https://doi.org/10.48048/tis.2023.6672

Correlation Among Tumor Size, Histopathological Grade and the Expression of RAC1 Protein with Breast Cancer Metastasis to Ipsilateral Axillary Lymph Nodes

Jafan Argya Pradana Heriady[1], Kiki Achmad Rizki[1], Yohana Azhar[1], Maman Abdurahman[1], Bethy Suryawathy Hernowo[2], Ahmad Faried[3], Yusuf Heriady[4] and Ika Adhani Sholihah[5,*]

[1]Department of Surgery, Faculty of Medicine, Universitas Padjadjaran, Dr. Hasan Sadikin Central General Hospital, West Java 40132, Indonesia
[2]Department of Pathological Anatomy, Faculty of Medicine, Universitas Padjadjaran, Dr. Hasan Sadikin Central General Hospital, West Java 40132, Indonesia
[3]Oncology and Stem Cell Working Group, Faculty of Medicine, Universitas Padjadjaran, Dr. Hasan Sadikin Central General Hospital, West Java 40132, Indonesia
[4]Departement of Surgery, Faculty of Medicine, Universitas Islam Bandung, Al-Ihsan Hospital, Baleendah, Bandung, West Java, 40375, Indonesia
[5]School of Life Sciences and Technology, Institut Teknologi Bandung, Jawa Barat 40132, Indonesia

([*]Corresponding author's e-mail: ikaadhani18@gmail.com)

Received: 25 January 2023, Revised: 9 March 2023, Accepted: 10 March 2023, Published: 21 March 2023

Abstract

Breast cancer (BC) is the most common cancer affecting women worldwide. Mastectomy is the standard treatment for breast cancer. The purpose of axillary lymph nodes (ALN) dissection in mastectomy is to examine the status and the presence of metastasis in ALN. This study is an analytic observational cross-sectional study coupled with a categorical comparative study in 60 BC patient's with- and without-ALN metastasis. The data that used in this study were histopathological data from patients medical record and immunohistochemistry (IHC) of RAC1 expression data. The immunohistochemistry was performed with a cutoff point based on the result of a receiver operating characteristics (ROC). This study combined BC tumor size, histopathological grade, and expression of RAC1 protein in correlation with known risk factors on metastasis to ALN. From 60 subjects, there were 63.3 % (38) ALN (+) and 36.7 % (22), ALN (−). BC of T2 tumor size, grade 2 and RAC1(−), the ALN metastasis chance was 0.6 %. T2 tumor size, grade 2 and RAC1(+), the ALN metastasis chance was 4.5 %. T2 tumor size, grade 3 and RAC1(−), the ALN metastasis chance was 3.0 %. T2 tumor, grade 3 and RAC1(+), the ALN metastasis chance was 18.8 %; T3 tumor size, grade 3 and RAC1(−), the ALN metastasis chance was 12.7 %. T3 tumor size, grade 3 and RAC1(+), the ALN metastasis chance was 52.2 %; T4b tumor size, grade 3, RAC1(−) and RAC1(+), the ALN metastasis chance was 40.8 and 83.8 %, respectively. We conclude that smaller tumor size and lower histopathological grading with RAC1(−) has lesser possibility to ALN metastasize.

Keywords: Breast cancer, Axillary lymph nodes, Tumor size, Histopathologic grade, RAC1

Introduction

Breast carcinoma is a malignant neoplasm originating from epithelial cells of the ducts and breast glands in the area of the lobules and terminal ducts known as the terminal duct lobular unit (TDLU) [1]. In 2020, there were 2.3 million women diagnosed with breast cancer and 685,000 deaths globally. As of the end of 2020, there were 7.8 million women alive who were diagnosed with breast cancer in the past 5 years, making it the world's most prevalent cancer [2]. In the US, the mortality rate is 14.8 to 30.8 per 100,000 population [3]. In Indonesia, breast carcinoma is the number one cancer among the 10 most common cancers in women [4]. According to Globocan data 2020 the breast carcinoma ranks first with cervical cancer [5].

Based on histopathological examination, breast carcinoma is catagorized into *in situ* type and invasive type. The distribution of breast carcinoma from all types of histology shows that *in situ* type amounts to 15 - 30 % while the invasive carcinoma is about 70 - 85 % [1]. Of all invasive carcinomas, invasive breast

carcinoma of no special type (NST) is the largest part of the breast cancer subtypes, by 79 % [1]. Based on the TNM stage classification from the Union International Contra Le Cancer (UICC) or the 8th American Joint Committee on Cancer Staging and End Results Reporting (AJCC), breast carcinoma consists of 4 stages (I, II, III, and IV) [6]. Breast carcinoma is classified into operable (BCO) and inoperable. Breast carcinoma that is included in the BCO group is stage I, IIA, IIB, and IIIA [7]. The surgery performed for breast carcinoma is breast-conserving treatment or mastectomy. There is 2 kind of mastectomy procedure; (i) radical mastectomy and (ii) modified radical mastectomy; the above procedure is accompanied by axillary lymph nodes (ALN) dissection. Mastectomy procedure is still a standard protocol of breast carcinoma in many countries, including Indonesia. However, the procedure increases the risk of postoperative morbidity such as the lymphedema, seroma, hematoma, surgical wound infection, and postoperative pain [8].

RAC1 is a member of the RHO-GTPase family that plays an important role in the migration process, loss of adhesions between cells, and tumor metastasis. Studies have reported on the association of several genes encoding certain proteins with the motility and direction of migration of cancer cells. Higher expression of RAC1 protein in breast cancer (BC) cells and not expressed in the normal breast cells, whereas RAC gene amplification1 was not found in breast cancer cells, and associated with the occurrence of ALN metastases [9]. Heriady *et al*. in their study, concluded that RAC1(+) had the highest value compared to RHOA and CXCR4 in terms of distribution to tumor cells and acted as a risk factor for carcinoma infiltration into the papilla areola complex with an infiltration risk of 5.76 times [10]. Based on the above reason, our study was aimed at determining an assessment of combination factors such as tumor size, histopathological grading, and the expression of RAC1 protein in breast cancer to observe the correlation with its metastasis to the ALN.

Materials and methods

Study design

The accessible population in this study was breast cancer patients in all grades; with- and without-axillary lymph nodes (ALN) metastases in Dr. Hasan Sadikin Hospital, Bandung, from January 2016 to December 2018. This study protocol was approved by the Faculty of Medicine Ethics Committee Review Board, Universitas Padjadjaran and all study participants gave informed consent with number LB.02.01/X.6.5/99/2020. All authors hereby declare that all patients have been examined following the ethical standards laid down in the 1964 Declaration of Helsinki.

The research subjects were patients that met the inclusion criteria and exclude from the exclusion criteria. The inclusion criteria were patients with and without breast cancer metastasis to ALN who can be operated on diagnosed histopathologically invasive carcinoma of no special type also has complete medical record data as needed in the study. On the other hand, the exclusion criteria were patients that has recidive breast carcinoma and invasive breast carcinoma with variants lobular, tubular, mucinous, medullary, and papillary.

Research experiment

This research experiment uses cross-sectional. Study included 60 breast cancer patients were obtained consisting of 38 subjects with breast cancer metastasis to ALN and 22 subjects without breast cancer metastasis to ALN. Various factors like patient's age, tumor size, histological grade, RAC1 intensity, and RAC1 distribution were correlated with the ALN positivity. The data was collected from available clinical and histopathology records.

Immunohistochemistry (IHC) on breast cancer tissue

Immunohistochemistry (IHC) analysis was done using an anti-RAC1 antibody. Staining was performed using a streptavidin-biotin method as described previously [10]. In brief, paraffin blocks were processed in 4-μm-thick sections and were then incubated with fresh 0.3 % hydrogen peroxide in methanol for 30 min at room temperature. After rinsing in phosphate-buffered saline (PBS; in pH 7.4), non- specific binding sites were blocked by incubating with 10 % normal serum for 30 min. The specimens were then incubated with anti-RAC1 (ab33186, Abcam, USA) monoclonal antibody.

The staining was scored semi-quantitatively as follows: Total staining was scored as the staining intensity (on a scale of 0, 1+ to 4+) and distribution of staining; level 4+ was the strongest-staining intensity and 1+ the lowest-staining intensity, and 0 for negative-staining. A distribution of < 20 % was scored 1, 20 - 50 % was scored 2, 50 - 80 % was scored 3 and > 80 % was scored 4. The value obtained of the staining intensity (i) and distribution (d) were combined into a histoscore value (the positivity value of the intensity

plus the number one then multiplied by the positivity value of the distribution). Then, the cutoff point was determined based on the receiver operating characteristics (ROC) curve in both groups.

Data analysis

Statistics analysis was performed using SPSS, ver. 16.0. A chi-square and correlation regression test was performed to determine the correlation among these variables; the p-values < 0.05 were considered as significant.

Results and discussion

Our study aims to observe the correlation of tumor size, histopathological grading, and the expression of RAC1 protein with the breast cancer metastasis to ipsilateral axillary lymph nodes (ALN). Until recently, the protocol for the management of breast cancer (BC) was to perform a radical mastectomy. In a radical mastectomy, ALN dissection is performed. Radical mastectomy reminds a standard procedure in the treatment of breast cancer in many countries, including Indonesia. However, this procedure tends to increase postoperative morbidity such as lymphedema, seroma, hematoma, surgical wound infection, postoperative pain [8].

On the other hand, lymph nodes metastases in breast cancer is an important factor to see the risk of recurrence and life expectancy of patients with breast cancer [11]. In order to find out the presence the breast cancer metastasis to ipsilateral lymph nodes; lymph nodes dissection should be performed. Another action to see if breast cancer metastases to lymph nodes are sentinel lymph nodes biopsy (SLNB), but this procedure still not the main protocol in breast cancer therapy, especially at our center in Dr. Hasan Sadikin Hospital (RSHS), Bandung. Considering that radical mastectomy reminds a standard therapy for breast cancer management in our country, it's necessary to know what factors influence breast cancer metastasis to ipsilateral ALN, to determine the need of lymph nodes dissection according to its stage to reduce postoperative complications and avoiding lymph nodes dissection in breast cancer not requiring ALN dissection.

Out of 147 breast cancer cases in our center, there are 60 study subjects meet the inclusion criteria, with patient's distribution of 38 (63.3 %) breast cancer cases with metastases to ALN and 22 (36.7 %) cases of breast cancer without metastases to ALN with stage I, II (II A-B), III (IIIA-B) and histopathologically no special type. The tumor size T2 was 14 (23.3 %), the T3 was 28 (46.7 %) and T4b was 18 (30 %). For histopathological gradations: grade 2 was 13 (21 %) patients and grade 3 was 47 (78.3 %) patients (**Table 1**).

Table 1 Characteristic of the study subject.

Variable	Breast Cancer (N=60)
Age (Mean ± SD)	51.68 ± 10.55
Metastasis	
Lymph nodes positive	38 (63.3 %)
Lymph nodes negative	22 (36.7 %)
Tumor size (T)	
T2	14 (23.3 %)
T3	28 (46.7 %)
T4b	18 (30.0 %)
Histopathological grade	
Grade 2	13 (21.7 %)
Grade 3	47 (78.3 %)
RAC1 intensity (i)	
Strong	27 (45.0 %)
Moderate	23 (38.3 %)
Weak	10 (16.7 %)
RAC1 distribution (d)	
< 20 %	3 (5.0 %)
20 - 50 %	6 (10,0 %)
50 - 80 %	10 (16.7 %)
> 80 %	41 (68.3 %)

The correlation of age, tumor size and histopathological grade with the status of ALN metastases was shown in **Table 2**. Based on the Chi-square test analysis, the age ($p = 0.976$) has no correlation with status of ALN metastases, meanwhile for the breast cancer tumor size ($p = 0.021$) and histopathological gradation ($p = 0.036$) correlated with the status of ALN metastases.

Table 2 Correlation of age, tumor size, and histopathological grade with the status of ALN.

Characteristic	Axillary lymph node (ALN)		*p*-Value*
	Positive (N = 38)	Negative (N = 22)	
1. Age (years old):			0.976
30 - 39	6	4	
40 - 49	9	6	
50 - 59	15	8	
≥ 60	8	4	
Mean ± SD	52.3 ± 10.4	50.7 ± 10.9	
Range	34 - 73	33 - 77	
2. Tumor size (T):			0.021¶
T2	5	9	
T3	18	10	
T4b	15	3	
3. Histopathological grade:			0.036¶
Grade 2	5	8	
Grade 3	33	14	

*based on Chi-square test; ¶significant

From many studies that have been published, it was found that various factors influenced breast cancer metastasis to ALN. The role of the familial RHO-GTPase and chemokines in the process of breast cancer metastasis to ALN has never been reported; a study was conducted on the tumor size, histopathological grading, and the expression of RAC1 protein, as risk factors for breast cancer metastasis to ALN. Familial RHO-GTPases, particularly the RAC1 protein, are involved in cell motility. The RAC1 protein facilitates the formation of lamellipodia and membrane ruffles [9]. During these processes, cell migration occurs and the formation of filopodia, lamellipodia, stress fibers and actomyosin contractility is initiated. Actomyosin contractility requires actin reorganization to trigger cell movement. Control of actin reorganization is regulated by RAC1 and RHOA [12-14].

RAC1 was chosen because among the proteins in the RHO family GTP'ase the expression RAC1 is the strongest protein to show the invasion; thus, RAC1 expression is more sensitive in breast cancer compared to the other RHO family GTP'ase member [10]. The correlation between tumor size and breast cancer metastasis to ALN from this study, is that tumor size has a significant correlation with the incidence of breast cancer metastasis to ALN. For T3 tumor size with a POR of 4.30; mean that T3 tumor size has a risk of breast cancer metastasis to ALN by 4.30 times when compared to T2 tumor size ($p = 0.013$), and for T4b tumor size has a risk of breast cancer metastasis to ALN of 22.82 times when compared with the tumor size T2 ($p = 0.035$). Then the larger the size of the breast cancer tumor, the higher the risk to metastasis to ALN.

The correlation between the expression of RAC1 protein with the status of ALN metastases was shown in **Table 3** and **Figure 1**. Brown color in the breast cancer tissue was indicated the presence of RAC1 expression. In the breast cancer with ALN metastases (+) group, the strength of RAC1 staining intensity (i) was 57.9 % (22/38); the highest of RAC1 distribution (d) was 71.1 % (27/38). On the other hand, in the breast cancer with ALN metastases (−) group, the strong RAC1 intensity was 22.7 % (5/22). The cutoff point for RAC1 expression is > 9 (AUC = 0.696; $p = 0.006$). The expression of RAC1 protein is positive if the histoscore value is > 9 and negative if ≤ 9; the patient with a histoscore score > 9 will be diagnosed as ALN metastases (+).

Table 3 Correlation of the RAC1 protein expression with the status of ALN.

Expression of RAC1 protein	Axillary lymph node (ALN)		*p*-Value*
	Positive (N = 38)	Negative (N = 22)	
1. Intensity (i):			
Weak	2	8	0.003
Medium	14	9	
Strong	22	5	
2. Distribution (d):			
< 20 %	0	3	0.095
20 - 50 %	5	1	
50 - 80 %	6	4	
> 80 %	27	14	
3. Histoscore (HS):			
Median	12	9	0.009
Range	4 - 16	2 - 16	

*For intensity and distribution using Chi-square test; for histoscore using Mann-Whitney test

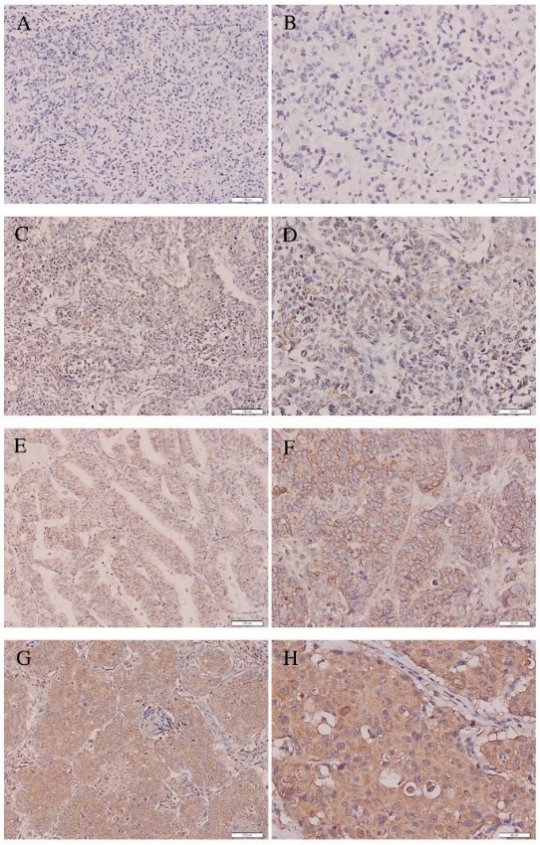

Figure 1 Immunohistochemical (IHC) detection of RAC1 expression in breast cancer tissue. (A) Expresion of RAC1 protein (−), magnification 100x; (B) Expresion of RAC1 protein (−), magnification 200x; (C) Expresion of RAC1 protein (+) weak, magnification 100x; (D) Expresion of RAC1 protein (+) weak, magnification 200x; (E) Expresion of RAC1 protein (+) moderate, magnification 100x; (F) Expresion of RAC1 protein (+) moderate, magnification 200x; (G) Expresion of RAC1 protein (+) strong, magnification 100x; (H) Expresion of RAC1 protein (+) strong, magnification 200x. Scale bar: 100 µm.

The expression of RAC1 protein was significantly correlated with the occurrence of breast cancer metastasis to ALN ($p = 0.003$; **Table 4**). The risk for breast cancer metastasis to an ALN on the expression of RAC1 protein (+) was 2.02 times compared with the expression of RAC1 protein (−). The prevalence of ALN (+) for the expression of RAC1 protein was 78.95 % (30/38).

Table 4 Correlation of *cut off* point the RAC1 protein expression with the status of ALN.

***Cut off point* histoscore RAC1**	**Axillary lymph node (ALN)**		***p*-Value***	**POR (CI 95 %)**
	Positive (N = 38)	**Negative (N = 22)**		
> 9	30	9	0.003	2.02 (1.14 - 3.58)
≤ 9	8	13		

Note: POR (IC 95 %) is Prevalency Odds Ratio (confidence interval 95 %)
*Based on Chi-square test; sensitivity 78,9 %; specificity 59.1 %; accuracy 71.7 %.

A study conducted by Sandoughran *et al.* found that tumor size was one of the predictive factors for lymph nodes metastases in patients with breast cancer [11]. Additionally, Chand *et al.*, shows that the larger the tumor size, the higher the incidence of lymph nodes metastases [15]. A study conducted in Pakistan by Hadi *et al.* showed that tumor size affects lymph nodes metastases, the larger the tumor size, the higher the risk of metastasis to the lymph nodes [16]. A study of Yenidunya *et al.* shown that metastasis to lymph nodes in breast cancer, was found mostly in tumors with a size > 2 cm [17]. Lymph nodes metastases were highly correlated with tumor size as mentioned in Mohamed's study [18]. From the results of the analysis of this study, the histopathological gradation of the tumor was significantly associated with the incidence of breast cancer metastasis to ipsilateral ALN. Histopathologic grade (Grade 3) had a risk of developing breast cancer metastasis to ALN by 4.96 times when compared to grade 2 ($p = 0.032$).

Table 5 showed the prevalence of T3 tumor size with RAC1 (+) was 39.5 % (15/38 with $p = 0.035$) and had a risk of metastasis to ALN 2.37 times compared to RAC1 (−). The prevalence of grade 3 with RAC1 (+) was 71.05 % (27/38); there was a significant correlation between histopathological gradation and the expression of RAC1 protein with breast cancer metastasis to ALN ($p = 0.008$) and a risk of ALN was 1.91 times compared to RAC1 (−). In a study conducted by Hadi *et al.*, conducted in Pakistan, grade 1 had a lower risk of metastasis to the lymph nodes compared to grade 2 and grade 3.16 From the analysis of this study, it was also found that the expression of RAC1 protein was significantly associated with the incidence of breast cancer metastasis to ipsilateral ALN. The RAC1 histoscore value > 9 had the risk of breast cancer metastasis to ALN 7.53 times when compared with the RAC1 histoscore ≤ 9 ($p = 0.003$). The RAC1 protein played an important role in cell growth and metastasis in in vivo experimental animals. In experimental experiments with Northern and Western blots, both of RAC1 RNA and RAC1 protein showed higher expression in the breast cancer tissue compared to the normal breast tissue. Immunohistochemical examination on RAC1 showed weak expression of RAC1 protein in the normal breast but showed high expression in ductal carcinoma-in-situ, invasive breast cancer, and lymph nodes metastases. In addition, breast cancer cells from patients with recurrent disease cases showed the expression of RAC1 protein on the plasma membrane, which indicates activation of the RAC1 protein in patients with aggressive breast cancer [9].

Table 5 Correlation of tumor size (T), histopathological grade, and RAC1 protein expression with the status of ALN.

	RAC1 expression	**Axillary lymph node (ALN)**		***p*-Value**	**POR (CI 95 %)**
		Positive (N = 38)	**Negative (N = 22)**		
Tumor size (T):	> 9	5	4	0.086	-
T2	≤ 9	0	5		
T3	> 9	15	4	0.035	2.37 (0.91 - 6.14)
	≤ 9	3	6		
T4b	> 9	10	1	0.528	1.27 (0.77 - 2.11)
	≤ 9	5	2		
Histopathological	> 9	3	3	0.592*	1.75 (0.42 - 7.22)
grade: 1 - 2	≤ 9	2	5		
grade: 3	> 9	27	6	0.008**	1.91 (1.02 - 3.57)
	≤ 9	6	8		

Note: POR (IC 95 %) is Prevalency odds ratio (confidence interval 95 %)
*Based on fisher exact test
**Chi-square

By multiple logistic regression analysis showed that the tumor size, histopathological grading, and histoscore of RAC1 were significantly correlated with the incidence of breast cancer metastasis to lymph nodes (**Table 6**). The size of the T3 tumor with a Prevalency Odds Ratio (POR) was 4.30; meant that the T3 tumor has a risk of metastasis to ALN 4.30 times higher when compared to the T2 tumor; for the T4b tumor, the risk of metastasis to ALN was 22.82 times higher compared to the T2 tumor. The histopathological grade (Grade 3) had a risk of developing breast cancer to ALN 4.96 times higher compared to grade 2. Histoscore RAC1 > 9 had a risk of metastasis to ALN 7.53 times compared to histoscore RAC1 ≤ 9. It was found that R^2 of the 3 variable was 0.430; means that 43 % of the ALN (+) incidence was influenced by the tumor size, histopathological gradation, and histoscore of RAC1 expression, the remaining 57 % was affected by other factors. Of the 3 variables above, based on POR, T4b tumor size and the expression of RAC1 protein were risk factors with the strongest association with the incidence of breast cancer metastasis to ALN.

Table 6 Multivariable analysis of correlation among tumor size, histopatological grade and Histoskore RAC1 protein with BC metastasis to the ALN based on double logistic regression.

Variable	Coefisien B	SE (B)	*p*-value	POR_{adj} (CI 95 %)
Tumor size (T):				
T3	1.458	0.802	0.013	4.30 (1.15 - 16.07)
T4b	3.128	1.062	0.035	22.82 (3.98 - 131.02)
Histopathological Grade:				
Grade 3	1.602	0.858	0.032	4.96 (1.21 - 20.35)
Histoscore RAC1:				
> 9	2.018	0.731	0.003	7.53 (2.26 – 25.03)

Note: Accuracy = 75 %; R^2(Nagelkerke) = 0.430; POR_{adj} (IK 95 %): Prevalency odds ratio $_{\text{adjusted}}$ and confidential interval 95 %. Tumor size T2; histopathological grade 2, and histoskore RAC1 ≤ 9

The probability of breast cancer metastasis to ALN based on the correlation of tumor size, histopathological gradation, and histoscore of RAC1 protein was shown in **Table 7**. Breast cancer with tumor size T2, grade 2 histopathological grading with RAC1 (−) showed 0.6 % chance of breast cancer metastasis to ALN. Breast cancer with tumor size T2, grade 2 histopathological gradation with RAC1 (+), the chance for breast cancer metastasis to ALN was 4.5 % times. Breast cancer with tumor size T2, grade 3 histopathologic gradation with RAC1 (−) the chance of breast cancer metastasis to ALN was 3.0 %. Breast cancer with tumor size T2, grade 3 histopathological gradation with positive RAC1 (+), the chance for breast cancer metastasis to ALN was 18.8 % times. Breast cancer with tumor size T3, grade 2 histopathological grading with RAC1 (−) the chance of breast cancer metastasis to ALN was 2.9 %. Breast cancer with tumor size T3, grade 2 histopathological gradation with RAC1 (+), the chance for breast cancer metastasis to ALN was 18.2 % times. Breast cancer with tumor size T3, grade 3 histopathological with RAC1 (−), the chance of breast cancer metastasis to ALN was 12.7 %. Breast cancer with tumor size T3, grade 3 histopathological gradation with RAC1 (+), the chance of breast cancer metastasis to ALN was 52.2 % times. Breast cancer with tumor size T4b, grade 2 histopathological grading with RAC1 (−), the chance of breast cancer metastasis to ALN was only 12.3 %. Breast cancer with tumor size T4b, grade 2 histopathological gradation with expression of RAC1 (+), the chance for breast cancer metastasis to ALN was 51.4 % times. Breast cancer with tumor size T4b, grade 3 histopathological grading with RAC1 (−), the chance for breast cancer metastasis to ALN was 40.8 %. On the other hand, breast cancer with tumor size T4b, grade 3 histopathological gradation with RAC1 expression (+), the chance of breast cancer metastasis to ALN was 83.8 % times.

Table 7 Chances of BC metastatic to the axillary lymphoma based on the correlation of tumor size, histopathological gradation, and histoscore of RAC1 protein.

Tumor size	Histopathology Grade	Histoscore RAC1 protein	Chance of BC Metastasis to ALN	Percentage
				Chance of BC Metastasis to ALN
T2	2	≤ 9	0.006	0.6 %
T2	2	>9	0.045	4.5 %
T2	3	≤ 9	0.030	3.0 %
T2	3	> 9	0.188	18.8 %
T3	2	≤ 9	0.029	2,9 %
T3	2	> 9	0.182	18.2 %
T3	3	≤ 9	0.127	12.7 %
T3	3	> 9	0.522	52.2 %
T4b	2	≤ 9	0.123	12.3 %
T4b	2	> 9	0.514	51.4 %
T4b	3	≤ 9	0.408	40.8 %
T4b	3	> 9	0.838	83.8 %

Note: Tumor size: 1. T2; 2. T3; 3. T4b; Histopathology grade: 1. Grade 2; 2. Grade 3; Histoscore RAC1: 0. ≤ 9; 1. > 9.

Conclusions

This study found that having a smaller tumor size and a lower histopathological gradation of the tumor with negative RAC1 expression was associated with a decreased risk of breast cancer metastasis to axillary lymph nodes (ALN). The positive expression of RAC1 protein significantly increased the chance of breast cancer metastasis to ALN, ranging from 2 - 8x vs. the negative expression of RAC1 protein. Sentinel lymph nodes biopsy (SLNB) could determine the status of ALN with low morbidity by performing sentinel lymph nodes biopsy as the first part of breast lymphatic drainage, however, this is not yet a fixed protocol in various hospitals in our country. From our data obtained in these study, a significant correlation was observed and we can predicted the percentage probability of breast cancer metastasis to ALN by assessing the tumor size, histopathological grading, and the expression status of RAC1 protein; considering the possibility that our result could be used as a tool for assessing the posibility of the breast cancer metastasis to ALN before the surgery and could be considered as an alternative non invasive assessment method other than SLNB, which until recently could not be done routinely in our country, including in our center in Bandung.

Acknowledgements

AF supported by the Grants-in-Aid from the ALG Universitas Padjadjaran, West Java, Indonesia.

References

[1] V Kumar, AK Abbas and C Jon. *Aster: Robbins and cotran pathologic basis of disease*. Elsevier, Amsterdam, Netherlands, 2020.
[2] WHO, Breast Cancer, Available at: https://www.who.int/news-room/fact-sheets/detail/breast-cancer, accessed January 2023.
[3] American Cancer Society. *Breast cancer facts & figures 2013-2014*. American Cancer Society, Inc, Atlanta, Georgia, 2013.
[4] Perpustakaan Badan Kebijakan *Pembangunan Kesehatan. Profil pengendalian penyakit dan penyehatan lingkungan tahun*. Perpustakaan Badan Kebijakan Pembangunan Kesehatan, Jakarta, Indonesia, 2008.
[5] International Agency for Research on Cancer, Available at: https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf, accessed April 2021.
[6] LH Sobin, MK Gospodarowicz and C Wittekind. *TNM Classification of malignant tumours*. John Wiley & Sons, Inc., New York, 2016.
[7] VT DeVita, TS Lawrence and SA Rosenberg. *DeVita, Hellman, and Rosenberg's Cancer: Principles & practice of oncology*. Lippincott Williams & Wilkins, Pennsylvania, 2008.

[8] AT Mancino. Diseases of the breast. *Ann. Surg.* 2001; **233**, 594.
[9] C Eiden and H Ungefroren. The ratio of RAC1B to RAC1 expression in breast cancer cell lines as a determinant of epithelial/mesenchymal differentiation and migratory potential. *Cells* 2021; **10**, 351.
[10] Y Heriady, D Achmad, BS Hernowo, A Faried, D Ismono and D Hilmanto. Expression of the RAC1, RHOA and CXCR4 proteins and their interaction as risk factors for infiltration to the nipple areola complex in operable breast carcinoma. *Breast Canc.* 2019; **26**, 172-9.
[11] S Sandoughdaran, M Malekzadeh and MEM Eismael. Frequency and predictors of axillary lymph node metastases in iranian women with early breast cancer. *Asian Pac. J. Canc. Prev.* 2018; **19**, 1617-20.
[12] AJ Ridley. Rho GTPases and cell migration. *J. Cell Sci.* 2001; **114**, 2713-22.
[13] TC Tu, M Nagano, T Yamashita, H Hamada, K Ohneda, K Kimura and O Ohneda. A chemokine receptor, CXCR4, which is regulated by hypoxia-inducible factor 2α, is crucial for functional endothelial progenitor cells migration to ischemic tissue and wound repair. *Stem Cell. Dev.* 2016; **25**, 266-76.
[14] DR Welch and DR Hurst. Defining the hallmarks of metastasis. *Canc. Res*. 2019; **79**, 3011-27.
[15] P Chand, S Singh, G Singh, S Kundal and A Ravish. A study correlating the tumor site and size with the level of axillary lymph node involvement in breast cancer. *Niger. J. Surg.* 2020; **26**, 9-15.
[16] NI Hadi and Q Jamal. Comparison of clinicopathological characteristics of lymph node positive and lymph node negative breast cancer. *Pakistan J. Med. Sci.* 2016; **32**, 863-8.
[17] S Yenidunya, R Bayrak and H Haltas. Predictive value of pathological and immunohistochemical parameters for axillary lymph node metastasis in breast carcinoma. *Diagn. Pathol.* 2011; **6**, 18.
[18] AA Mohammed. Predictive factors affecting axillary lymph node involvement in patients with breast cancer in Duhok: Cross-sectional study. *Ann. Med. Surg.* 2019; **44**, 87-90.