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Correlation between Microvessel Density Based on CD31 Immunohistochemical Expression and Clinicopathology of Invasive Breast Carcinoma

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ABSTRACT

Background
Breast carcinoma is the most common malignancy in women worldwide. Tumor size, metastases, lymph node involvement (KGB), stage and histopathological grade are significant prognostic factors in invasive breast carcinoma. Microvessel density (MVD) assessment is used to measure angiogenesis which helps predict tumor behavior and the effect of antiangiogenic therapy. MVD is a quantitative calculation of intratumoral capillary blood vessels which reflects the results of the process of angiogenesis. The aim of this study was to analyze the relationship between MVD based on CD31 immunohistochemical expression and the clinicopathology of invasive breast carcinoma.

Method
Cross sectional analytic study with 42 samples diagnosed with invasive breast carcinoma at H. Adam Malik General Hospital Medan. MVD is the number of small blood vessels in terms of size and diameter (arterioles and venules) with a round lumen that is positively stained with CD31 at 10 large visual fields, categorized as low if <45 blood vessels/10 LPB and high if ≥45 blood vessels/10 LPB. The relationship between MVD and several clinicopathological parameters was analyzed using the Chi-Square, Fisher's Exact and Mann-Whitney tests.

Results
There was a significant relationship between tumor size (p=0.029), metastasis (p=0.014), stage (p=0.002), histopathological grade (p=0.0043), LVI (p=0.002), TILs (p=0.007). Large tumor size, metastases, high histopathological stage and grade, positive LVI and high TILs were significantly associated with MVD. However, there was no significant relationship between KGB involvement (p=0.570) and MVD**.**

Conclusion
MVD is significantly related to tumor size, metastases, stage, histopathological grade, LVI and TILs, and not significantly related to lymph node involvement. MVD plays a role in the angiogenesis of invasive breast carcinoma clinicopathology.

Keywords: CD31, breast cancer, stage, grade, LVI, TILs

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INTRODUCTION

Breast carcinoma is a health problem with an increasing incidence in most countries in the last few decades.[1] Based on the 2020 Global Burden of Cancer data, the incidence of breast carcinoma has now surpassed lung carcinoma as the most frequently diagnosed carcinoma, namely 2,261,419 (11.7%).[2] The prognosis of invasive breast carcinoma depends on several clinicopathological parameters including tumor size, lymph node (KGB) status, stage, subtype and histopathological grade, vascular invasion and estrogen receptor status.[3]

MVD is a quantitative calculation of intratumoral capillary blood vessels which reflects the results of the process of angiogenesis. The process of forming new blood vessels in tumor development is known as angiogenesis. Folkman and colleagues reported that solid tumors cannot grow larger than 2-3 mm in diameter without inducing growth of new capillary vessels from the host.[3-5,7] This study used CD31 monoclonal antibody immunohistochemistry. Selection of CD31 antibody for MVD assessment as a prognostic factor is more accurate than other endothelial cell markers such as CD34 and factor VIII, it is said that CD31 is more sensitive as an endothelial cell marker.

This MVD is not fully understood so that MVD as a marker for angiogenesis still needs to be re-identified. response to therapy.[9] The results of studies on MVD assessment have not been homogeneous, caused by several factors such as methodological variations, the type of antibody used, selection bias in using different tumor areas, the subjectivity of researchers in interpreting and calculating different MVD and the lack of patient follow-up data. in retrospective studies.[10-12] The purpose of this study was to determine the relationship between MVD based on CD31 Immunohistochemical Expression and Invasive Breast Carcinoma Clinicopathology.

METHOD

The research design is a cross sectional analytic study. The study was conducted at the Laboratory of Anatomic Pathology H. Adam Malik General Hospital Medan using a paraffin block which had been diagnosed histopathologically as invasive breast carcinoma. Sample calculation was carried out with a total sample of 42 samples including the inclusion criteria, namely the completeness of clinical data and the origin of the mastectomy surgery tissue and the exclusion criteria, namely recurrence patients, biopsy results and incomplete medical record data.

Tumor size (T) based on the AJCC/UICC TNM system where T1=≤2 cm, T2=>2-≤5 cm, T3=>5 cm and T4=tumor of any size, direct extension to the chest wall/skin. KGB involvement (N) using the AJCC/UICC TNM system where N0=no regional lymph node metastases, N1=metastases to level I and II ipsilateral axillary lymph nodes and N2=metastases to level I and II ipsilateral axillary lymph nodes that are clinically fixed. Metastases (M) based on the AJCC/UICC TNM system were categorized into M0=no distant metastases and M1=presence of distant metastases. Stages of invasive breast carcinoma tumors according to the TNM system of AJCC/UICC are categorized into Stages I, II, III and IV. The histopathological subtypes of invasive breast carcinoma are architectural, cell morphological and stromal features. In this study they were categorized into Infiltrating ductal carcinoma NOS, Oncocytic carcinoma, Lobular carcinoma NOS, Mucinous adenocarcinoma. Histopathological grade is a microscopic assessment of three parameters, namely tubule/gland formation, nuclear pleomorphism and mitotic count which are categorized into Grades 1, 2 and 3. Lymphovascular invasion (LVI) is found in tumor cells in lymphatic vessels and blood vessels lined by endothelial cells, assessed in the peritumoral area on the H&E slide which was categorized as negative=no invasion and positive=there was invasion. Tumor infiltrating lymphocytes (Tils) to be assessed are in the stroma using the recommendations of the International Tils Working Group. Stromal tils are mononuclear immune cells in the stroma between tumor cells and do not come into direct contact with tumor cells. In this study, stromal Tils was assessed on H&E slides at 200-400 times magnification. In this study, the ordinal scale was categorized as low=<50% stromal Tils and high=≥50% stromal Tils.

CD31 immunohistochemical expression was an assessment of CD31 endothelial cell protein, primary antibody diluent K004, clone JC/70A, Diagnostic biosystem polyvue with a dilution of 1:25. The positive control used was tonsil tissue with a positive brown smear expression pattern on the vascular endothelial cell membrane. MVD assessment is the number of small blood vessels that are seen from the size

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and diameter (arterioles and venules) with a round lumen, counted visually with an Olympus CX 23 light microscope. It is done by the hot spot method where the slide is initially viewed with 100x magnification to identify the area with the highest microvessel. Microvessel density counts were calculated at 400 times magnification in 10 intratumoral hotspot areas and accumulated the sum of all microvessel densities expressed by CD 31 as mitotic counts in breast carcinoma. which are categorized as Low=<45 Vascular/10 Large Visual Fields and High=≥45 Vascular/10 Large Visual Fields.[11,13] The relationship between MVD and several clinicopathological parameters will be analyzed by the Chi-Square test, Fisher's Exact and Mann-Whitney. The statistical test is significant if the p value <0.05.

RESULTS

In this study, there were 42 samples of invasive breast carcinoma. Age data obtained Mean=49.83, Median=48±sd 11.16 with the youngest age being 23 years old and the oldest being 75 years old where most were found in the 41-50 year age group of 16 samples (38.1%). Tumor size grouping based on the TNM system. Tumor size, KGB involvement and metastases were assessed based on the AJCC/UICC TNM system, found that the largest tumor size was found on T4 with 26 samples (61.9%) followed by T3 with 11 samples (26.2%), T2 with 3 samples (7, 1 %) and T1 of 2 samples (4.8%). Based on the KGB involvement data, it was found that the most samples were N1 with 20 samples (47.6%), followed by N0 with 19 samples (45.2%) and N2 with 3 samples (7.2%). Based on the metastatic data, the highest number was found in M0 with 35 samples (83.3%) and M1 with 7 samples (16.7).

Based on the stage, the most found was stage III with 24 samples (57.1%), followed by stage II with 9 samples (21.4%), stage IV with 7 samples (16.7%) and stage I with 2 samples (4, 8%). The most common histopathological subtype in this study was infiltrating ductal carcinoma NOS in 33 samples (78.6%). The histopathological grade found the highest grade II with a total of 26 samples (61.9%). Positive LVI scores slightly more than negative LVI and high TILs more than low TILs. High MVD is more common than low MVD.

Table 1. Frequency distribution of clinical characteristics including age, tumor size, lymph node involvement, metastases, stage, subtype, grade, LVI and Tils.

Characteristics	Total (n=42)	(%)
Age		
Mean=49,83 Median=48±sd 11,16		
21-30 years old	1	2.4
31-40 years old	7	16.7
41-50 years old	16	38.1
51-60 years old	11	26.2
61-70 years old	5	11.9
>70 years old	2	4.8
Tumor size		
T1	2	4.8
T2	3	7.1
T3	11	26.2
T4	26	61.9
Lymph node involvement		
N0	19	45.2
N1	20	47.6
N2	3	7.2
Metastases		
M0	35	83.3
M1	7	16.7
Stage		
Stage I	2	4.8
Stage II	9	21.2
Stage III	24	57.1
Stage IV	7	16.7
Subtype		
Infiltrating ductal carcinoma NOS	33	78.6
Oncocytic carcinoma	1	2.3
Lobular carcinoma NOS	5	11.9
Mucinous adenocarcinoma	3	7.2
Grade		
I	6	14.3
II	26	61.9
III	10	23.8
LVI		
Negatif	9	21.4
Positif	33	78.6
TILs		
Low	9	21.4
High	33	78.6
MVD		
Low	18	42.9
High	24	57.1

Lymphovascular invasion (LVI), Tumor infiltrating lymphocytes (Tils), Microvessel density (MVD).

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Table 2. Correlation between tumor size, lymph node involvement, metastases, stage, grade, LVI and TILs with MVD.

Variable	MVD Low n (%)	MVD High n (%)	p-value
Tumor size			
T1	2 (11.1)	0 (0)	
T2	2 (11.1)	1 (4.2)	0,029*
T3	6 (33.3)	5 (20.8)	
T4	8 (44.5)	18 (75.0)	
Lymph node involvement			
N0	9 (50.0)	10 (41.7)	
N1	8 (44.4)	12 (50.0)	0,570*
N2	1 (5.6)	2 (8.1)	
Metastases			
M0	18 (51.4)	17 (48.6)	0,014**
M1	0 (0)	7 (100)	
Stage			
I	2 (11.1)	0 (0)	
II	6 (13.3)	3 (12.5)	0,002*
III	10 (55.6)	14 (58.3)	
IV	0 (0)	7 (29.2)	
Grade			
I	5 (27.8)	1 (2.2)	
II	12 (66.7)	14 (58.3)	0,004*
III	1 (5.5)	19 (37.5)	
LVI			
Negative	8 (88.9)	1 (11.1)	
Positive	10 (30.3)	23 (69.7)	0,002**
Tils			
Low	12 (66.7)	6 (33.3)	0,007***
High	6 (25.0)	18 (75.0)	

Lymphovascular invasion (LVI), Tumor infiltrating lymphocytes (Tils), Mann-Whitney test*, Fisher's Exact test**, Chi-Square test***.

From the table above it can be seen that there is a relationship between tumor size and MVD which was statistically analyzed using the Mann-Whitney test with a value of p=0.029 (p<0.05). There was no relationship between the involvement of the KGB and the MVD which was statistically analyzed using the Mann-Whitney test with a value of p=0.570 (p>0.05). There was a metastatic relationship with MVD which was statistically analyzed using the Fisher's Exact test with a value of p=0.014 (p<0.05). There is a staging relationship with MVD which was statistically analyzed using the Mann-Whitney test with a value of p=0.002 (p<0.05). There is a relationship between histopathological grade and MVD which was statistically analyzed using the Mann-Whitney test with a value of p=0.004 (p <0.05). There is a relationship between LVI and MVD which was statistically analyzed using the Fisher's Exact test with a value of p=0.002 (p<0.05). There is a relationship between Tils and MVD which was statistically analyzed using the Chi-Square test with a value of p=0.007 (p<0.05).

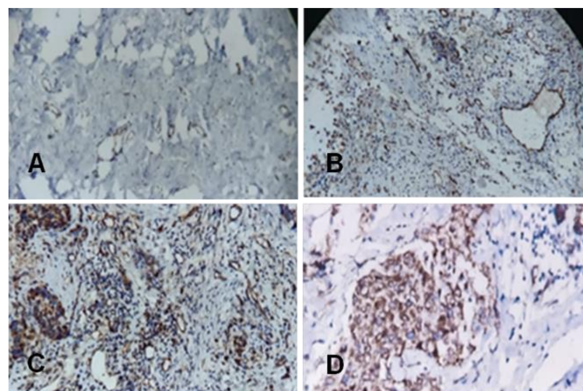

Figure 1. Microvessel density in invasive breast carcinoma. A and B. MVD <45/10 LPB (400 times). C. and D. MVD ≥45/10 LPB (400 times).

DISCUSSION

Clinicopathological factors in invasive breast carcinoma can affect the biological behavior of tumors such as patient age, tumor size, axillary lymph node involvement, metastases, stage, vascular invasion, grade and histopathological subtype. These prognostic factors are used to identify poor clinical outcomes and to select patients for adjuvant therapy. Angiogenesis is required for tumor growth, invasion and metastasis. The incidence of breast carcinoma increases with age, this carcinoma is rarely found at the age of <25 years old where in this study the youngest age was found at 23 years old in the histopathological subtype of oncocytic carcinoma and the oldest was 75 years old found in invasive lobular carcinoma. Some literature states that the age difference found is caused by inherited genes or susceptibility genes, germline mutations in tumor suppressor genes or sporadic, menache age <11 years old, hormonal exposure and other etiological factors.

Krishnapriya S et al reported the age group of 41-50 years old, namely 59 cases (43.7%) found the most[10]. Niasari M et al in his research, he reported that the youngest age was 35 years old and the oldest was 84 years old with an average age of 52.33 years old and the most common age of invasive breast carcinoma NST grade 3 tumor patients was in the age range 51-60 years old, namely 16 cases (44.4 %).[12] Alwan NA et al reported the age group of 35-49 years old with the most number of 497 cases (42.4%).[14]

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The TNM classification is used in clinical practice and in various studies for the prognostic assessment of patients and for clinical decision making in planning treatment regimens. Research by Krishnapriya S et al found tumor size >5 cm in 89 cases (66%), metastases to level I and II ipsilateral axillary lymph nodes in 96 cases (71%) and stage IIIA in 98 cases (72%).[9] Alwan NA et al reported tumor size >2-≤5 cm in 351 cases (59.3%), metastases to level I and II ipsilateral axillary nodes in 185 cases (31.8%) and stage II in 250 cases (47.5%).[14] Research Novrial D et al also reported tumor size >2-≤5 cm in 56 cases and stage I in 47 cases.[15] Research by Chen Z et al reported stages I and II in 35 cases, tumor size >2 cm in 33 cases, positive lymph node metastases in 27 cases.[16] Sener et al reported a tumor size of 2-5 cm in 60 cases and found no KGB involvement in 28 cases.[17] Bujor IS et al reported tumor size ≥2 cm in 40 cases, negative lymph nodes in 44 cases and stage II in 31 cases.[11] Some literature states that infiltrating ductal carcinoma NOS is the most widely reported histological subtype.[1] Research by Krishnapriya S et al also reported a subtype of infiltrating ductal carcinoma in a total of 126 cases (93.3%).[9] The same was also in the study Novrial D et al reported histopathological subtype of invasive ductal carcinoma in 53 cases (91.4%).[15]

The differences that occur are due to differences in research locations related to socio-economic, education, environment and people's lifestyles. The awareness of the Indonesian people in having their health checked, especially breast health, is still very low, in addition to the low economy and the health insurance system that does not fully protect so that they experience difficulties in carrying out breast screening. Involvement of the axillary lymph nodes is an important step before metastases occur, so it is used to predict the prognosis with the presence or absence of distant metastases in breast carcinoma.

In this study, immunohistochemical examination with CD31 monoclonal antibody was used. The selection of CD31 antibodies was carried out based on some library literature which said that MVD as a prognostic factor was more accurate when examined with CD31 than CD34 and factor VIII, it was said that CD31 was more sensitive as a marker of endothelial cells.[4,14]

Sener et al research found that there was no relationship between KGB involvement and MVD and found a relationship between tumor size and MVD. 17 Niasaria M et al found that there was no association between lymph node involvement and metastasis with MVD.[12] As is well known, angiogenesis plays a key role in tumor growth, invasion and metastasis. In recent years, the treatment of carcinoma has highlighted treatment target options that can inhibit angiogenesis. Angiogenesis inhibitors slow and inhibit tumor growth and metastasis through different mechanisms.

Low MVD is a marker to identify patients with a good prognosis. However, contradictions and inconsistencies were also found in several studies that have been conducted. The majority found that high MVD, high histopathological grade and distant metastases correlated with a poor prognosis. In the study of Krishnapriya S et al found the highest grade III in 66 cases (49%).[9] Novrial D et al also reported grade II with the most number of 69 cases.[15] Agnani B et al reported grade I and grade II with the same number of 15 cases each.[19]

Sener et al found the most LVI in 82 cases.[17] Research by Agnani B et al reported the most LVI in 39 cases with high MVD in 27 cases and low MVD in 12 cases and found a relationship between LVI and MVD.[19] LVI is associated with an increase risk of axillary lymph nodes and distant metastases and LVI refers to invasion of the lymphatic space, blood vessels in the peritumoral area by tumor emboli. The mechanism of LVI has not been clearly proven, it is said that LVI can reflect the surrounding microenvironment processes in predicting tumor aggressiveness and a worse prognosis.

In several literatures it is explained that the presence of Tils is important evidence regarding the immune response between tumor cells and effector immune cells. TILs serve as a prognostic marker where a high TILs count is associated with better outcome and better response to neoadjuvant therapy. In invasive breast carcinoma, the role of TILs varies in each histopathological subtype, TILs is associated with a good prognostic in the TNBC subtype and conversely high TILs is a poor prognostic in the luminal subtype.

CONCLUSION

In this study, large tumor size, metastases, stage, histopathological grade and high TILs and positive LVI were also found to have high MVD. MVD is significantly related to tumor

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size, metastases, stage, histopathological grade, LVI, TILs and not significantly related to lymph node involvement based on CD31 immunohistochemical expression in patients with invasive breast carcinoma.

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