risk of developing distant metastases.

There was no significant relationship between the clinical stage and the incidence of distant metastases in this study (p=0.052). Joseph et al., (2020) found that the clinical stage does not have a significant relationship with MMP-9 cytoplasmic expression. A study conducted by Hardisman et al., (2021) found that the clinical stage is a dominant predictor of recurrence and survival in BC. This study found that patients who came at a locally advanced stage would have a greater risk of developing distant metastases nine times greater than patients who came at an early stage (Dasman et al., 2021).

The MMP-9 expression in this study was found to be more dominant with MMP-9 expression (+3) in 21 patients (60%). This figure is not much different from that obtained by Yousef et al. where the study found high MMP-9 expression (50%) in HER2 Enriched (Yousef et al., 2014).

The results of this study are also consistent with several studies; namely a high MMP-9 expression was associated with a poor prognosis in TNBC and its clinical pathological features. (Wang et al., 2018; Jiang and Li, 2021). Wu et al., (2019) showed a significant relationship between high MMP-9 expression and lymph node metastases, high Ki-67 expression; however, there was no significant relationship with chemotherapy status, menstrual status, P53 expression, CEA and CA 15-3 values before therapy. This study was also supported by Wang's study, who showed a significant relationship between MMP-9 expression and lymph node metastasis; however, MMP-9 expression was not associated with age (Wang et al., 2018).

As the results indicated, MMP-9 can be used as a prognostic factor and based on the Log-rank analysis test, there was a significant relationship between MMP-9 expression and DFS (p = 0.023). Twenty-eight meta-analyses showed that MMP-9 expression in serum and tissues was a poor prognostic predictor of BC (Sullu et al., 2011). Kaplan Meier's analysis found that the mean DFS of five years for High MMP-9 expression (+3) was lower than DFS for Low MMP-9 expression (+1 and +2). The mean DFS on High expression of MMP-9 (+3) was 37.3 months. DFS Low expression of MMP-9 (+1 and +2) was 45.3 months (Figure 3). From these data, it can be seen that the stronger the expression of MMP-9, the lower the DFS of HER2 Enriched BC patients. From Kaplan Meier's analysis, it can be seen that the Hazard Ratio in patients with MMP-9 High expression has a risk of metastases 7.5 times higher compared to patients with MMP-9 Low expression. Several meta-analyses have also reported that overexpression of MMP-9 is a predictor of risk for DFS and OS (Sullu et al., 2011).

Based on OS assessment, there was no significant relationship (p=0.093) between increased MMP-9 expression and OS. Based on Kaplan Meier's statistical analysis, there was a decrease in the mean OS of High MMP-9 expression (+3) to low MMP-9 expression (+1 and +2). The mean OS value for High Expression MMP-9 (+3) was 37.6 months, with the mean value for OS Low expression MMP-9 (+1 and +2) equal to 42.7 months. The same results were obtained from a study conducted by Yousef who showed that an increase in MMP-9 expression was not associated with OS in BC (Yousef et al., 2014).

Author Contribution Statement

Daan K, Denni JP and Heldrian DS designed the study.

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