Research Article

Morphometric Characteristics and Features of Metachronous Breast Cancer

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Abstract
An important morphological criterion for the development of the degree of malignancy of breast cancer is the change in the structure of cell nuclei. At the same time, both the morphometric parameters of metachronous breast cancer and their connection with the molecular biological characteristics of these tumors are currently unknown.

The objective of the research was to identify metachronous malignant disease in the patients with breast cancer.

Materials and methods. The study is based on a retrospective analysis of tumors in 63 patients with breast cancer, who developed uterine cancer or ovarian cancer within 5 years after diagnosis of breast cancer. All the patients received treatment at the Ivano-Frankivsk Regional Clinical Oncology Center. The criterion for selecting patients in the study was the presence of gynecologic cancer after the treatment of breast cancer. The age of patients with breast cancer involved in the study ranged from 24 to 83 years, averaging 56.8±1.5 years.

Results and discussion. The maximum values of the perimeter, radius, and area of cell nuclei (19.21±0.7, 3.05±0.4 and 36.3±0.9, respectively) were observed in tumors of the patients with metachronous breast cancer. The improvement of the perimeter, radius, and area of tumor cell nuclei in the patients with metachronous breast cancer was associated with an increase in the size of the tumors and the presence of metastases in regional lymph nodes.

Conclusions. The morphometric sign of the high risk of developing metachronous breast cancer is the increase in the perimeter, radius, and cell nucleus area on the background of increased proliferative activity of low-grade cancer in the presence of large tumors and metastases in regional lymph nodes.

Keywords
cancer; morphometric characteristics

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Problem statement and analysis of the latest research

Breast cancer (BC) is the most common cancer in women in Ukraine and worldwide with high morbidity and mortality rates and a tendency for steady growth. According to the American Cancer Society (2015), there are two peak periods of developing one more tumor in patients with BC: 1 month - 1 year, 3 - 4 years after the treatment of BC [1].

In 73% of BC cases, two mammary glands are involved in the pathological process; in 18% of the patients, another tumor is localized in the genitals; in 7% of the cases, tumor develops in the gastrointestinal tract; in 4% of the patients, tumor is localized in the skin and other organs. The etiology of BC is multifactorial. Among the well-established etiological factors, the impairment of endocrine interconnections plays a critical role. Mammary gland tumors are hormonal and hormone dependent. To such tumors, we include a significant part of BC, as well as tumors of the uterus and...
ovaries. The main common factor for all of the above-mentioned tumors, apart from the factors of metabolic nature, is latent or apparent chronic hyperestrogenemia [1, 2, 3].

Metachronous BC accounts for 69.6% of all primary multiple tumors and ranks first among them. According to various authors, the frequency of its occurrence in relation to the total number of the patients with BC varies from 12 to 28% [3, 4].

The lack of the objective criteria for predicting the onset of secondary illness in the patients with BC requires further investigation of the biological characteristics of this pathology in order to identify the risk factors and predict the course of the disease [5].

In recent years, the prognosis of the course of metachronous BC has been carried out based on studying the expression of a number of molecular markers. Change in the structure of cell nuclei is known to be an important morphological criterion for the development of the degree of BC malignancy. At the same time, the morphometric parameters of metachronous BC are currently unknown and their connection with the molecular biological characteristics of these tumors is not determined [6, 7, 8].

The nuclei of malignant cells usually exhibit a variation in the size that is known as pleomorphism. According to the theory of mutant phenotype, genetic instability occurs at the early stages of carcinogenesis, and leads to the formation of subclones of cells with different mutations. As a consequence, heterogeneous population of cells with variations in the size and shape of the nucleus is observed. The determination of these variations has both diagnostic and prognostic value for predicting oncological diseases [7, 8, 9].

The objective of the research was to study the morphometric characteristics of metachronous BC and to find out the possibility of using these indicators to predict the course of the tumor process.

### 1. Materials and Methods

The study is based on a retrospective analysis of tumors in 63 patients with BC, who developed uterine cancer (UC) or ovarian cancer (OC) within 5 years after diagnosis of breast cancer. All the patients received treatment at the Ivano-Frankivsk Regional Clinical Oncology Center. The criterion for selecting patients in the study was the presence of gynecologic cancer after the treatment of breast cancer. The age of patients with breast cancer involved in the study ranged from 24 to 83 years, averaging 56.8 ± 1.5 years.

The characteristics of the patients by age are presented in Table 1. The patients at the age of 50-59 years prevailed amounting for 28.6% of the cases; the patients at the age of 60-69 years accounted for 23.8% of the cases. The average age of the patients was 56.8 ± 1.5 years.

| Age, years | Patients with BC |
|------------|------------------|
|            | abs. | %     |
| 20-29      | 1    | 1.6   |
| 30-39      | 6    | 9.5   |
| 40-49      | 12   | 19.0  |
| 50-59      | 18   | 28.6  |
| 60-69      | 15   | 23.8  |
| 70-79      | 9    | 14.3  |
| 80-89      | 2    | 3.3   |
| Total      | 63   | 100   |

**Table 1. Distribution of the patients with BC by age.**

Histological preparations of tumors were used for the measurement [9]. The indicators such as the perimeter, radius, and area of tumor cell nuclei were selected [10].

A microscope LM Carl Zeiss (PrimStar) was used to visualize the preparations [9]. The pictures were taken using a Canon camera. The measurements were carried out using the microscope software AxioVision. For the calculation of statistical indicators, the sampling size was 100 measurements for each geometric index [9, 10].

Statistical processing of the results obtained was carried out by means of the mathematical program of biomedical statistics STATISTICA 6.0. Student’s t-test was used to assess the reliability of the differences in the expression of the examined markers.
and other clinical and pathological parameters. The difference was considered statistically significant at \( p < 0.05 \).

## 2. Results and Discussion

Among the patients with BC, 47 (74.6%) patients developed UC, and 16 (25.4%) patients were diagnosed with OC.

Most patients with BC (47 individuals - 74.6%) developed UC after treatment (Fig. 1).

![Figure 1. Distribution of the patients with BC according to the development of metachronous disease.](image)

We also analyzed the patients according to the stage of BC (TNM classification, 2002) (Table 2).

| BC stage, FIGO staging | Patient with BC, % |
|------------------------|--------------------|
| Stage I: \( T_1N_0M_0 \) | 17 (27.0)          |
| Stage II: \( T_{0-3}N_{0-1}M_0 \) | 32 (50.8)          |
| Stage III: \( T_{0-4}N_{0-3}M_0 \) | 12 (19.0)          |
| Stage IV: \( T_{0-4}N_{0-3}M_1 \) | 2 (3.2)            |
| Total                  | 63 (100)           |

Among these patients, stage I and II BC were detected most often - 27.0% and 50.8% of the cases, respectively; stage III BC was found in about 19.0% of the cases.

By the degree of differentiation, tumors were divided into well differentiated (G1), moderately differentiated (G2) and poorly differentiated (G3) tumors (Table 3).

### Table 3. Distribution of the patients by the degree of tumor differentiation.

| Stage differentiation | G, (%) |
|-----------------------|--------|
| G1                    | 18 (28.6) |
| G2                    | 37 (58.7) |
| G3                    | 8 (12.7)  |
| Total                 | 63 (100)  |

Among the patients with BC, moderately differentiated tumor was most frequently detected - 58.7% of the cases; well differentiated tumor was found in 28.6% of the cases; poorly differentiated tumor was less common - 12.7% of the patients.

When analyzing the morphometric characteristics of cell nuclei, the maximum values of the studied parameters, perimeter, radius, and area of tumor cell nuclei were determined (Table 4).

### Table 4. Morphometric characteristic of nuclei of tumor cells in patients with breast cancer.

| Parameter                              | Perimeter, \( \mu m \) | Radius, \( \mu m \) | Area, \( \mu m^2 \) |
|----------------------------------------|------------------------|---------------------|---------------------|
| Patients with BC                       | 19.21±0.7              | 3.050±0.4           | 36.3±0.9            |

The correlation of the morphometric parameters of tumor cell nuclei with clinical and pathological characteristics of BC such as tumor size, the presence of metastatic lesions of regional lymph nodes and the degree of differentiation was determined.

An increase in tumor size was found to be associated with an increase in the perimeter, radius and area of tumor cells in the patients. In the patients with T3 tumors, the perimeter, radius, and area of tumor cell nuclei were \( 21.6±1.8, 3.4±1.0 \) and \( 45.9±1.3 \), while in the patients with T2 and T1 tumors, they were \( 13.8±0.9, 2.2±0.8 \) and \( 18.1±0.9 \) and \( 14.3±1.1, 2.2±0.8 \) and \( 18.5±1.2 \), respectively (Table 5).
In addition, an increase in the perimeter, radius, and diameter of BC cell nuclei was found to be associated with the presence of metastatic involvement of regional lymph nodes. In the patients without metastatic involvement of regional lymph nodes, the perimeter, radius, and area of tumor cell nuclei were 10.9±0.8, 1.7±0.6 and 9.7±1.0, while in the patients with N1 and N2 BC, they were greater - 14.02±1.3, 2.2±0.3, 17.2±0.9 and 19.3±1.0, 3.07±0.7, 37.6±1.1, respectively (Table 6).

| Metastatic lesions of regional lymph nodes | Parameter       |
|-------------------------------------------|-----------------|
|                                           | Perimeter, µm   | Radius, µm | Area, µm² |
| N0                                        | 10.9±0.8*       | 1.7±0.6*   | 9.7±1.0*  |
| N1                                        | 14.02±1.3       | 2.2±0.3    | 17.2±0.9  |
| N2                                        | 19.3±1.0        | 3.07±0.7   | 37.6±1.1  |

Note: * – p<0.05 as compared to N1 and N2 tumors.

A decrease in the degree of BC differentiation was found to be accompanied by an increase in all investigated parameters of tumor cell nuclei in the patients of the studied group (Table 7). Thus, in the patients with well, moderate and poor degree of BC differentiation, the perimeter of tumor cell nuclei was 12.5±0.7, 18.9±0.7 and 19.9±0.7 µm. The decrease in the degree of BC differentiation was associated with the increase in the radius and the area of tumor cells as well.

| Degree of differentiation | Parameter       |
|---------------------------|-----------------|
|                           | Perimeter, µm   | Radius, µm | Area, µm² |
| G1                        | 12.5±0.9*       | 1.9±0.9*   | 15.2±0.8* |
| G2                        | 18.9±1.0        | 3.0±0.4    | 36.2±0.8  |
| G3                        | 19.9±0.8        | 3.1±0.7    | 39.1±1.2  |

Note: * – p<0.05 as compared to moderately and poorly differentiated tumors.

When analyzing the dependencies of the morphometric indices of tumor cell nuclei on their receptor status and proliferative activity, certain differences were determined. The studied morphometric parameters were found not to depend on the presence of the expression of estrogen and progesterone receptors in tumor cells of the patients (Table 8). High proliferative activity of BC in the patients was found to be associated with an increase in the perimeter, radius and cell nucleus area by 1.5-2 times.

The next stage of the research was to study the correlation between the morphometric indices of BC cell nuclei according to the period of occurrence and localization of another tumor. There was no statistically significant difference between the morphometric characteristics of BC and the period of occurrence of secondary illness (Table 9).

The maximum indicators of the perimeter, radius and area of cell nuclei were determined in the patients with BC and localization of another neoplasm in the ovary (Table 10).

### 3. Conclusions

The maximum values of the perimeter, radius, and area of cell nuclei (19.21±0.7, 3.05±0.4 and
Table 8. Morphometric characteristics of cell nuclei depending on the degree of differentiation of breast cancer cells.

| Expression of molecular markers | Parameter | Perimeter, µm | Radius, µm | Area, µm² |
|--------------------------------|-----------|---------------|------------|-----------|
| ER (-)                         |           | 15.5±0.9      | 2.4±0.9    | 24.0±0.8  |
| ER (+)                         |           | 19.9±1.0      | 3.1±0.4    | 24.4±0.8  |
| PR (-)                         |           | 15.5±0.8      | 2.4±0.7    | 24.7±1.2  |
| PR (+)                         |           | 19.9±0.9      | 3.1±0.9    | 27.0±0.8  |
| Ki-67(-)                       |           | 13.5±1.0      | 2.1±0.4    | 24.4±0.8  |
| Ki-67 (+)                       |           | 29.9±0.8*     | 3.6±0.7*   | 40.7±1.2* |

Note: * – p<0.05 as compared to Ki-67 (-) tumors.

Table 9. Morphometric characteristics of cell nuclei depending on the degree of differentiation of breast cancer cells.

| Another tumor occurrence | Parameter | Perimeter, µm | Radius, µm | Area, µm² |
|--------------------------|-----------|---------------|------------|-----------|
| Within 1 year            |           | 17.4±1.9      | 2.7±0.9    | 29.1±1.8  |
| Within 3 years           |           | 18.3±1.0      | 2.9±0.6    | 35.9±1.6  |
| More than 3 years        |           | 19.5±1.8      | 3.1±0.7    | 36.5±1.2  |

Table 10. Morphometric characteristics of cell nuclei depending on localization of another tumor in the patients with BC.

| Localization of another tumor | Parameter | Perimeter, µm | Perimeter, µm | Perimeter, µm |
|-------------------------------|-----------|---------------|---------------|---------------|
| UC                            |           | 20.1±2.1      | 3.2±0.6       | 40.2±2.8      |
| OC                            |           | 12.2±1.0      | 1.94±0.4      | 14.6±1.6      |

High proliferative activity of BC in the patients was found to be associated with an increase in the perimeter, radius, and cell nucleus area by 1.5-2 times.

The morphometric sign of the high risk of developing disease secondary to BC is the increase in the perimeter, radius, and cell nucleus area on the background of increased proliferative activity of low-grade cancer in the presence of large tumors and metastases in regional lymph nodes.

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