

Original Research Article

Comparison of diagnostic validity between mitotic activity index and Ki-67 expression as predictor for breast cancer in distant metastasis

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Received: 26 April 2019

Accepted: 16 May 2019

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ABSTRACT

Background: One particular cause of death from breast cancer is distant metastasis. In this study, we calculate and compare diagnostic value of Mitotic Activity Index (MAI) and Ki-67 expression in predicting distant metastasis.

Methods: Study was conducted in Sanglah Hospital from January 2017 to February 2019. All histopathology results from open biopsy are examined, thus MAI and Ki-67 values were obtained. We divided this into 2 groups: MAI high (≥ 20 /HPF), low (< 20 /HPF) and Ki-67 high ($\geq 20\%$) and low ($< 20\%$). We compared with distant metastasis event as gold standard, obtained from radiology examination. We count all diagnostic characteristics (sensitivity, specificity, positive and negative predictive value, accuracy, and likelihood ratio). We compared these diagnostic validities from data area under curve (AUC) with p value < 0.005 considered to be statistically significant.

Results: A total of 173 breast cancer patients were participated in this study, 92 of them had distant metastasis (53.2%) and 81 patients didn't have any distant metastasis (46.8%). MAI had relative high specificity (82.7%) and Ki-67 had fair sensitivity values (69.6%). There are 0.08 point AUC differences between these two variables. With p value higher than 0.05 (0.06), it can be summarized that these two variables are not different significantly and statistically.

Conclusions: There is not any statistically significant difference between these two markers in predicting distant metastasis in breast cancer. We hope other researcher interest into exploring more about these markers and their function.

Keywords: Breast cancer, Distant metastasis, Diagnostic trial, Mitotic activity index, Ki-67

INTRODUCTION

Breast cancer is the most common cancer found in women worldwide. According to Global Cancer Incidence, Mortality and Prevalence (GLOBOCAN) in 2012, the prevalence of breast cancer in woman worldwide is 25.1% compared to other malignancies. There are 1.671.149 new cases of breast cancer in 2012 and it was predicted in 2012, that death caused by breast cancer could reach to more than 500.000 deaths. Most of

these numbers can be found in developing countries, and Indonesia is one of them.¹

In years to come, Indonesia could become a country with the highest number of woman patients with breast cancer, even highest death counts related to breast cancer in South East Asia. Numbers of patient suffered from breast cancer in Indonesia are approximately 48.988 patients and total death caused by breast cancer are approximately 19.750 deaths in 2012.² The total of breast cancer patients seek for medical attention in Sanglah Hospital are

increasing from 253 to 322 patients from 2015 to 2016, where 192 patients are stage IV breast cancer patients.³

Distant metastasis is still the leading cause of death in patient with breast cancer.⁴ The majority of breast cancer patients with distant metastasis have lower survival rate although these patients were provided with appropriate treatment. According to American Cancer Society, the survival rate of most patients with stage III breast cancer are 84%, meanwhile patients with stage IV breast cancer only have 19% survival rate. In Indonesia, stage III breast cancer patients have 72% five-year survival rates, while patients with stage IV only have 22% five-years survival rates.⁵

The proliferation of tumor cells are counted to be cancer “hallmark” and one of reliable factors which affects cancer prognosis. Tumor cells proliferation caused matrix remodelling and neo-angiogenesis which can confirm tumor aggressiveness.⁶ Simple methods to determine the degree of proliferation are counting Mitotic Activity Index (MAI) and Ki-67 expression. MAI can be used to determine breast cancer prognosis.⁷ High mitotic activity indicates an increased risk of death and relapse marker although the size of tumor is small and although there isn't any spread to nearest regional lymph node.⁸⁻¹⁰ Ki-67 expression is a better marker for tumor proliferation activity compared to MAI.¹¹ Breast cancer patients with Luminal B subtype more often had distant metastases compared to Luminal A subtype.¹² Breast cancer patients with high Ki-67 expression had higher relapse event and lower survival rate.¹³

Nowadays, studies regarding the relationship between high MAI, high Ki-67 expressions and distant metastases event in breast cancer have been explored. But there are still lack of studies concentrating on MAI and Ki-67 sensitivity, specificity, accuracy, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio in predicting distant metastases in breast cancer. In this study, we would like to examine the diagnostic validity between MAI and Ki-67 in predicting breast cancer distant metastases.

METHODS

This study is a diagnostic trial between two markers in predicting distant metastases in breast cancer patients who were admitted to Sanglah Hospital for advanced medical treatment. Sanglah hospital is center referral hospital, particularly patients from around Bali and Nusa Tenggara islands. Patients with moderate to severe degrees are usually referred here for better medical treatment. There are huge numbers of oncology patients seek better treatment options, particularly chemotherapy due to availability of chemotherapy agents in Sanglah Hospital. For that reasons, Sanglah Hospital became one of the biggest cancer treatment center in Bali and Nusa Tenggara islands. Sanglah Hospital is also a teaching hospital of Udayana University Faculty of Medicine.

This study was conducted from January 2017 to February 2019. There were total of 173 patients participating in this study, all of these patients are breast cancer patients with or without distant metastases. The inclusion criteria for our study includes female patient who had a breast cancer seeking medical treatment in Sanglah Hospital during our study period. The exclusion criteria for our study consist of breast cancer patients whose medical records were not found or incomplete and didn't have any open biopsies histopathology results from Pathological Anatomy laboratory. Our sample study is recruited with simple random sampling method in breast cancer patients who meet our inclusion criteria.

Data and variables required for this study are obtained retrospectively from SIMARS, our medical record system in Sanglah Hospital, written medical records and histopathology results from Sanglah Hospital Department of Pathological Anatomy. Variables collected from our study sample in medical records consist of patient's age, distant metastasis event, distant metastasis target organ, amount of organ inflicted by metastasis, primary tumor size and lymph node spreading event. Variables obtained from patient's histopathology results consist of histopathology grade, MAI level, and Ki-67 expression level.

Mitotic Activity Index (MAI) is total of mitosis activity found on histopathology slide with High Power Field microscopic view. MAI are divided based on how many mitotic activities found in High Power Field view. MAI values are considered low when total of mitotic activities found in microscope are below than 20. MAI values are considered high when 20 or more mitotic activities found. Ki-67 expression is cancer cell antigen expression from single cell cycle. Ki-67 expressions are presented in percentages. Ki-67 expression 20% or more are considered high and Ki-67 expression below 20% are considered low. Histopathology grade consist of scoring system which present how atypical some cancer cells compared with normal cell. This score consists of 3 categories, grade 1 for low grade/well differentiated, grade 2 for intermediate grade/moderately differentiated and grade 3 for high grade/poorly differentiated or undifferentiated. All of these variables are taken from histopathology result, obtained from open biopsy.

For the statistical analyses, we used STATA statistic software to analyze our study variables. All numerical variables were presented as mean \pm standard deviation and all categorical variables were shown in percentages. Diagnostic study was presented in 2x2 tables, consist of MAI levels and Ki-67 expression values listed in row sections and metastatic event listed in columns. We calculated the sensitivity value, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio positive (LR+) and negative (LR-). We used radiology examination in determining metastasis event as a gold standard. Once we had these diagnostic values, we compare between MAI and Ki-67

expression's diagnostic trial results compare both validities using are under curve (AUC) comparative test with confidence interval of 95% and p value <0.05.

RESULTS

A total of 173 breast cancer patients were participated in this study, 92 of them had distant metastasis (53.2%) and 81 patients didn't have any distant metastasis (46.8%). Patients are divided by 2 based on their age, below 40 years old and 40 years or above. There are 27 patients (15.6%) aged below 40 and 146 patients (84.4%). Based on target organ affected from metastasis, 52 patients (56.5%) had lung metastasis, 37 patients (40.2%) had bone metastasis and 22 patients had liver metastasis (23.9%). Based on primary tumor cells size, 126 patients (72.8%) had primary tumor cell with size above 5 cm, while 47 patients (27.2%) had primary tumor cells with size 5 cm or smaller (Table 1).

Table 1: Patient's characteristic and histopathology status.

Variables	Frequencies (%)
Age (years)	
<40	27 (15.6)
≥40	146 (84.4)
Distant Metastasis	
Positive	92 (53.2)
Negative	81 (46.8)
Target Organ Affected	
Bone Metastasis	37 (40.2)
Liver Metastasis	22 (23.9)
Lung Metastasis	52 (56.5)
Total Patients With (...) Metastasis	
1	76 (82.6)
2	13 (14.1)
3	3 (3.3)
Primary Tumor Size (cm)	
>5	126 (72.8)
≤ 5	47 (27.2)
Lymph Node Involvement	
Positive	143 (82.7)
Negative	30 (17.3)
Histopathology Grade	
1	6 (3.5)
2	96 (55.5)
3	71 (41)
MAI	
High	42 (24.3)
Low	131 (75.7)
Ki-67	
High	123 (71.1)
Low	50 (28.9)

There are 143 breast cancer patients (82.7%) with regional lymph node involvement, and only 30 patients (17.3%) didn't have any spread into regional lymph node. According to histopathology grades, 96 patient (55.5%) had grade 2 malignant cells, 71 patients (41%) had grade 3 malignant cells, and only 6 patients had grade 1 tumor cells. From MAI examination, 42 patients (24.3%) considered having high levels of MAI, and 131 patients (75.7%) had low MAI levels. From Ki-67 expression observation, 123 patients (71.1%) had high expression of Ki-67 and 50 patients (28.9%) had low Ki-67 expression (Table 1).

Data obtained from MAI and Ki-67 expression examinations are presented in 2x2 tables and compared to distant metastasis event. Results regarding metastatic event from radiologic examination (gold standard) are compared with MAI examination from histopathology results. There are 28 patients (30.4%) patients with high MAI and had distant metastasis, while 64 patients (69.6%) had low MAI and distant metastasis positive. There are 14 patients (17.3%) with high MAI but didn't have any distant metastasis, and 67 patients had low MAI and didn't have any distant metastasis (Table 2).

Table 2: Cross tabulation between MAI variables and distant metastasis event.

Variable	Distant Metastasis		Total
	Positive	Negative	
MAI			
High	28 (30.4%)	14(17.3%)	42
Low	64 (69.6%)	67(82.7%)	131
Total	92	81	173

Results regarding metastatic event from radiologic examination (gold standard) are compared with Ki-67 expressions from histopathology results. There are 64 patients (69.6%) patients with high Ki-67 expressions and had distant metastasis, while 28 patients (30.4%) had low Ki-67 expressions and distant metastasis positive. There are 59 patients (72.8%) with high Ki-67 expressions but didn't have any distant metastasis, and 22 patients had low Ki-67 expressions and didn't have any distant metastasis (Table 3).

Table 3: Cross tabulation between Ki-67 expression and distant metastasis event.

Variables	Distant Metastasis		Total
	Positive	Negative	
Ki-67 expression			
High	64 (69.6%)	59(72.8%)	123
Low	28 (30.4%)	22(27.2%)	50
Total	92	81	173

We calculated every diagnostic value regarding MAI values and Ki-67 expressions compared to distant metastasis event, consist of sensitivity, specificity,

positive predictive value (PPV), negative predictive value (NPV), accuracy (in percentages), likelihood ratio positive (LR+) and negative (LR-) (Table 4).

Our next analysis is to compare two variables validity using area under curve (AUC) with 95% confidence

interval and α score 0.05. Table 5 shows diagnostic validity comparison between MAI and Ki-67 expressions. There are 0.08 point AUC differences between these two variables. With p value higher than 0.05 (0.06), it can be summarized that these two variables are not different significantly and statistically (Table 5).

Table 4: Diagnostic validity presentation in MAI and Ki-67 expression.

Diagnostic variables	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	LR+	LR-
MAI	30.4	82.7	66.7	51.1	54.9	1.76	0.84
Ki-67	69.6	27.2	52	44	49.7	0.95	1.12

Table 5: Diagnostic validity comparison between MAI and Ki-67 expression in predicting distant metastasis.

Variable	AUC	IK 95%	AUC Difference	p value
MAI	0.56	0.50-0.63	0.08	0.06
Ekspresi Ki-67	0.48	0.42-0.55		

DISCUSSION

Total patients age below 40 years old are 27 people (15.6%), and 13 patients (14.1%) had distant metastasis. Patients age 40 years and above are counted for 146 patients (84.4%) where 79 patients (85.9%) had distant metastasis. A study conducted by Narisuari at Oncology Surgery Clinic Sanglah Hospital in 2012 support this number by stating majority of breast cancer patients seek medical attention are patients with age ranged from 41 to 50 years old (42.18%).¹⁴ Partini and associates also did a study regarding breast cancer patients in Department Surgery Oncology in Sanglah Hospital between 2014 to 2016.¹⁵ The study shown patients with breast cancer aged below 40 years old were 14 patients (11.3%), while breast cancer patients aged 40 years old and above were 110 patients (88.7%).¹⁵

In this study, the most organ affected from distant metastasis were bone, lungs, and liver, where lungs are the most common distant metastasis target organ in out breast cancer patients, counted for 52 patients or 56.5%. We didn't found any case where distant metastasis affect patient's brain. With this result, it doesn't relate to a study conducted by Pulido and associates, where they stated that bone was the most common distant metastasis target organ found in breast cancer patients.¹⁶ Similar outcome also presented from a study conducted by Chen, stating that bone was the most common distant metastases target organ in breast cancer patients.¹⁷

Our study showed several patients, particularly patient aged 40 years and above suffered distant metastasis for more than one target organ. Thirteen patients (13.1%) had two target organs affected from distant metastasis breast

cancer and 3 patients (3.3%) had three target organs affected. These phenomenon probably can be explained from "seed and soil" hypothesis. This hypothesis describes that different tumor cell subpopulations can be more comfortable in particular micro environment, thus these cell tumors can invade and proliferate in other area or multiple areas.¹⁸

In diagnostic trial, we are familiar with these 2 terms, pre-test probability and post-test probability. Whether we found a change in probability or how big/small changes in probability depends on LR value.¹⁹ Pre test probability is a prevalence value which is obtained from data analyses result. High or low MAI pre test probability value is 53%, which means before MAI were obtained, there is a 53% probability of a patient will suffer or not suffering distant metastasis. High MAI post test probability value according to data analysis are 66.7%, meaning after MAI values were obtained, there is a 66.7% probability of a patient will suffer distant metastasis. Low MAI post test probability value according to data analysis are 51.1%, meaning after MAI values were obtained, there is a 51.1% probability of a patient will not suffer distant metastasis. Likelihood ratio values of MAI are below than 2 or above 0.5 which means changes of probability are very unlikely.¹⁹

High or low Ki-67 expression pre test probability value is 53%, which means before Ki-67 expression variable were obtained, there is a 53% probability of a patient will suffer or not suffering distant metastasis. High Ki-67 expression post test probability value according to data analysis are 52%, meaning after Ki-67 expression values were obtained, there is a 52% probability of a patient will suffer distant metastasis. Low Ki-67 expression post test probability value according to data analysis are 44%,

meaning after Ki-67 expression values were obtained, there is a 44% probability of a patient will not suffer distant metastasis. Likelihood ratio values of Ki-67 expression are below than 2 or above 0.5 which means changes of probability are very unlikely.

Based on comparison analysis between MAI and Ki-67 diagnostic validity in predicting distant metastases, there was not a significant statistical difference between these two, with p value of 0.06 (>0.05).

High activity in tumor cell proliferation is associated with tumor aggressiveness, particularly the presence of distant metastasis. However, these potential malignant cells have to pass through several stages or cascades to evolve from primary tumor into spreading into other distant target organ, one example, tumor have to pass through defensive mechanism such as tumor infiltrating lymphocytes (TIL).

Metastasis are also determined by genetic factor, such as Human epidermal growth factor receptor 2 (HER2), a genome that has major part in growth process, differentiation, and survival in these cancer cells. HER2 overexpression is associated with metastasis, recurrence and lower survival rate.²⁰ Other studies concerning diagnostic value and its comparison between MAI and Ki-67 expression in predicting breast cancer distant metastasis has not yet found as far as our knowledge.

There are some limitations we found on conducting this study. First, we used only radiology examinations, such as CT-Scan, Ultrasonography and X-Ray to diagnose distant metastasis as our gold standard. In Sanglah Hospital, we didn't have appropriate resources in detecting or diagnosing distant or micro metastasis such as PET-Scan. Second, there are more than one institute which provide histopathology results, resulting bias in diagnosing malignancies. Lastly, we didn't observe Pathological Anatomy Department on how they utilized our specimens, such as staining and buffering. We hope for further study related to put these factors into attention, since it can alter the diagnosis result.

CONCLUSION

In predicting distant metastasis in breast cancer, MAI and Ki-67 have several advantages and disadvantages. MAI values have high specificity in diagnosing distant metastasis, with 82.7%, while Ki-67 expressions have a relatively high sensitivity in diagnosing distant metastasis with 69.6%. There is not any statistically significant difference between these two modalities in predicting distant metastasis in breast cancer. We hope that these markers can encourage other researcher into exploring more about these markers and their function. We also encourage for other researchers who intend conducting prospective studies to put these markers into their main objectives.

ACKNOWLEDGEMENTS

Authors would like to thank Prof. Dr. I. Gede Raka Widiana, Sp. PD-KGH for review before submit and Dr. Wayan Gede Artawan Eka Putra, M.Epid and Erma Pradnyani, SKM for data analysis.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee of Medical Faculty of Udayana University and Sanglah General Hospital, Denpasar, Indonesia (ethical Approval number: 2220/UN14.2.2.VII.14/LP/2018 date: 18 October 2018)

REFERENCES

1. Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and mortality and epidemiology of breast cancer in the World. *Asian Pacific J Care Prevention.* 2016;17:43-6.
2. Youlden DR, Cramb SM, Yip CH, Baade PD. Incidence and mortality of female breast cancer in the Asia-Pacific region. *Cancer Biol Med.* 2014 Jun;11(2):101.
3. Nirvana IW, Dewi NP. Gambaran stadium dan jenis histopatologi kanker payudara di Subbagian Bedah Onkologi RSUP Sanglah Denpasar tahun 2015-2016. *Intisari Sains Medis.* 2018;9(1):80-4.
4. Hermansyah D, Albar ZA, Purwanto DJ, Sari L, Putri RI. CD 105 as prognostic factors in advanced stage breast cancer patients. *Bali Med J.* 2017 Jan 1;6(1):227-35.
5. Haroon S, Hashmi AA, Khurshid A, Kanpurwala MA, Mujtuba S, Malik B, et al. Ki67 index in breast cancer: correlation with other prognostic markers and potential in pakistani patients. *Asian Pac J Cancer Prev.* 2013 Jan 1;14(7):4353-8.
6. Devianti L, Aswiyanti A, and Edison. Peningkatan Ekspresi Ki-67 tidak Berhubungan Dengan Parameter Prognostik Histopatologik Karsinoma Payudara Invasif di Sumatera Barat. *Majalah Patologi Indonesia.* 2012;21(3):10-6.
7. Buhmeida A, Al-Maghrabi J, Merdad A, Al-Thubaity F, Chaudhary A, Gari M, Abuzenadah A, et al. Prognostic value of mitotic counts in breast cancer of Saudi Arabian patients. *Anticancer Res.* 2011 Jan 1;31(1):97-103.
8. Stuart-Harris R, Caldas C, Pinder SE, Pharoah P. Proliferation markers and survival in early breast cancer: a systematic review and meta-analysis of 85 studies in 32,825 patients. *The Breast.* 2008 Aug 1;17(4):323-34.
9. Baak JP, Van Diest PJ, Janssen EA, Gudlaugsson E, Voorhorst FJ, van der Wall E, et al. Proliferation accurately identifies the high-risk patients among small, low-grade, lymph node-negative invasive breast cancers. *Annals Oncol.* 2007 Nov 27;19(4):649-54.

10. Baak JP, van Diest PJ, Voorhorst FJ, van der Wall E, Beex LV, Vermorken JB, et al. Prospective multicenter validation of the independent prognostic value of the mitotic activity index in lymph node-negative breast cancer patients younger than 55 years. *J Clin Oncol.* 2005;23(25):5993-6001.
11. Kramer E, Herman O, Frand J, Leibou L, Schreiber L, Vaknine H. Ki67 as a biologic marker of basal cell carcinoma: a retrospective study. *IMAJ.* 2014 Apr;16(4):229-32.
12. Kobayashi K, Ito Y, Ogiya A, Gomi N, Horii R, Takahashi S, Hatake K, Akiyama F, Iwase T. Relationship of Ki-67 proliferative index and metastatic tumor of breast cancer. *J Clin Oncol.* 2013;31(15):1-4.
13. De Azambuja E, Cardoso F, de Castro Jr G, Colozza M, Mano MS, Durbecq V, et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12 155 patients. *Brit J Cancer.* 2007 May;96(10):1504.
14. Narisuari. Gambaran Karakteristik Penderita Kanker Payudara di Poliklinik Bedah Onkologi RSUP Sanglah tahun 2016. Skripsi, Fakultas Kedokteran Universitas Udayana, Denpasar Bali; 2017. Available at: https://sinta.unud.ac.id/uploads/dokumen_dir/5f295d28521e77f2c72cd4b83e3e33be.pdf
15. Partini PD, Niriyana IW, Adiputra PA. Karakteristik kanker payudara usia muda di Subbagian Bedah Onkologi Rumah Sakit Umum Pusat Sanglah tahun 2014-2016. *Intisari Sains Medis.* 2018;9(1):76-9.
16. Pulido C, Vendrell I, Ferreira AR, Casimiro S, Mansinho A, Alho I, Costa L. Bone metastasis risk factors in breast cancer. *E Cancer Medical Sci.* 2017;11:715.
17. Chen MT, Sun HF, Zhao Y, Fu WY, Yang LP, Gao SP, et al. Comparison of patterns and prognosis among distant metastatic breast cancer patients by age groups: a SEER population-based analysis. *Scientific Reports.* 2017 Aug 23;7(1):9254.
18. Ribelles N, Santonja A, Pajares B, Ll acer C, Alba E. The seed and soil hypothesis revisited: current state of knowledge of inherited genes on prognosis in breast cancer. *Cancer Treatment Reviews.* 2014 Mar 1;40(2):293-9.
19. Tjokrowidjaja A, Lee CK, Houssami N, Lord S. Metastatic breast cancer in young women: a population-based cohort study to describe risk and prognosis. *Inter Med J.* 2014 Aug;44(8):764-70.
20. Mitri Z, Constantine T, O'Regan R. The HER2 receptor in breast cancer: pathophysiology, clinical use, and new advances in therapy. *Chemotherapy research and practice.* 2012 Dec 20;2012.

Cite this article as: Ginting A, Setiawan IGB, Sudarsa IW, Widiana IGR. Comparison of diagnostic validity between mitotic activity index and Ki-67 expression as predictor for breast cancer in distant metastasis. *Int J Res Med Sci* 2019;7:2072-7.