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The Correlation of Immunohistochemistry Expression of Matrix Metalloproteinase-9 with Peritumoral Budding and Molecular Subtype in Invasive Breast Carcinoma of No Special Type

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ABSTRACT

Background
Breast cancer ranks first most often in the world and is a malignant cancer that often occurs in women. Matrix metalloproteinase-9 (MMP-9) has been the focus of attention in several previous studies in the field of breast cancer. MMP-9 plays a role in the degradation of the extracellular matrix. High expression of MMP-9 is associated with tumor aggressiveness, metastatic potential and poor prognosis. The presence of tumor budding and molecular subtypes also indicates tumor aggressiveness, one of which is the potential for metastasis through extracellular matrix degradation. The aim of this study was to analyze the association of MMP-9 mmunohistochemistry expression with peritumoral tumor budding and molecular subtypes in IBC-NST.

Methods
This study is an analytic study with a cross sectional approach on 41 samples of paraffin block cases of IBC-NST which had been diagnosed histopathologically and then performed with MMP-9 mmunohistochemistry staining. The correlation between the immunohistochemical expression of MMP-9 with peritumoral tumor buds and molecular subtypes in IBC-NST was tested statistically using Somers'd test and Eta test.

Results
There was a significant relationship between the immunohistochemistry expression of MMP-9 with peritumoral tumor budding and molecular subtypes.

Conclusion
Reporting the results of MMP-9 expression on IBC-NST can be one of the prognostic criteria that can be applied in routine examinations. However, the relationship with prognosis cannot be concluded from this study because it is not associated with survival rate

Keywords: MMP-9, breast cancer, tumor budding, prognosis.

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INTRODUCTION

Breast cancer ranks first in the world in 2020, found 2,261,419 new cases (11.7%) with a mortality rate of 684,996 (6.9%).[1] Data from the American Cancer Society (ACS) in 2022, it is estimated that around 287,850 new cases of invasive breast cancer will be diagnosed and around 43,250 women are expected to die from breast cancer.[2] GLOBOCAN shows 68,858 cases (16.6%) of the total 396,914 new cancer cases in Indonesia with the number of deaths reaching 22,430 people (9.6%).[3]

Breast cancer is a heterogeneous disease that has a number of different histological and molecular subtypes, which show differences in behavior and propensity to metastasize. Identification of the distinct biological subtypes occurs primarily through the use of techniques including immunohistochemistry and gene expression profiling. Currently, the treatment and care provided for individual patients is based on several clinicopathological parameters including the assessment of molecular subtypes. The different molecular subtypes describe the biological diversity of breast cancer. The molecular subtypes of invasive breast cancer are based on the genes expressed by the cancer cells. In general, these subtypes include luminal A, luminal B, HER2-enriched, and basal-like.[4,5]

Tumor budding is an important factor in prognosis and cancer-free survival. Tumor budding describes the presence of a single cancer cell or group of cells with fewer than five tumor cells, observed in the front of an invasive tumor. Tumor budding is an independent predictor and correlates with tumor size, lymphatic invasion, lymphovascular invasion and lymph node metastasis. There is no consensus regarding standard tumor budding evaluation methods and clinical applications that are not uniform in breast cancer, so further research is needed on a large scale to determine the cut off point of tumor budding in breast cancer in order to obtain uniformity in the assessment of tumor budding.[6-8]

MMP-9 plays a role in the degradation of the extracellular matrix, which is secreted by various cells. MMP activity is also associated with angiogenesis which facilitates tumor growth and facilitates spread via hematogen. MMP-9 has been the focus of attention in several previous studies in the field of breast cancer, for its role in ECM remodeling. Increased expression of MMP-9 may be associated with poor outcome in breast cancer. The presence of budding tumors and molecular subtypes is associated with poor clinicopathological characteristics. The presence of high grade buds and molecular subtypes such as HER2 Enriched and TNBC combined with MMP-9 expression by stromal cells in the invasive tumor front suggest a more aggressive tumor phenotype. Based on the previous description, the researchers wanted to know the relationship between MMP-9 immunohistochemical expression and peritumoral budding tumors and molecular subtypes in IBC-NST.

METHODS

This research was conducted as an analytical study with a cross-sectional approach which aimed to assess the relationship between MMP-9 immunohistochemical expression and peritumoral budding tumors and molecular subtypes in IBC-NST. The sample size for this study was 41 samples who were diagnosed histopathologically as IBC-NST in the Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra and the Anatomic Pathology Unit of Adam Malik Hospital, Medan, which met the inclusion criteria. Inclusion criteria with clinical data included age, tumor size and ER, PR, HER-2 and Ki-67 immunohistochemical panel examination to determine molecular subtype. Samples were assessed from paraffin blocks and HE slides.

Slides that had been diagnosed as IBC-NST by HE staining, were reviewed by the investigators with guidance to assess histopathological grade and peritumoral budding tumors. The tumor budding assessment was peritumoral, referring to studies where the number of tumor buds was categorized as follows, if ≤10 buds/10 LPB (low grade buds) and >10 buds/10 LPB (high grade buds).[6] The grading system refers to the 2019 WHO classification for breast tumors.[11]

The IBC-NST paraffin block was re-cut for MMP-9 immunohistochemical staining to be used as a research sample. Immunohistochemistry of MMP-9 (BZ-0898450-AP Rabbit Anti MMP-9 Polyclonal Antibody, dilution 1:200) with positive control from colon cancer tissue. Assessment of MMP-9 immunohistochemical expression using an Olympus CX23 microscope. Assessment of MMP-9 immunohistochemical expression was categorized into negative, weak and strong based on the percentage of tumor cells and the intensity of brown stained cells in the cytoplasm of tumor cells and/or cells in the stroma. The assessment of MMP-9 expression scores was categorized as follows, percentage 0= no cells stained, 1= stained <10%, 2= stained 10-50%, 3= stained 51-80%, 4= stained >80%. For

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staining intensity, the value is 0 = negative, 1 = weak intensity, 2 = medium intensity, 3 = strong intensity. MMP-9 expression was calculated by multiplying the perc entage of stained cells by the staining intensity. The multiplication results show a total score of 0-1 = negative, a total score of 2-3 = +1, a total score of 4-8 = +2, and a total score of 9-12 = +3, then the MMP-9 expression is categorized as follows:[12]

a. 1= negative (score 0-1)
b. 2= weak expression (score +1 dan +2)
c. 3= strong expression (score +3)

RESULT

In this study, 41 IBC-NST samples were obtained.

Table 1. *IBC-NST* frequency distribution based on age, tumor size dan moleculer subtype.

Variable (n=41)	Frequency (f)	Percentage (%)
Age		
<30	2	4.9
30-39	4	9.8
40-49	13	31.7
50-59	12	29.3
>59	10	24.4
Tumor size		
<2 cm (*T1*)	9	22.0
2-5 cm (*T2*)	22	53.7
>5 cm (*T3*)	10	24.4
Tumor of any size with direct extension to the chest wall and/or skin (ulceration or skin nodule) (T4)	0	0
Moleculer subtype		
Luminal	22	53.7
HER2 positif (non luminal)	11	26.8
TNBC	8	19.5

From the results of this study, most IBC-NST sufferers occurred at the age of 40-49 years, namely 13 samples (31.7%). The mean age of IBC-NST patients in this study was 46.24 years, where the youngest was 27 years old and the oldest was 73 years old. The largest tumor size was T2 (53.7%), and the most molecular subtype obtained was luminal in 22 samples (53.7%).

Table 2. IBC-NST frequency distribution based on histopathological variables.

Variable (n=41)	Frequency (f)	Percentage (%)
Grade		
Grade 1	10	24.4
Grade 2	15	36.6
Grade 3	16	39.0
Peritumoral budding		
Low grade buds	16	39.0
High grade buds	25	61.0

Invasive Breast Carcinoma of No Special Type (IBC-NST)

Based on the evaluation results to assess histopathological grading, grade 3 was the most common grade found in 16 samples (39.0%) and peritumoral budding tumors obtained the most were high grade buds, namely 25 samples (61.0%).

Table 3. Frequency distribution of MMP-9 immunohistochemical expression in IBC-NST.

MMP-9 expression	Frequency	Percentage (%)
Weak expression	14	34.1
Strong expression	27	65.9
Total	41	100.0

Matrix metalloproteinase-9 (MMP-9), Invasive Breast Carcinoma of No Special Type (IBC-NST)

Assessment of MMP-9 immunohistochemical expression in IBC-NST based on the table above, found the highest expression with strong expression, namely 27 samples (65.9%).

Table 4. The correlation between MMP-9 immunohistochemical expression and peritumoral budding in IBC-NST.

Variable	Peritumoral budding		Total f(%)	p-value*
	Low grade buds f (%)	High grade buds f(%)		
MMP-9 expression				
Weak	13 (31.7)	1 (2.4)	14 (34.1)	0.0001
Strong	3 (7.3)	24 (58.5)	27 (65.9)	0.0001
Total	16 (3.0)	25 (61.0)	41 (100.0)	

*Matrix metalloproteinase-9 (MMP-9), Invasive Breast Carcinoma of No Special Type (IBC-NST), Somers'd test**

From the table above it can be seen that there is a significant relationship between MMP-9 immunohistochemical expression and peritumoral budding tumors which were statistically analyzed using the Somers'd test with a p-value of 0.0001 (p-value <0.05).

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Table 5. The correlation between MMP-9 immunohistochemical expression and molecular subtypes in IBC-NST.

Variabel	Moleculer subtype			Total f (%)	*p-value**
	Luminal f (%)	*HER2* positif f (%)	*TNBC* f (%)		
Ekspresi *MMP-9*					
Weak	13 (31.7)	1 (2.4)	0 (0.0)	14 (34.1)	<0,05
Strong	9 (22.0)	10 (24.4)	8 (19.5)	27 (65.9)	
Total	22 (53.7)	11 (26.8)	8 (19.5)	41 (100.0)	

Matrix metalloproteinase-9 (MMP-9), Invasive Breast Carcinoma of No Special Type (IBC-NST), Uji Eta*

From the table above it can be seen that there is a significant relationship between MMP-9 immunohistochemical expression and molecular subtypes which were statistically analyzed using the Eta test with a p-value <0.05.

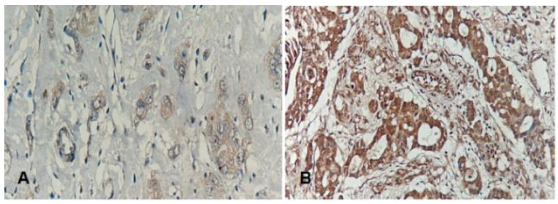

Figure 1. Microscopy of MMP-9 immunohistochemical expression in IBC-NST. A. Weak expression of MMP-9. B. Strong expression of MMP-9.

DISCUSSION

Breast cancer is the most commonly diagnosed cancer in women. IBC is a heterogeneous group of diseases, having differences in morphological, molecular and clinical features.[13,14] The etiology of breast cancer is still not known with certainty. There are risk factors associated with the occurrence of breast cancer, including older age, genetic factors, early menarche (<12 years), late menopause (>55 years), age at first pregnancy over 30 years, infertility and not having children , contraceptive use, hormonal treatment after menopause, and no history of breastfeeding.[15] As many as 10% of breast cancers are associated with inherited mutations. About one third of women with hereditary breast cancer have mutations in BRCA1 or BRCA2. The incidence rate of breast cancer increases with age, from 1.5 cases per 100,000 in women aged 20 to 24 years to a peak in women aged 75 to 79 years. In this study, the youngest was 27 years old and the oldest was 73 years old. Sriwidyani et al reported that the mean age of patients was 48.6 years with an age range of 23-74 years.[16] Ardian et al reported that the age of most IBC-NST patients was 35-49 years. [17] Chen et al reported that the ages of patients ranged from 25-75 years, with an average of 50.79 years.[18]

Based on the literature that the incidence rate of breast cancer is around 95% of new cases occurring in women aged 40 years or more and is in line with this study. The presence of young patients in this study may be caused by the influence of gene mutations. Breast cancer at a young age is often familial, and about half of young women with breast cancer under the age of 30 have germline mutations BRCA 1, BRCA 2, or TP53.[7]

Tumor size refers to the TNM classification which can assist in prognosis and therapy. Masilamani et al reported a larger tumor size at T2 (49.8%).[19] Saragih et al reported that the highest tumor size was at T2 (50.0%).[20] Chen et al in their study reported that the largest tumor size was found in T2, which was 71.08%.[18] Badowska-Kozakiewicz et al reported that the highest tumor size was at T1 size (44.72%).[21] Kumarguru et al reported that the largest tumor size was T3 (38%).[22]

The most common tumor size found in this study was T2 (2-5 cm), this indicates that most patients still come to health services when their tumors are large enough which may be due to ignorance, worry or not understanding how to detect early. The role of health workers in this case is also very necessary, such as counseling about the importance of self-examination of the breast . This can help prevent delays in treatment, which so far patients usually come at an advanced stage.

Molecular subtype in breast cancer is a strong prognostic and predictive factor. Histological grade is significantly related to the molecular subtype of breast cancer. It was reported in several studies that grade I was associated with luminal A, whereas grade III was associated with positive HER2 and TNBC.[23] Luminal molecule subtype A is generally associated with a good prognosis and usually shows less frequent and less extensive involvement of the lymph nodes. Luminal molecular subtype B is associated with a more moderate prognosis when compared with luminal molecular subtype A. TNBC is associated with a poor prognosis when compared with other breast cancer subtypes.[24]

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Masilamani et al reported that the highest molecular subtype was luminal A (39.3%).[19] Budzik et al reported that IBC-NST cases were often associated with positive hormone receptor expression, namely ER (63.7%) and PR (59.3%).[25]

Determination of histopathological grading is used to assess tumor behavior and prognosis in IBC and identify the possibility of a poor outcome in patients. Histopathological grading is a simple and inexpensive method for routine breast cancer reports. Agarwal et al reported that the most common grade 3 cases were 82%.[26] Saragih et al reported that the highest grade was grade 3 (47.6%).[20] Okcu et al reported that the highest grade was grade 2 (71.3%).[27] Singh et al reported that grade 2 was the most common, 67.64%.[7]

The most abundant molecular subtype found in this study was the luminal subtype, this is related to the limited number of samples for other molecular subtypes, such as TNBC. Meanwhile, the most graded assessment is grade 3, this shows that most of the cases have aggressive tumor behavior. In addition, there are differences in socio-economic, education, research location, and environment as well as awareness to check themselves early if a lump is found in the breast, causing patients who come to have tumors with a high grade.

Tumor budding is considered to be the first step process of tumor metastasis and is related to EMT. Tumor budding can be used to predict unfavorable survival rates. Masilamani et al reported that the highest number of budding tumors were high grade buds, namely 80.4% [19] Saragih et al reported that the most tumor budding was high grade buds 85.7%.[20] Singh et al reported of 99 IBC cases, the most common category was low peripheral tumoral budding, namely 54.9% and high peripheral tumoral budding 45.10%.[6]

Standards for tumor budding evaluation and clinical application are still not uniform in breast cancer.[28] The most tumor budding found in this study were high grade buds, this shows that most cases have a higher invasive potential and this is associated with an unfavorable prognosis.

MMP-9 plays an important role in ECM remodeling and protein cleavage, hence it is found to be widely associated with cancer pathology. The development of MMP-9 inhibitors is an important research area to achieve this goal. However, until now there has been no specific MMP-9 inhibitor that has been used successfully in the clinic, especially in cases of breast cancer.[29] Fouad et al reported that the highest expression of MMP-9 was a strong expression of 57.6% and a weak expression of 42.4%.[30] This study was also in line with Khambri et al who reported that the highest expression of MMP-9 was found to be strong expression, which was 58.8%.[13]

Sriwidyani et al's study reported that there was a significant relationship between high grade tumor budding and MMP-9 expression.[16] A study by Gonzalez et al reported several associations between MMP and tumor budding grade which significantly affect prognosis. That said, high-grade tumor budding and expression of MMP-9, -11, and -14 is associated with a poor prognosis, whereas a low-grade tumor budding and non-expression relationship is associated with a better outcome.[31] Khambri et al reported that MMP-9 expression was found to be more dominant with strong expression in 21 patients.[13]

MMP-9 can also be used in combination with other cancer biomarkers to achieve high specificity. In recent years, there have been many advances in cancer biomarker research regarding the exploration of MMP-9 as a biomarker for various types of cancer including breast cancer.[29] Research shows that there is a significant increase in MMP-9 expression in breast cancer cells. Moreover, it was found that MMP-9 is differentially expressed in different molecular subtypes. Yousef et al reported where in their study found strong expression of MMP-9 (50%) in HER2 Enriched.[32] *Overexpression MMP-9* can also occur in TNBC molecular subtypes.[32] MMP-9 biomarkers with HER2 and Ki-67 are expected to provide more specificity in prognosis for better treatment options in breast cancer.[12]

CONCLUSION

In this study, it can be concluded that based on the frequency distribution of IBC-NST sufferers, the highest results were obtained in the 40-49 year age group, the tumor size T2, grade 3 and the most molecular subtypes were luminal. MMP-9 immunohistochemical expression was significantly associated with peritumoral budding tumor and molecular subtypes. Reporting the results of MMP-9 expression in IBC-NST can be one of the prognostic criteria that can be applied in routine examinations. However, its relation to prognosis cannot be concluded from this study because it is not associated with survival rate.

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