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Differentiation of Microvessel Density Based on The Breast Cancer Patient Characteristics Including Age, Stage, Tumor Size, and Lymph Node Metastasis

Tasya Salsabila [1], ***Sari Eka Pratiwi*** [2*], ***Heru Fajar Trianto*** [3,4], ***Muhammad In'am Ilmiawan*** [2], ***Desriani Lestari*** [2], ***Iit Firianingrum*** [2], ***Henky Hartono*** [5]

[1] Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia
[2] Department of Biology and Pathobiology, Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia
[3] Department of Histology, Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia
[4] Anatomical Pathology Laboratory, Universitas Tanjungpura Hospital, Pontianak, Indonesia
[5] Anatomical Pathology Laboratory, Soedarso Hospital, Pontianak, Indonesia

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*Corresponding author:
Sari Eka Pratiwi
Department of Biology and Pathobiology, Faculty of Medicine, Universitas Tanjungpura, Pontianak, West Kalimantan, Indonesia
sariekapratiwi@medical.untan.ac.id

ABSTRACT

Background: Breast cancer is the most common malignant tumor in the world and Indonesia. One well-known prognostic marker is microvessel density (MVD), the numerical value of angiogenesis. In recent years, it has been recognized that tumor growth depends on angiogenesis. Therefore, this study aimed to determine the differentiation of MVD based on the breast cancer patients' characteristics including age, stage, tumor size, and lymph node metastasis in the Anatomical Pathology Laboratory of Soedarso Hospital.

Methods: This research was an observational study with a cross-sectional approach. The study was conducted by observing the slides of Hematoxylin-Eosin (HE) in breast cancer patients. Samples were taken using the total sampling technique. The samples were observed by two observers. 51 tissue preparations met the inclusions and exclusions criteria. MVD cut-off points are taken by calculating the median. Research analysis was using the Kruskal-Wallis test in SPSS version 24.

Results: All samples of this study were women and had invasive ductal carcinoma. Breast cancer tends to occur in patients aged 48-53 years, has stage III, lymph node metastasis (N2). The patients have a low MVD rate but have a large tumor size (T4). Kruskal Wallis test showed that there was a differentiation of MVD based on age (p = 0.029). While, there was no differentiation of MVD based on stage (p=0.974), tumor size (0.069), and lymph node metastasis (0.571).

Conclusions: There was a differentiation of MVD based on the age of breast cancer patients in the Anatomical Pathology Laboratory at Soedarso Hospital.

INTRODUCTION

Breast cancer is the most common type of cancer in the world and Indonesia [1]. Based on Global Burden of Cancer statistics in 2020, the cases of breast cancer in the world have reached 2.3 million cases and 685,000 deaths, while in Indonesia it has reached 68,858 cases and 22 thousand deaths [2,3]. At Soedarso Hospital Pontianak, there was an increment in the frequency of breast cancer patients by 13.3% from 2010 until 2014, and most of the patients were in the advanced stage. [4]. In 2021, the incidence of breast cancer at Soedarso Hospital Pontianak reached 169 cases. The earlier the detection and treatment of breast cancer, the higher the patient's recovery rate [5]. One well-known prognostic factor in breast cancer is tumor size which is directly proportional to the formation of blood vessels (angiogenesis). The correlation between tumor size and patient survival is linear and does not depend on nodal status. Tumor size (T) was made based on the clinical size of the longest tumor diameter in centimeters [6].

The numerical value of tumor angiogenesis is known as Microvessel Density (MVD). The high MVD in premalignant lesions is strongly associated with the risk of breast cancer incidence. Microvessel density is closely

related to lymph vessels and metastases in primary tumors and has been commonly used to assess tumor angiogenesis in relation to tumor recurrence and metastases. Increased MVD is associated with a shortened free-relapse period and survival in patients with breast cancer without lymph node spread [6,7]. Research conducted in Norway in 2019 showed that MVD proved high in invasive lesions. Microvessel density is further associated with increased expression of Vascular Endothelial Growth Factor (VEGF) [6].

In West Kalimantan Province, there has been no research on MVD with age, stage, tumor size, and lymph node metastasis in breast cancer patients. Based on this phenomenon, researchers are interested in conducting research that aims to determine the differentiation of MVD based on the breast cancer patients' characteristics including age, stage, tumor size, and lymph node metastasis at the Anatomical Pathology Laboratory of Soedarso Hospital.

METHODS

This study used an observational research design with a cross-sectional approach. This study used slides of breast cancer tissue from the Anatomical Pathology Laboratory at Soedarso Hospital that had been stained with Hematoxylin and Eosin (HE). The inclusion criteria include invasive breast cancer patients who had complete histopathological examination data records and undergone breast cancer surgery in 2021. The exclusion criteria include patients suffering from metabolic and inflammatory disorders, patients who have or are undergoing cancer therapy, and Neoadjuvant Chemotherapy (NAC) which will decrease MVD value.

The primary data was obtained by observing MVD in the stroma area. The observations were made in the Microbiology Laboratory of the Faculty of Medicine Tanjungpura University using a Zeiss Axio Scope A1 microscope by 2 observers. First, five stromal component areas with the highest number of microvessels (hot spots) were identified at 50x magnification. Then, each hot spot area is calculated at 100x magnification. After calculating the number and mean of microvessels, the median of MVD was determined as the cut-off value. Patients with microvessel count ≤5 are classified as low MVD and patients with microvessel count >5 are classified as high MVD.

The secondary data was obtained from the results of histopathological examination at the Anatomical Pathology Laboratory of Soedarso Hospital Pontianak which were medical record number, number of patients, patient age, cancer stage, tumor size, and lymph node metastasis. The statistical analysis used in this study includes the Kruskal-Wallis test using Statistical Product and Service Solution (SPSS) 24.0 software.

RESULTS

Microvessel density was observed in the observation area (**Figure 1**). Arrows indicate the MVD identified in the stromal region because it supports and is essential for neoplasm growth. Samples with microvessel count ≤5 are classified as low MVD and patients with microvessel count >5 are classified as high MVD.

The characteristics of breast cancer patients at Soedarso Hospital in 2021 using univariate analysis assessment can be seen in **Table 1**. It was found that all study samples were female patients, who had invasive ductal carcinoma, was dominated by patients aged 48-53 years (23.5%), staged III (78.4%), had a low MVD (54.9%), T4 tumor size (70.6%), and N2 lymph node metastasis (49.0%).

Kruskal-Wallis analysis in **Table 2** shows that there is only significant differentiation of MVD based on age because the p-value (0.029) is less than 0.05. Meanwhile, there is no significant differentiation of MVD based on stage (0.974), tumor size (0.069), and lymph node metastasis (0.571) because the p-value is greater than 0.05.

DISCUSSION

All study samples were female and had invasive ductal carcinoma. Research conducted by Wangsa et al. [8] showed that patients were dominated by women with 573 (99.65%) patients. Research conducted by

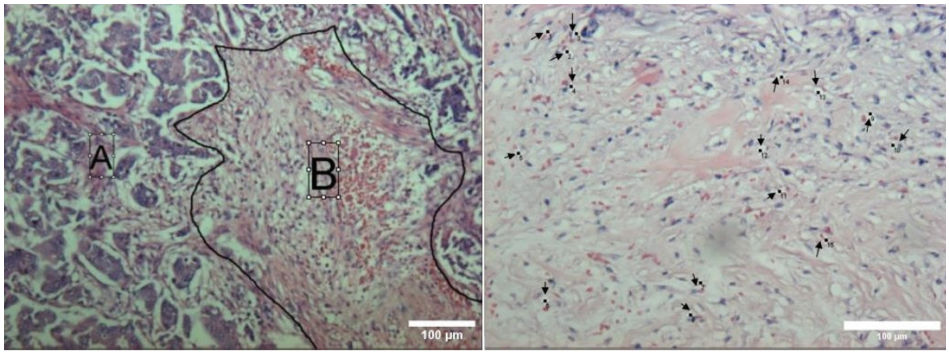

Figure 1. Depiction of microvessel density observational area in breast cancer slides. The observation in the left area: (A) Tumor area; (B) Stroma (HE, 50x magnification); the right picture is MVD area in stroma (HE, 100x magnification).

Table 1. Characteristics of breast cancer patients

Characteristics	Total	
	N (N=51)	%
Age (years)		
24–29	2	3.9
30–35	8	15.7
36–41	8	15.7
42–47	10	19.6
48–53	12	23.5
54–59	6	11.8
60–65	5	9.8
Stage		
I	4	7.8
II	7	13.7
III	40	78.4
Microvessel Density		
Low	28	54.9
High	23	45.1
Tumor size		
T2	9	17.6
T3	6	11.8
T4	36	70.6
Lymph node metastasis		
N1	17	33.3
N2	25	49.0
N3	9	17.7

Table 2. Distribution of Microvessel Density Based on Patient Characteristics

Characteristics	Microvessel Density			p-value
	Low	High	Total	
Age (years)				
24–29	2	0	2	**0.029***
30–35	2	6	8	
36–41	3	5	8	
42–47	9	1	10	
48–53	4	8	12	
54–59	4	2	6	
60–65	4	1	5	
Total	28	23	51	
Stage				
I	2	2	4	0.974
II	4	3	7	
III	22	18	40	
Total	28	23	51	
Tumor Size				
T2	7	2	9	0.069
T3	1	5	6	
T4	20	16	36	
Total	28	23	51	
Lymph node metastasis				
N1	11	6	17	0.571
N2	12	13	25	
N3	5	4	9	
Total	28	23	51	

*Significantly different if p<0.05

Alfalah [9] showed that breast cancer cases dominantly occurred in women with 52 people (98.1%), and there was 1 case (1.9%) in men. Women are known to have breast cancer 100 times more often than men because of estrogen exposure, menstruation, pregnancy, and breastfeeding [10]. Invasive ductal carcinoma refers to neoplastic proliferation that has penetrated the basement membrane, stroma, and blood vessels that are expected to detect microvessels [11].

The Kruskal-Wallis analysis showed that there was only significant differentiation of MVD based on age in breast cancer patients (p=0.029). Meanwhile, there is no significant differentiation of MVD based on stage (p=0.974), tumor size (p=0.069), and lymph node metastasis (0.571). According to the American Cancer Society in 2015, breast cancer incidence starts growing at the age of 40 years and progressively rises to a peak around the age of 70 to 80 years [12]. Research conducted by Khrisnapriya et al. [13] stated that 59 (43.7%) patients were found over the age of 41 years. This is also supported by the Firasi & Yudhanto [14] research at the H. Adam Malik General Hospital, Medan, in 2009. The youngest age of breast cancer patients in this study was 24 years. Young patients (<35 years) have certain oncogenic changes that can promote tumorigenesis. Breast cancer that occurs at a young age usually has a poor prognosis, is more aggressive, and is a higher stage because estrogen receptor positivity in young breast cancer patients was relatively lower, while HER2 expression tended to be higher [15].

The clinical staging of breast cancer patients was in stage III with a total of 40 (78.4%) patients. Stage III indicates that the tumor has spread from the breast to lymph nodes close to the breast, to the skin of the breast, or the chest wall [16]. This is supported by Windarti [17] in Lampung, with 24 (77.4%) patients, and research by Stankov et al. [18] in Mexico, with 29 (48.33%) patients.

In this study, the MVD cut-off value was 5, obtained from the median value. There were 23 breast cancer patients (45.1%) who had high MVD and 28 patients (54.9%) who had low MVD. The results of this study are directly proportional to the research by Krishnapriya in 2019 where the cut-off value obtained from the median is 5.5 with a high MVD of 45 (48.9%) patients

and a low MVD of 47 (51.1%) patients [13]. Another study by Agnani et al. [19] in 2020 obtained a cut-off value from the median value of 14.78 with high MVD in 32 (49.2%) patients and low MVD in 33 (50.8%) patients. MVD was evaluated by counting anti-CD34 positive microvessels and calculated using a light microscope [19]. High MVD can increase the supply of nutrients and oxygen for proliferating tumor cells and provide vascular routes for tumor cell metastasis. Meanwhile, a low MVD will compensate for tumor cell proliferation and trigger compensatory changes in vascular properties, such as increased vascular permeability and changes in blood flow properties. These changes ultimately promote tumor development [20].

The most common tumor size distribution in breast cancer patients was T4 with a total of 36 people (70.6%) indicating that the tumor had extended directly to the chest wall or skin. Tumor size is one of the most powerful predictors of tumor behavior in breast cancer that constitutes the basis of major staging systems. The growth of a large tumor indicates the growth of cancer cells has entered its final phase and aggressive growth. This is proportional to the research conducted by Marpaung et al. [21], where T4 was found in 72 (52.9%) patients with lung metastases in 34 (25%) patients, Septiawati et al. [6], where T4 was found in 34 (54.8%) patients, and Jamnasi et al. [22] at Cipto Mangunkusumo Hospital, where T4 was found with 60.4% percentages. The results of this study are also in line with studies in China which found that most patients with distant metastases in breast cancer had T4 tumors with a percentage of more than 50%. It is known that higher tumor sizes tend to have a greater risk of developing distant metastases [23].

Low MVD was found to be more than high MVD. This contradicts the research conducted by Septiawati et al. [6]. This study has a cut-off value of 34 which is obtained from the results of the Receiver Operating Characteristics (ROC) analysis. However, the study did not specify the location of the MVD observations, whether in the stroma or intratumoral area. There has not been any other research discussing similar topics in Indonesia [24]. This also contradicts the Muhammad [25] study in Egypt ($P<0.04$). However, research conducted by Comsa et al. [26] in Romania reported that there is no differentiation of intratumoral MVD based on tumor size. The study found that a high MVD was associated with tumor necrosis. This can be explained by hypoxia which can stimulate the expression of VEGF and MVD [26]. In addition, previous studies have showing that there is a relationship between higher vascular density and necrosis. Aggressive tumors rapidly outstrip the vascular supply resulting in prolonged areas of hypoxia within the tumor. Tumor hypoxia-induced altered gene expression, suppressing apoptosis, or promoting autophagy. These cause necrosis and attract macrophages into the tumor which contribute to the angiogenic process giving rise to the link between high levels of angiogenesis and extensive necrosis [27]. The main factor limiting the strength of the differentiation is the high interobserver variability in microvessel counts.

In this study, it was found that tumors with T4 size had the most MVD, with 36 (70.6%) cases, followed by T2 in 9 (17.6%) cases and T3 in 6 (11.8%) cases. This is consistent with studies by Septiawati et al. [6], Muhammad [25], and Biesaga et al. [28], where most MVD was found in larger tumors. It was reported that tumor growth decreased, while the density of blood vessels increased. Blood vessels have poor perfusion leading to tumor cell necrosis [29]. Necrotic tumors may have increased neoangiogenesis which could be due to an increased inflammatory response. For the diffusion of oxygen and nutrients to occur, cells can be up to 110 mm from the microvessel, a size achieved with low microvascular density. Breast cancer that has grown beyond the stroma, such as stage III breast cancer, is called invasive. Breast cancer can spread to other parts of the body through the blood or lymph vessels in the stroma. If left untreated, the primary tumor may extend beyond the lobule wall or tract into the stroma [30]. Usually, breast cancer spreads through the lymph, a clear fluid that provides cells with water and food. It also has white blood cells that fight germs. Most of the lymph in the breast drains into the axillary lymph nodes. Once in the axillary glands, cancer cells can multiply and form secondary tumors [30]. The highest frequency distribution of lymph node metastases in this study was N2 with a total of 25 (49.0%) patients, then N1 with 17 (33.3%) patients, and N3 with 9 (17.7%) patients. The criteria for N2 metastases were found 4-9 metastases in the axillary lymph nodes [31].

CONCLUSIONS

There was only significant differentiation of MVD based on the age of breast cancer patients in the Anatomical Pathology Laboratory at Soedarso Hospital Pontianak. Meanwhile, there was no significant differentiation of MVD based on stage, tumor size, and lymph node metastasis.

Observations using HE-stained breast cancer histopathological preparations showed a low MVD of 28 (54.9%) samples and a high MVD of 23 (45.1%) samples. Breast cancer patients had a tumor size distribution of T4 in 36 (70.6%) patients, T2 in 9 (17.6%) patients, and T3 in 6 (11.8%) patients. Besides that, breast cancer patients had lymph node metastasis, N1 in 17 (33.3%) patient s, N2 in 25 (49%) patients, and N3 in 9 (17.7%) patients.

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DECLARATIONS

Competing interest
The authors declare no competing interest in this study.

Ethics approval and consent to participate
This study was approved by the Health Research Ethics Committee at Soedarso Hospital Pontianak through Letter of Ethical Approval (Ethical-Clearance) No. 74/ RSUD/KEPK/IX/2022. This research is guided by the principles of research ethics, one of which respects the privacy and confidentiality of research subjects. The researcher did not display the patient's name in the data and research results.

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