Peritumoral Lymphangiogenesis in Breast Carcinoma

S.K. Gantsev, S.R. Kzyrgalin* and K.S. Gantsev

Research Institute of Oncology, Bashkir State Medical University, Ufa, Russian Federation

Abstract: *Objective*: to assess the correlation between microscopically discernible peritumoral lymphangiogenesis and breast carcinoma survival rates; to reveal the regularities of macroscopic changes in the structure of axillary lymphatic system in breast cancer.

Materials and Methods: We carried out morphometric examination of axillary tissue complexes that had been taken from 100 women during surgery for breast carcinoma, stage $T_{1-3}N_{1-2}M_0$ and had been treated with sonolipodistruction. The density of realized lymphatic vessels was determined by counting the number of lymphangiogenesis "points". An anastomosis between individual lymphatic vessels was taken as a "point". Correlation of macroscopically distinguishable peritumoral lymphangiogenesis expression and survival rate was determined using Pearson's parametric analysis.

Results: The analysis of lymphatic maps allowed us to reveal some patterns of axillary lymphatic collector reorganization in breast carcinoma and to distinguish the following types: "Sequential", "Magistral", "Uniform", "Radial" and "Reticulate". Based on the results of prospective observation, 16 patients from the observation group died within the period of 5 years. According to the Pearson correlation analysis, the correlation coefficient was 0,87, the determination coefficient was 0,7488, the value of p = 0,000015, which indicates that in the breast carcinoma, stage T_{1-3} $N1_{-2}$ M_0 there is a statistically significant direct correlation between the number of "points" of macroscopically distinguishable peritumoral lymphangiogenesis and lifespan after a radical surgical treatment. Lymphatic vessels in the process of peritumoral lymphangiogenesis are the indicator of compensatory-protective resources condition.

Keywords: Breast carcinoma, lymphangiogenesis, metastasis, lymphatic vascular density.

INTRODUCTION

Lymphatic system is one of the main routes for metastasis of most human malignant tumors. The most typical metastatic area for breast carcinoma is the lymph nodes of axillary region.

Differences in the degree of lymphangiogenesis manifestation in patients with breast carcinoma have become prerequisites for studying the relationship between lymphatic vascular density and patient survival. However, at present, the researchers' methodology of quantitative evaluation of lymphangiogenesis is still characterized by significant differences.

Metastases are the main cause of death from carcinoma. In the process of anoikis, tumor cells are separated from the primary tumor and subsequently spread to distant organs. [1]; transfer of these cells happens either through their invasion into vascular or lymphatic system or in some condition both. The movement of biological fluids along the vessels has its own peculiarities [2, 3], it differs from classical hydrodynamics which cannot be ignored in the study of metastasis processes.

Vascularization of the tumor is widely used as a reliable indicator of tumor growth, metastasis and patient survival. In 1996, Vermeulen *et al*. published the first international consensus regarding the methodology and criteria for quantitative evaluation of solid tumor angiogenesis, and after 5 years, they published the second one, where new concepts and mechanisms of tumor vascularization were integrated [4, 5]. Both consensuses were aimed at improving standardization of the quantification of the degree of manifestation of intratumoral angiogenesis in order to be used as a prognostic indicator and, moreover, as a reliable predictor as a risk factor for malignant transformation of precancerous lesions. In contrast to intratumoral angiogenesis, the formation of lymphatic vessels *de novo* (lymphangiogenesis) and its role in the metastasis of tumor cells has relatively recently become the center of carcinoma research with an increasing number of studies showing the relationship between patient survival and lymphatic vessel density of various tumor types. Determination of the prognostic potential of lymphangiogenesis for the course of the tumor process can be of particular importance in the individual tactics of treating patients with breast carcinoma, and also to expand the understanding of its role in carcinogenesis.

Purpose of the Study

To study the manifestation degree effect of macroscopically distinguishable peritumoral lymphangiogenesis on the course of the tumor process in breast cancer.

*Address correspondence to this author at the Research Institute of Oncology, Bashkir State Medical University, Ufa, Russian Federation;
E-mail: ufa.shamil@gmail.com

ISSN: 1929-2260 / E-ISSN: 1929-2279/20

MATERIALS AND METHODS

The study group included 100 women who received treatment for breast carcinoma.

The criteria for inclusion in the study group were: morphologically confirmed breast carcinoma of T_{1-3} $N_{1-2}M_0$ stages; lateral location of the primary tumor; regional lymph node metastases only in the axillary region; absence of distant metastases; absence of severe concomitant diseases.

The criteria for exclusion from the study group were: medial location of the primary tumor; metastases in groups of lymph nodes other than the axillary nodes; primary-multiple synchronous and metachronous carcinoma; presence of distant metastases; presence of severe concomitant diseases; refusal of patients from further observation.

The patients underwent mastectomy accompanied by axillary lymphatic dissection.

The subject of the study was the biological material (axillary tissue complexes), removed from 100 women (according to inclusion and exclusion criteria), during surgical intervention for breast carcinoma.

The biological material was subjected to ultrasonic treatment ex vivo. Isolation of lymph nodes and vessels from the axillary tissues was performed with the help of LySonix 3000® ultrasound device with PulseSelect ™ (Byron Medical, Inc., USA) using the method developed by Professor Sh. Kh. Gantsev: layer-by-layer removal of adipose tissue with external moistening (saline), the time of continuous contact of the probe with one surface area did not exceed 3 seconds in order to exclude damaging the histological structure of the studied tissues by a mechanical wave; When deeper structures were reached, the movement of the probe corresponded to the direction of the vessels.

As a result of ultrasonic treatment ex vivo, a substrate was obtained in the form of preparations devoid of adipose tissue for subsequent results documentation.

The documentary fixation of preparations of the axillary lymphatic collector was carried out with a mirror digital camera EOS 5D Mark II with a macro lens (Canon, Inc., Japan): 21,1-megapixel CMOS-matrix with a physical size of 24 × 36 mm, maximum ISO sensitivity up to 25600. Automatic calibration of ISO indicators was used during the shooting. The flash was not used in order to minimize glare.

The degree of expression of the realized lymphatic vessels in the process of lymphangiogenesis was determined by counting the number of "points" of peritumoral lymphangiogenesis and comparative quantitative analysis of the "points" data. An anastomosis between individual lymphatic vessels was taken as a “point”. Point markers were applied to a separate graphic layer in Adobe Photoshop CC (Adobe Systems Software, Ltd., Ireland) and then extracted into a separate file with a white background. The number of "points" was counted in automatic mode using the program "Image Pro plus 6.0" (Media Cybernetics, Inc., USA).

Statistical processing of the research results was carried out using the standard software products "Excel 2013" (Microsoft, Corp., USA) and programs "Statistica 10.0" (StatSoft, Inc., USA).

For statistical processing we used a sample of patients who died of breast carcinoma within 5 years after surgery. The correlation coefficients were calculated by the Pearson parametric analysis method in order to study the interrelation of attributes. The Pearson method is more adequate in this case, since the data analyzed are quantitative. The normality of the data series was determined using the "Statistica 10.0" package (StatSoft, Inc., USA).

RESULTS AND DISCUSSION

According to the results of the study, the package of primary documentation consisted of: case histories, outpatient cards, histological micro-preparations, more than 7000 photographs of axillary lymphatic collector subjected to sonolipodestruction.

Special technology for processing post-surgical material allowed obtaining unique images of axillary lymphatic network. The advantage of this research is the possibility for complex studying of lymphatic vessels: their communication and position to each other and to lymph nodes.

The condition of each lymph node influenced the condition of the surrounding vessels. Palpation and histology revealed that blocked lymph nodes had a more developed network of vessels, afferent vessels were characterized by dilatation evidencing on the violation of lymph flow.

The following types of restructured axillary lymphatic collector have been identified: "Sequential", "Magistral", "Uniform", "Radial" and "Reticulate".

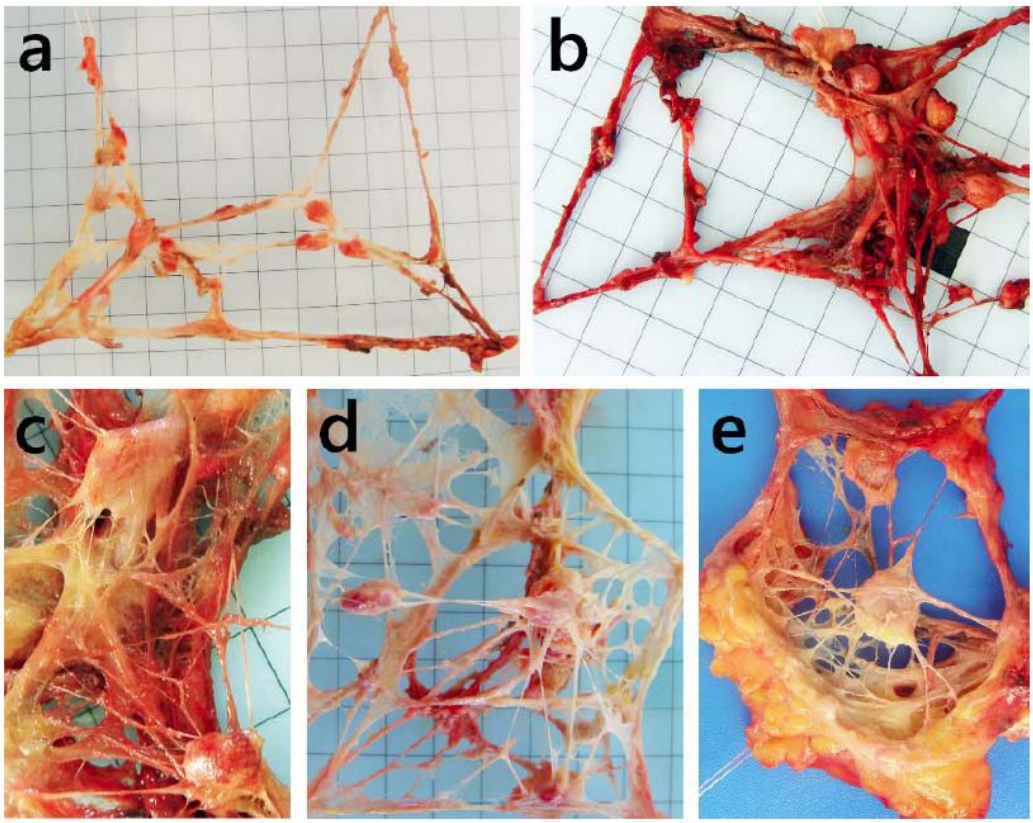

Figure 1: Types of axillary lymphatic collector.

The main criteria for attribution to one or the other type were the number, caliber and length of the interanastomous segments of the lymphatic vessels; number, size and functional status of lymph nodes; and the arrangement of these structural elements relative to each other in the entire tissue complex.

The "Sequential" type of axillary lymphatic collector was characterized by "elongation", predominance of long and thus developed vessels, as well as the presence of a large number of relatively small lymph nodes as they go along. Also, a distinctive feature was the presence of large-sized vascular -free "windows", a small number of intervascular anastomoses. This type of lymphatic apparatus was more often detected in initially small sized and massed tissue complexes removed during surgery (Figure **1a**).

The "Magistral" type was characterized by the presence of several main "magistrals" represented by large vessels, with a developed network of smaller vessels and a small group of different-sized lymph nodes (Figure **1b**).

The "Reticulate" type was characterized by a developed thin network of repeatedly branching micro vessels, the presence of a large number of anastomoses, and relatively small lymph nodes uniformly located throughout the entire tissue complex (Figure **1c**).

The "Uniform" type was characterized by a relatively uniform lymphatic vascularization without a significant preponderance in this or that region, small vessel – free “windows of blood vessels (Figure **1d**).

The "Radial" type was characterized by the presence of one or a group of large lymph nodes (or a conglomerate of lymph nodes) occupying a predominantly central position and radially located afferent and efferent vessels (Figure **1e**).

Most often there was a "Uniform" type of lymphatic collector - 30 complexes out of 100 (30%), least often "Sequential" (8%) while the frequency of occurrence of "Magistral", "Radial" and "Reticulate" types was comparable (20%, 21 % and 21% respectively). The analysis of five-year survival shows that the proportion of deceased patients depending on the type of lymphatic reservoir fluctuated within 12,5-23,33%, except for a group of patients with a "reticular" type of transformed lymphatic apparatus, where within a five-year period all the patients were alive.

The proposed classification is conditional, possible transition of one axillary lymphatic collector type into

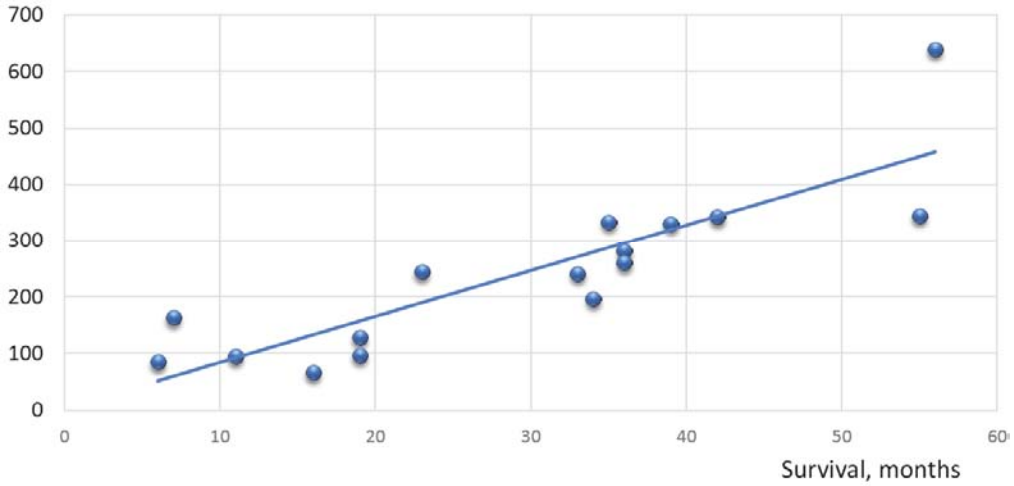

Figure 2: Correlation field. The distribution of the "points" number of macroscopically distinguishable peritumoral lymphangiogenesis and overall survival rate.

another is not excluded, that is, it may be possible that these are the stages of one process fixed at various instants of time. From a researcher's point of view, it is interesting to note the absence of deceased patients in a group with a "Reticular" type of lymphatic reservoir. It can be assumed that it could happen due to the fact that this type is characteristic for the initial stages of lymphatic reorganization, when the probability of cancer cells invasion is lower. The very arrangement of each lymphatic vessel is rather chaotic and does not lend itself to the classical description, canonically accepted in anatomy; however, certain regularities are traced.

The main difficulty in the quantitative evaluation of peritumoral macroscopically distinguishable lymphangiogenesis was three-dimensional and dissimilar location of the lymphatic vessels, which could lead to the omission of certain "points" due to overlapping by surrounding structures. The problem was solved by comparing the images of one macro preparation in different angles; it was achieved by using a metal framework to fix the macro preparation and achieving the possibility of its rotation in volume.

Archiving the data of the axillary lymphatic collector condition allowed a prospective study of the patients' survival with breast cancer. The follow-up period was 5 years. During the observation period, 16 patients out of 100 taken to the observation group died from the progression of the main disease (breast carcinoma).

Based on the survival data, the patients were divided into 2 groups: group I - deceased patients, group II - living patients. The "points" of peritumoral lymphangiogenesis were marked and life expectancy was calculated for patients of group I. The indicators of overall survival and the number of "points" varied widely - 6-56 months (observation period - 60 months) and 85-639 "points", respectively. The distribution of the number of peritumoral lymphangiogenesis "points" and life expectancy in breast carcinoma in the correlation field is shown in Figure **2**.

Thus, 16 pairs of features were subjected to the Pearson parametric correlation analysis, the correlation coefficient was equal to (r) -0,87, the determination coefficient (R2) -0,7488, the value p = 0,000015, which indicates a high direct correlation dependence of patient survival on the number of "points" of peritumoral lymphangiogenesis in breast carcinoma.

CONCLUSION

The results obtained differ from the world opinion "the higher the vascular density of the lymphatic network is, the worse is the prognosis" [6] and demonstrate the opposite picture. In our opinion, the calculation of the "points" number is closer to assessing the vascular density of the lymphatic network as such, in contrast to the indirect sign of prolymphogenic factors density. At the same time, it is impossible to firmly state the direct dependence of the lymphatic network vascular density on overall survival rate using the method of counting "points" due to multifactor regulation of the tumor process. Selective (in view of the high laboriousness) calculation of the "points" number in 5 living patients revealed indexes that do not agree with the correlation in the group of deceased patients: 121, 145, 547, 670, 683. Thus, indexes of prolymphogenic factors used to assess the degree of lymphangiogenesis can not be indicative of

the degree of lymphatic vessels realization, but at the same time they are associated with survival.

The classification of the restructuring of the peritumoral lymphatic collector developed in the course of this study is conditional, the variant of the transition of one axillary lymphatic collector type into another one is possible, that is, these may be the stages of one process at various fixed times. We would like to note that moderate patients in the group with the “reticular” type of lymphatic reservoir were absent. We believe that there is a connection between the absence of moderate patients with a “reticular” type of lymphatic reservoir and the low likelihood of invasion of carcinoma cells, accepted throughout the anatomical community, but certain patterns are observed.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

REFERENCES

[1] Coates JM, Galante JM, Bold RJ. Cancer therapy beyond apoptosis: autophagy and anoikis as mechanisms of cell death. J Surg Res 2010; 164: 301-308. https://doi.org/10.1016/j.jss.2009.07.011

[2] Swartz MA. Lymphatic and interstitial flow in the tumormicroenvironment: Linking tumor mechanobiology with lymph node immunity. Lund. Nature Rev Cancer 2012; 12: 210-219. https://doi.org/10.1038/nrc3186

[3] Swartz MA, Skobe M. Lymphatic function, lymphangiogenesis, and cancer metastasis. Microsc Res Tech 2001; 55: 92-99. https://doi.org/10.1002/jemt.1160

[4] Vermeulen PB, Gasparini G, Fox SB, *et al*. Quantification of angiogenesis in solid human tumours: an international consensus on the methodology and criteria of evaluation. Eur J Cancer 1996; 32A: 2474-2484. https://doi.org/10.1016/S0959-8049(96)00379-6

[5] Vermeulen PB, Gasparini G, Fox SB, *et al*. Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumours. Eur J Cancer 2002; 38: 1564-1579. https://doi.org/10.1016/S0959-8049(02)00094-1

[6] Paduch R. The role of lymphangiogenesis and angiogenesis in tumor metastasis. Cellular Oncology 2016; 39(5): 397-410. https://doi.org/10.1007/s13402-016-0281-9

Received on 18-04-2020 Accepted on 16-05-2020 Published on 19-05-2020

DOI: https://doi.org/10.30683/1929-2279.2020.09.02