

**BREAST CANCER RECURRENCE CLINIC-PATHOLOGICAL RISK FACTORS**Suk Jin Choi<sup>1</sup>, Nigora Atakhanova<sup>2</sup>, Nariman Shayusupov<sup>3</sup>, Doniyor Ishakov<sup>3</sup> and Dr. Alisher Kahharov\*<sup>2</sup><sup>1</sup>Inha University Hospital.<sup>2</sup>Tashkent Medical Academy.<sup>3</sup>Tashkent Medical Academy, Specialized Scientific Center of Oncology and Radiology.**\*Corresponding Author: Dr. Alisher Kahharov**

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**INTRODUCTION**

Breast cancer is the most common malignant tumor among women worldwide.<sup>[1]</sup> By its nature, breast cancer is a heterogeneous disease, including breast cancer with a different clinical course, response to treatment and prognosis.

The most common histological form of breast cancer is a heterogeneous group of epithelial malignant neoplasms with the general name of invasive carcinoma. The traditional histological classification of invasive cancer presents limited prognostic information about the biological nature of the tumor, such as: tumor size, histological type, degree of differentiation, degree of lymph node involvement, lymphovascular invasion, and others. It is interesting to note that the prognosis of a tumor with the same traditional histological characteristics, as well as in patients with the same stage of the disease, varies widely. With the identification of new biological markers, the classification and approach to treating patients begins to be more differentiated.<sup>[2,3,4,5]</sup> In addition, early detection of breast cancer, with minimal tumor size and extramammary spread, gradually reduces the prognostic and clinical significance of the TNM classification, and requires a search for new approaches to the classification of cancer, with a transition from organ and tissue levels to cellular and molecular genetic levels.<sup>[2,3,4,5]</sup> Stratification of the risk of recurrence and metastasis in patients, depending on the presence and absence of new prognostic significant biological, genomic and genetic factors, will improve the results of treatment and the quality of life of patients, avoid excessive use of special therapy.<sup>[2]</sup>

Predicting the response to treatment is the most important element in making a clinical decision; however, to date, the problem of individual prediction remains unresolved. Avoiding unnecessary, excessive and ineffective treatment should be one of the main goals of modern cancer treatment.<sup>[2]</sup>

In fact, to build an accurate forecast, it is necessary to consider all the prognostic factors in the aggregate, and not separately. Ideal from the point of view of many

researchers are prognostic and predictive factors that can be determined on the basis of immunohistochemical studies of paraffin blocks, as they are easily implemented in clinical practice.

The main problem in conducting experimental and clinical studies is their high cost. Independent and young researchers face a shortage of funding, which is an obstacle to the conduct of original scientific research in the field of medicine.

In order to process a large amount of histological material in developed countries, tissue matrix technology (tissue microarray) is used, which consists in collecting biopsy material (material from histological blocks) of many patients on one histological block.<sup>[6,7,8]</sup> This method allows with a high accuracy, as well as low financial and time costs to simultaneously perform analysis of a large amount of data. Histological material in the form of microblocks can be studied using various techniques, such as IHC, in situ hybridization reactions, etc. The tissue matrix allows for scientific research to study new biomarkers, as well as to perform prognostic modeling of disease outcomes.<sup>[6,7,8]</sup>

**MATERIALS AND METHODS**

A retrospective analysis of the archival material of 95 patients with breast cancer T2N0M0, who received complex treatment from 2011 to 2013 under the conditions of the Tashkent branch of the National institute of oncology and radiology was made. Paraffin blocks of these patients were used as donor units to create a tissue matrix.

The fabric matrix fabrication process includes: planning the study, making the recipient block, preparing donor blocks (histological blocks of patients included in the

study), creating a tissue matrix map, sampling and implanting the areas of histological blocks of interest (the material subjected to the fence should contain tumor tissue) in the recipient unit. For the manufacture of the recipient block, agar agar dissolved in distilled water (0.5–5%) was used, followed by pouring into a mold for paraffin blocks and slowly cooled. The optimal concentration of agarose gel is 2% with a thickness of 2 mm. After that, the resulting gel was placed on a standard plastic fabric cassette. Subsequently, the gel using an automatic tissue processor was subjected to a fixation procedure with 10% formalin, dehydration with alcohol of different strength (30%, 50%, 70%, 80%, 95%, 100%), cleaning with xylene and impregnated with paraffin.

After preparation of the paraffin-agar block, its surface was treated with a microtome. In order to mark future holes, mesh paper was glued onto the surface of the block (Figure 1). A self-made bioptic handle with a diameter of 2 mm was used to make holes in the tissue matrix for collecting the histological material. Also, holes can be made using the apparatus for drilling. Using a 11 G modified needle for a bone marrow biopsy and a metal ink cartridge of a ballpoint pen with an internal diameter of 2 mm, the bioptic handle made holes in the recipient unit. The fabric matrix contained 108 holes (12x9). Subsequently, using the same handle, the histological material was collected from the donor unit, followed by implantation into the recipient unit. The site of implantation is determined according to a previously

prepared map. In order to orient the tissue matrix in 2 or 4 corners, tissue is marked with other organs. After the implantation of histological material into the recipient unit, the tissue matrix must be subjected to thermal effects in a thermostat in order to merge donor material with the recipient unit, thus preventing the loss or displacement of the donor histological material during subsequent work with it. Subsequently, the finished tissue matrix is frozen, followed by cuts using a microtome.

The thickness of the microtome sections for the purpose of hematoxylin staining with eosin and immunohistochemical reagents is 4 microns.

The tissue matrix was stained with hemotoxylin-eosin, as well as the following reagents: estrogen (ER), progesterone (PR), HER2neu, proliferation index (Ki67), endothelial tumor growth factor (EGFR), cytokeratin CK 5/6, according to standard methods based on Diagnostic Clinic Mediofarm LLC Premium Diagnostics.

Such histological indicators as the degree of differentiation, lymphovascular invasion, extensive intraductal component, the ratio of the parenchyma to the stroma of the tumor, infiltration of the tumor by lymphocytes were studied.

The study included patients of different age groups from 26 to 78 years. The distribution of patients by age was uniform.

The main characteristics of the sample are shown in Table 1.

Risk factors	Number	%
Menstrual function:		
Premenopausal	27	28,4
Menopausal	30	31,6
Postmenopausal	38	40
Tumor grade:		
G1	15	15,8
G2	53	55,8
G3	27	28,4
Lymphovascular invasion		
Yes	54	56,8
No	41	43,1
Extensive ductal component		
Yes	49	51,6
No	46	48,4
Tumor/stroma ratio		
Poor stroma	34	35,7
Severe stroma	61	64,2
Tumor infiltration with lymphocytes		
Yes	56	58,9
No	39	41,1
IHC status:		
Luminal A	13	13,7
Luminal B	42	44,2
HER2 neu negative luminal B type	33	37,8
HER2 neu positive luminal type B	9	9,4

HER2neu positive	5	5,3
Triple negative	31	32,6
Unknown	4	4,2
Ki67		
High	23	24,2
Middle	42	44,2
Low	26	27,4
Unknown	4	4,2
EGFR		
Positive	25	26,3
Negative	66	69,5
Unknown	4	4,2

Radical mastectomy was performed in 80 (84.2%) patients, of which in 33 (34.7%) cases was carried out by the second stage after verification of the diagnosis through sectoral resection with urgent histological examination. In 5 (5.3%) cases, the operation was limited to the volume of sectoral resection, in 10 (10.5%) patients, a radical resection of the mammary gland was performed.

All patients were treated according to the standards.

Monitoring of patients after the completion of complex treatment was carried out according to the protocol.

Statistical data processing was performed using the IBM SPSS 18 program. The Kaplan-Meier method was used to analyze the survival rate. Cox regression analysis was used to analyze risk factors.

## RESULTS

In 8 (8.4%) cases, patients had relapses in the postoperative scar, of them in 7 cases after radical mastectomy, in 1 cases after sectoral resection of the mammary gland. The duration of the relapse-free period was  $51 \pm 7$  months. The minimum period of local relapse was 11 months, the maximum period of 53 months, respectively.

When analyzing risk factors, the following patterns were identified.

In premenopausal patients, the relative risk (HR = 0.9, CI 95%, 0.22-2.54) of the development of local recurrence was significantly higher than in the group of meno- and postmenopausal patients. The outcome was also negatively influenced by such indicators as the presence of a pronounced intraductal component (HR = 1.2, CI 95%, 0.20-3.67), lymphovascular invasion (HR = 3.7, CI 95%, 1.42-9, 85), poor stroma (HR = 5.3, CI 95%, 1.75-16.09) and the absence of lymphocyte infiltration of the tumor (HR = 5.7, CI 95%, 2.98-10.58). Infiltration with plasma cells or inflammatory cells increased the risk of local recurrence.

In patients with hormone-negative tumors (HR = 5.5, CI 95%, 3.92-10.71), local recurrence was more common than in patients with hormone-positive tumors. A high

level of proliferation index (HR = 5.4, CI 95%, 1.85-5.76), as well as a positive HER 2neu status (HR = 5.7, CI 95%, 1.93-6.59) were predictors of local recurrence.

## CONCLUSIONS

Adverse factors include: a high degree of differentiation, a common intraductal component, the presence of lymphovascular invasion, a high proliferation index, a poor stroma, hormone-negative and triple negative tumor status, HER 2neu expression and EGFR. Stratification of risk in patients depending on the presence and absence of new prognostically significant biological, genomic and genetic factors will improve the results of treatment and the quality of life of patients, to avoid excessive use of special therapy.

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