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High receptor activator of Nuclear Factor Kappa β (RANK) expression and luminal A subtype are associated with bone metastasis in patients with breast cancer

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ABSTRACT

Introduction: Bones are the most common distant metastasis site in breast cancer, especially in advanced stages. Bone metastasis involves continuous interaction between tumor cells, osteoblast, osteoclast, and bone matrix. There are many risk factors regarding distant metastasis sites in breast cancer patients, including breast cancer molecular subtypes and mediator known as Receptor Activator of Nuclear Factor Kappa β (RANK). In this study, we explore relationships between Luminal A breast cancer and RANK in association with bone metastasis site.

Method: This study is a cross-sectional analysis study conducted in Sanglah General Hospital, Bali Denpasar. The estimated sample size was measured using formula to hypothesize between two proportions and obtained 34 patients as our minimal sample needed in this study. Data will be presented in 2x2 tables, consist of RANK Protein expression and molecular cancer subtype (Luminal A / Non-Luminal A) in row section and Metastasis (Bone Metastasis or Non-Bone Metastasis) in column section. Univariate analysis was done using a comparative method between 2 categorical unrelated groups: Chi-Square and Fisher's Exact Test. OR values were measured, and p-value <0.05 considered to be significant statistically.

Result: From these 106 patients, we used nested sampling to randomize these patients into our study sample, with a total of 36 patients. The mean age of our patients is 48.64 ± 9.86 years. Luminal A subtypes tend to metastasize into bone component compared with Non-Luminal A subtypes with p-value 0.041 and OR: 3.5; with 95% CI (0.82 – 14.84). Tumor with high-RANK expressions tends to metastasize into bone component compared with low-RANK expressions with p-value 0.045 and OR 3.25; with 95% CI (0.81 – 13.03)

Conclusion: There is a significant difference statistically in molecular subtype breast cancer and RANK protein expressions between two patient groups (bone metastasis site vs. other metastasis sites)

Keyword: breast cancer, bone metastasis, Luminal A breast cancer, RANK Protein
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INTRODUCTION

Distant metastases in breast cancer are commonly found in bone, liver, and lungs, although it can spread to other organs, such as brain. Bones are the most common distant metastasis site in breast cancer, especially in advanced stages. Bone metastasis process with its related complication generally is termed as Skeletal Related Events (SRE). This SRE term is referring to five major complications found in bone with metastasis pathology, consist of pathologic fracture, necessities for radiotherapy in bones, necessary bone surgery, vertebral compression, and hypercalcemia. Bone radiotherapy and pathologic fracture are the most common complication found in SRE.[1]

Until today, Indonesia still unable to obtain the accurate incidence rate for breast cancer, particularly bone metastasis incidence in breast cancer patients. The latest incidence data in Indonesia revealed approximately 20.000 new breast cancer cases annually, with an incidence rate of 18.6 every 100.000 women in Jakarta, 50% of them already in an advanced state.[2,3] Based on the recent observation in Bali, more than 70% breast cancer patient who seeks medical treatment in Sanglah General Hospital were also already in advanced stages. Approximately 5-10 % of breast cancer patients had distant metastases and bones are the most common sites found in these patients.[4]

Based on XII St. Gallen International Breast Cancer Conference in 2011, breast cancer were further classified into 5 molecular groups, consist of Luminal A subtype, Luminal B subtype, Luminal BH subtype, HER2 subtype and Triple Negative Breast Cancer (TNBC) These molecular subtypes had different risk factors, tumor progressions, treatment responses, and prognosis. HER2 subtypes and TNBC had tendencies to metastasize into visceral organs. Meanwhile, luminal subtypes were usually metastasized into bone components.

Bone metastasis involves continuous interaction between tumor cells, osteoblast, osteoclast, and bone matrix.[1] These interactions will decide whether bone metastasizes osteolytic or osteoblastic. There are some mediators, which had a role in osteolytic bone metastasis (Parathyroid Hormone-related Protein / PTHrP, Receptor Activator of Nuclear Factor Kappa β) and some mediators had a role in osteoblastic bone metastasis (Endothelin-1, Transforming Growth Factor β / TGF- β, Fibroblast Growth Factor / FGFs). These interactions were described by Mundy as a 'vicious cycle' between tumor cells and bone.[5,6]

With this Receptor Activator of Nuclear Factor Kappa β (RANK), Receptor Activator of Nuclear Factor Kappa β Ligand (RANKL) and Osteoprotegerin (OPG) central roles had been discovered in bone physiology and their involvement in metastasis bone processes, these mediators were already become subjects in many studies regarding markers for breast cancer and distant metastases, especially for treatment purposes. In general, metastasis in bones will show increasing activities of RANKL and low OPG levels, resulting in the development of osteolytic lesions in a bone through osteoclast's activities.[7] These RANK-RANKL-OPG activities, not only had a role in bone physiology and bone metastasis involvement, they also had a role in breast lactation physiology. Therefore these mediators were suspected of playing a role in breast cancer carcinogenesis process.[8-10] With this information in mind, we intend to develop a study to find any relationship between high-RANK expression, luminal A subtype breast cancer (as they had tendencies to metastasize into bone components) with bone metastasis events in breast cancer patients.

METHODS

This study is a cross-sectional analysis study to analyze RANK expressions, luminal A subtype breast cancer and their relationships with bone metastasis event in breast cancer patients. This study was conducted in Sanglah General Hospital, central referral hospital located in Denpasar, Bali. Due to referral systems in Indonesia national health policies, patients with government health insurance will not be referred to our hospital, unless they need specific treatments or advanced medical procedures. There are numerous oncology patients, including breast cancer patients, seek advanced treatment modalities in our hospital, particularly chemotherapy due to availability of chemotherapy agents and can be covered by government health insurance in Sanglah General Hospital. Thus, Sanglah General Hospital becomes one of the biggest oncology centers in Bali and Nusa Tenggara (Lesser Sunda Islands).

This study was conducted from January until December 2016, with a total of 106 patients with breast cancer admitted into our hospital. The estimated sample size was measured using formula to hypothesize between two proportions and obtained 34 patients as our minimal sample needed in this study. Randomization methods are required, thus we used nested sampling methods in our sample study to achieve the number of samples intended. Our inclusion criteria consist of all breast cancer patients with distant metastasis which were confirmed by histopathology results and radiology examination, patients who had complete medical records (clinical condition, histopathology results and immunohistochemistry results) with variables information needed in our study. Male breast cancer patients are excluded from our research. Also, patients with incomplete medical records and histopathology examination result from our Pathological Anatomy laboratory.

All data and variables needed in this study are obtained from SIMARS, our computerized medical record system in Sanglah General Hospital, written medical records, histopathology results and immunohistochemistry results. All variables collected from our data pool consist of a degree of differentiation, stadium T according to TNM Classifications, distant metastasis organ sites and immunohistochemistry results (Estrogen Receptor / ER, Progesterone Receptor / PR, HER2 Subtype, Ki-67 Expressions). Tumor differentiation grade or degree were counted using Nottingham Combined Histologic Grade, modified from Scarf-Bloom Richardson by Elston-Ellis. These grading systems consist of tumor tubule formation, mitotic activity, and nuclear pleomorphism grade. These grading systems are further classified into 3 categories, Grade 1 (Well Differentiated), Grade II (Moderate Differentiated) and Grade III (Poorly Differentiated). We classified Grade I as low-grade tumor and Grade II and III as high grade tumor.

RANK Protein Expression data were also collected as our independent variables. RANK Protein Expressions are obtained from histopathology results from primary tumor specimen paraffin block, which were stained for immunohistochemistry. Semi-quantitative count was done using immunohistochemistry staining (Rabbit Polyclonal Antibody GTX11697, Genetex) and we concentrate our findings in cell nucleus inside microscopic high field view. This immunohistochemistry stain will be counted using H-score method. H-score are ranged between 0-300, with H-score 0-150 are considered

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low expression, and H-score 151-300 are considered high expression. From molecular cancer subtype variables, we divided these subtypes into 2 main groups, luminal A and non-luminal A subtypes. Non-luminal A subtypes are consist of luminal B cancer subtypes, HER2 subtypes and TNBC.

We analyzed our data using SPSS 21.0 software (New York). All numerical variables will be presented as mean ± standard deviation and all categorical variables were shown in percentages. Data will be presented in 2x2 tables, consist of RANK Protein expression and molecular cancer subtype (Luminal A / Non-Luminal A) in row section and Metastasis (Bone Metastasis or Non-Bone Metastasis) in column section. Univariate analysis was done using a comparative method between 2 categorical unrelated groups: Chi-Square and Fisher's Exact Test. We count the effect size with a confidence interval of 95% and p-value <0.05 considered to be statistically significant.

RESULTS

From January 2016 to December 2016, there was a total of 106 breast cancer patients who seek medical treatments in our hospital. From these 106 patients, we used nested sampling to randomize these patients into our study sample, with a total of 36 patients. The mean age of our patients is 48.64 ± 9.86 years, with the youngest patient is 27 years old, and the oldest is 76 years old. All of the variables collected from our data are presented in Table 1.

Table 1 Clinical, histopathology and immunohistochemistry profile

Variable	n	%
Differentiations Degree		
Low Grade	3	8,3%
High Grade	33	91,7%
Stadium T (Based on TNM)		
T_{0-2}	5	13,9%
T_{3-4}	31	86,1%
Estrogen Receptor		
Negative	14	38,9%
Positive	22	61,1%
Progesterone Receptor		
Negative	14	38,9%
Positive	22	61,1%
Her-2		
Negative	18	50%
Positive	18	50%
Ki67		
Negative (<14%)	9	25%
Positive (>14%)	27	75%
Breast Cancer Subtype		
Luminal A	13	36,1%
Luminal B	10	27,8%
Her2 Type	8	22,2%
Triple-Negative	5	13,9%

In this study, our data showed that majority of the breast tumors with distant metastasis had a high grade of tumor differentiation with 33 patients or 91.7% and only 3 patients (8.3%) had a low-grade tumor. From tumor size variables, 5 patients had a breast tumor with stage T_{0-2} (13.9%), and 31 patients had tumor with TNM staging T_{3-4} (86.1%). From molecular breast cancer categories, there are 13 patients with Luminal A subtypes (36.1%) and 23 patients with Non-Luminal A subtypes, consist of 10 Luminal B subtype patients (27.8%), 8 HER2 type patients (22.2%) and TNBC patients (13.9%). In this study, we also analyzed the association of tumor differentiation degree, primary tumor size (based on TNM staging) and molecular subtype in patients with bone metastasis and patients with metastasis other than bones. There is no significant difference found on tumor differentiation degree, primary tumor size (based on TNM staging) and molecular subtype between these two groups with a p-value of 0.546, 0.630 and 0.298 consecutively (Table 2).

From 36 study sample, 9 patients with Luminal A molecular breast cancer had distant metastasis with bone as their target organ sites (69.2%), where Luminal A subtypes cause 3.5 times more likely to metastasize into bone compared with Non Luminal A molecular subtypes (odds ratio: 3.500). There is significant difference statistically in molecular subtype breast cancer between two groups (bone metastasis site vs. another metastasis site) with p-value 0.041 (< 0.05) (Table 3).

Based on RANK Protein Expressions, a total of 13 breast cancer patients with bone metastasis had high-RANK Protein Expressions (61.9%), while only 5 patients had low-RANK Protein expressions (33.3%). There are 8 breast cancer patients with other metastasis sites had high-RANK Protein expressions (38.1%) and 10 patients had low-RANK Protein expressions. Patients were with high-RANK Protein expression cause 3.25 times more likely to metastasize into bone compared with low-RANK Protein expression (odds ratio: 3.25) with 95% confidence interval range 0.81 – 13.03. There is also significant difference statistically in RANK Protein expressions between two groups (bone metastasis vs. non-bone metastasize site) with p-value 0.045 (< 0.05) (Table 3).

Table 2 Association between breast cancer molecular subtype and metastasis target sites

	Metastasis Target Sites		95% Confidence Interval			
	Bone Metastasis	Non-Bone Metastasis	OR	Lower	Upper	p value
Luminal A	9 (69.2%)	4 (30.8%)	3.500	0.825	14.848	0.041
Non Luminal A	9 (39.1%)	14 (40.0%)				

Table 3 Association between RANK Protein Expressions and Metastasis Target Sites in Breast Cancer Patients

	Metastasis Target Sites		95% Confidence Interval			
	Bone Metastasis	Non-Bone Metastasis	OR	Lower	Upper	p value
High RANK	13 (61.9%)	8 (38.1%)	3.250	0.811	13.030	0.045
Low RANK	5 (33.3%)	10 (66.7%)				

DISCUSSION

All of our samples in this study are all breast cancer patients with distant metastasis who seek medical treatment in our surgical oncology polyclinics in Sanglah General Hospital, located in Denpasar, Bali. In this study, we found majority of these breast cancer patients had tumor with high grade differentiations (n=33, 91.7%), high tumor grade (T_{3-4}) based on TNM staging (n=31; 86.1%), and had Luminal A molecular subtype (n=13; 36.1%), followed by Luminal B molecular subtypes with 10 patients (27.8%). The mean age patients are 48,64 ± 9,86 years old, with the youngest patient is 27 years old, and oldest is 76 years old. The youngest patient in our study period had breast cancer with de novo metastasis, had T_{3-4} tumor staging, high differentiation grade, and had Luminal A molecular subtype. Our oldest patient also had a similar characteristic.

There are some clinical and histopathology factors which were studied and known to play a role in predicting metastasis pattern in breast cancer patients.[11] The duration time needed to develop metastasis process, first metastasis target organ sites, and breast cancer subtypes are one of the factors which were studied as a benchmark in delivering targeted therapy, oligometastatic surgeries and risk stratifications in distant metastasis patterns.[11-13] Based on this information. We included molecular subtype breast cancer as our independent variables. In this study, we found that the most molecular subtypes found in our sample study are patient with Luminal A subtypes with 13 patients (36.1%). This finding result is similar with studies conducted by Haque et al[13] in 2012 and Kast et al[11] in 2015. In patients without immunochemistry results or Ki-67 expressions, we used tumor differentiation grade systems as our substitute. The reason why we used this grading systems are Ki-67 expressions are already accepted as one of the markers used in breast cancer proliferation and there is a similar study using the same definitions and measuring tools just like our study.[14]

In general, we knew that breast cancer with Luminal A subtype (ER+ and PR+) tends to metastasize into bone compartment individually or together metastasize into another target organ, including visceral organ and breast contralateral. According to into our study, Luminal A subtypes have 3.5 times more likely to metastasize into bone component, a similar result also shown by Gerratana et al.[15] Process of developing metastasis in these patients occurs in relatively long duration, with consume approximately more than 5 years, and dormant.[16] There are some limitations regarding this result. First, we did not measure the time or duration needed from a tumor to develop into distant metastasize. Second, we did not notify which organs were affected first from this distant metastasis, nor discovered micro-metastasis process in these patients.

In this study, we also found a significant association in RANK protein expressions between patients with or without bone metastasis. Patients with high-RANK protein expressions are more likely to metastasize into bone component in patients with breast cancer patients (p=0.045). High-RANK protein expressions had 3.25 times more likely to metastasize into bone component compared with patients with low-RANK protein expressions. In our opinion and experience, this is the first study, discovering and describing RANK protein expressions (as shown in immunohistochemistry results) role in predicting distant metastasis site, particularly bone metastasis in patients with breast cancer. The similar results are also demonstrated by

Trinkaus[17] et al. in 2009 and Ney et al. in 2011, but the total cases are relatively few.

Our findings are also supported by previous data, which RANK-RANKL-OPG activity is a balance and dynamic process, found both in bone tissue and breast tissue.[18,19] There is a possibility that this chemotaxis mechanism between RANK-RANKL-OPG in breast tumor cells with osteoblast found in bone can be a new theoretical basis for further studies related. We hope for the next studies to include this mechanism, particularly RANK-RANKL-OPG, in determining treatment for breast cancer patients, also for predicting metastasis sites in breast cancer patients.

CONCLUSION

There are many studies focusing their attentions on how to predict outcome and deciding prognosis in malignancies, including clinical status, histopathology results, and other diagnostic tools. From this study, we can conclude that high-RANK protein expression had a statistically significant role in breast cancer patient with bone metastasis, with 3.25 times more likely to metastasize into bone component compared to patients with low-RANK protein expression. Molecular subtypes also had an association with bone metastasis. It can be concluded from our study that there is a statistically significant difference in Luminal A subtype breast cancer between bone metastasis with other sites metastasis, with odds ratio of 3.50. With these analyses, we hope that further study can apply these mediators in predicting outcomes, such as metastasis, and can improve global research for a better treatment approach for breast cancer patients.

CONFLICT OF INTEREST

The author declares there is no conflict of interest regarding publication of this article.

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