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CLINICAL SIGNIFICANCE OF THE DENSITY OF TUMOR MICROVESSELS IN BREAST CANCER IN MEN

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ABSTRACT

Breast cancer in men is a rare disease that accounts for less than 1% of all breast tumors. Currently, there is a need for research and search for new biomarkers for early diagnosis, monitoring of the course and prognosis of the disease, and in this aspect, the assessment of angiogenesis is of great importance. Taking into account the fact that the heterogeneity of tumor microvessels in breast cancer has not been practically studied, correlations of different types of vessels with clinical, morphological and molecular biological factors of disease prognosis, as well as with the severity of inflammatory infiltration of the tumor stroma and features of expression have not been studied, the purpose and objectives of this study have been determined.

Key words: breast cancer, microvascular density, prognostic factors, angiogenesis

INTRODUCTION

The rarity of BCM causes a considerable number of medical errors in its diagnosis and treatment. The incidence of breast cancer in men varies synchronously with the "female" breast cancer, which indirectly indicates the common causes of the disease.

Early detection of the disease and timely treatment undoubtedly improve the prognosis of the disease. Over 10 years, the proportion of early stages of BCM (I-II stages) in Russia increased by 10% and amounted to 69.7%, and the number of cases detected during preventive examinations increased from 21.9 to 38.7%. Nevertheless, it should be noted that, taking into account the external localization

of the BCM, the neglect figures for this pathology (stage III-IV) are still high (29.6%).

Currently, due to the existing difficulties of conducting differential diagnosis of BCM, and consequently, the delayed start of the treatment process, the need for research and search for new biomarkers with which early diagnosis, monitoring of the course and prognosis can be carried out is dictated.

It should be noted that today the assessment of angiogenesis is of great importance for the prognosis of the disease and the choice of methods of antitumor therapy, including in breast cancer. Various markers are used to assess the activity of tumor angiogenesis. The most frequently studied expression level of BC or its receptors, as well as PMS, which is studied by counting the number of vessels expressing CD31, CD34, CD133 D2-40 and other markers (Ener E et al, 2016; Svastav S et al, 2016). Lymphovascular invasion is a significant prognostic factor directly associated with an increased risk of local and long-term relapse and lower survival of patients, it is also associated with a high risk of local and systemic relapse of the disease, which is important to consider when planning treatment. Recent studies indicate that inflammatory infiltration of the tumor stroma plays an important role in the invasion and metastasis of malignant neoplasms due to increased secretion of cytokines, chemokines, growth factors and proteases by immune cells, as well as destruction of the connective tissue matrix and basement membranes of epithelium by proteolytic enzymes, activation of angiogenesis and induction of epithelial-mesenchymal transformation. Taking into account all of the above, the heterogeneity of tumor microvessels in BCM has not been practically studied, correlations of different types of vessels with clinical, morphological and molecular biological factors of disease prognosis have not been studied. Thus, the analysis of the literature data indicates that the severity of inflammatory infiltration of the tumor stroma is an important factor associated with tumor progression, which must be taken into account when assessing the prognosis of the disease and planning the treatment of patients with BCM.

THE AIM OF THE STUDY

To improve the results of treatment for breast cancer by a comprehensive assessment of the morphological features and clinical significance of different types of tumor microvessels.

MATERIAL AND METHODS OF RESEARCH

The study included 65 radically operated male patients with a newly diagnosed breast cancer aged 45 to 86 years. The average age of the patients was 57.9 ± 9.9 years (median - 57 years). Before the operation, the standard examination included mammography, ultrasound examination of the mammary glands,

morphological examination of the tumor punctate, X-ray examination of the chest organs, ultrasound examination of the liver, pancreas, kidneys, pelvic organs, examination by a general practitioner and urologist. A number of patients (n=28) additionally underwent osteoscintigraphy to exclude metastatic bone damage. Concomitant chronic diseases were detected in 45 patients.

Table 1.

Data on the concomitant pathology of the examined patients

Name of pathology	N	%
Pathology is absent	21	31,8
Hypertension	25	37,7
IHD	20	30,3
Chronic bronchitis	16	24,2
Anemia of mild and moderate severity	3	4,5
Varicose veins of the lower extremities	8	12,1
Chronic cholecystitis	5	7,6
Chronic pancreatitis	3	4,5
Chronic pyelonephritis	7	10,6
Gastritis and peptic ulcer in the anamnesis	2	3,1
Diabetes mellitus	5	7,6
Chronic hepatitis	1	1,5
VSD	2	3,1
Other	10	15,2

Table 2.

Distribution of patients with breast cancer depending on the clinical, morphological and molecular biological features of the disease.

Clinical and pathological data	N	%
T stage		
pT1	26	40,0
pT2	39	60,0
N stage		
pIII	37	56.9
pIII	15	23.1
pN	1	1.5
pN	12	18.5
The degree of differentiation of the tumor		
01	8	12.3
02	43	66.1
03	14	21.5

Status of estrogen receptors		
Negative	34	52.3
Positive	31	47.7
Status of progesterone receptors		
Negative	46	70.8
Positive	19	29.2
Status HER2/ neu		
Negative	52	80.0
Positive	13	20.0
Biological subtypes		
Luminal	38	58.5
Her2/neu positive	11	16.9
Thrice negative	16	24.6

In the study of tumor microvessels, vessels located in the tumor tissue were defined as intratumoral and vessels located outside the tumor, but within 2 mm from its edge, were defined as peritumoral. The following parameters were determined and evaluated in the tumor stroma and adjacent tissues with an increase of x200:

1. The severity of the tumor stroma. The assessment was carried out by visual-analog method (no, weakly expressed - diffuse infiltration or small focal clusters and pronounced - large focal infiltrates)
2. The presence of loose, non-fibrous, unformed connective tissue of the tumor stroma in the peritumoral region (no, there is)
3. The presence of characteristic cellular structures in loose unformed non-fibrous connective tissue in the tumor stroma (no, there is)

CD34 Ab-1 antibodies in 1:50 dilution, Podoplanin in 1:500 dilution and Vimentin in 1:100 dilution were used for immunohistochemical study. The material for the study by IHC methods was fixed with 10% neutral formalin for 24 hours, poured into paraffin wax, prepared sections with a thickness of 4 microns, which were placed on highly adhesive glasses and dried at a temperature of 37 ° C for 18 hours. UltraVision LP Detection System HRP Polymer & DAB Plus was used as a visualization system. The preparations were studied using an Optika B-350 microscope.

65 blocks were examined by the IHC method using antibodies to CD34, podoplanin and vimentin. In histopreparations stained with CD34 antibodies, the following indicators were evaluated:

1. *The density of microvessels* (DMV) was estimated at the points of maximum vascularization" on an area of 0.30 x 0.22 mm by counting the average number of capillaries and individual cells expressing CD34

2. *The number of dilated capillaries* (DC) in the peritumoral stroma, atypical dilated capillaries (ADC) in the intratumoral stroma and structures with partial endothelial lining were evaluated by a semi-quantitative method with an increase of x200. The 35th percentile was used to divide vessels and structures into single and multiple.

3. In histopreparations stained with antibodies to podoplanin, the localization of vessels, their diameter and features of the endothelial lining were evaluated.

Statistica 6.0. Statistical processing of the results of the study was carried out using the Statistica 6.0 application software package.

The results obtained: All patients underwent radical operations - radical mastectomy - in 50 (76.9%) cases, subcutaneous mastectomy - in 15 (23.1%). Clinical and pathological data, including age, tumor size and localization, degree of malignancy, as well as the status of regional lymph nodes were obtained from outpatient charts and patient histories. After the operation, radiation therapy for regional metastasis zones was performed in 36 (55.4%) patients, adjuvant chemotherapy was performed in 42 (64.6%) patients.

Different types of tumor microvessels differed not only in morphology, but also correlated with various clinical-morphological and molecular-biological features of the BCM. At the same time, attention is drawn to the presence of highly significant correlations within two groups of tumor microvessels and structures with endothelial lining:

1. Between ADCs and structures with partial endothelial lining ($p < 0.00001$);
2. Between DC and characteristic cellular structures in the peritumoral stroma ($p = 0.0001$).

It is important to note that ADCs and structures with partial endothelial lining correlated not only with each other, but also with the same characteristics of breast cancer. Multiple ADCs and structures with partial endothelial lining were significantly more often detected with a negative status of RE ($p = 0.01$ and $p = 0.03$), with CD34 cells in the vascular lumen ($p < 0.00001$ and $p = 0.00007$) and with a high density of vimentin expressing polymorphic stromal cells ($p = 0.002$ and $p = 0.004$).

As for DC and characteristic cellular structures in the peritumoral stroma, they were highly significantly correlated with the presence of loose unformed non-fibrous connective tissue in the peritumoral stroma ($p = 0.0001$ and $p < 0.0001$). It was also common for DC and characteristic cellular structures that they were mainly located in the peritumoral stroma and stained with antibodies to podoplanin. Unlike ADCs and

structures with partial endothelial lining, characteristic cellular structures in the peritumoral stroma.

The median follow-up period for patients with BCM was 68.2 months. During the follow-up period, 14 had relapses of BCM. Local relapse was detected in 1, metastatic bone lesion - in 6, metastases to the contralateral lymph nodes - in 1, metastases to the lungs - in 1 and dissemination to the liver, lungs, bones - in 5 patients. 4 patients died between 34.2 and 66 months after surgery.

CONCLUSION

In this study, special attention was paid to the presence of CD34 positive cells in the lumen of tumor vessels. Since only CD34 positive cells that were not associated with the walls of tumor vessels were taken into account, it can be assumed that they may be related to circulating endothelial cells or angioblasts. These cells are the main source of circulating tumor cells in the peripheral blood. The level of circulating tumor cells increases in many malignant tumors and correlates with the clinical characteristics and prognosis of the disease.

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