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CASE REPORT

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Resistance to neoadjuvant chemotherapy in breast cancer with proven intratumoral heterogeneity: a clinical case

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ABSTRACT

Breast cancer (BC) is the most common cancer in women in Bulgaria, with a frequency of 26.7% of all newly registered cancer cases in 2020 and ranks first in mortality. In recent years, research and studies have confirmed that breast cancer is a highly heterogeneous disease at the morphological, genomic, and transcriptomic levels, manifested clinically with different behavior and response to therapy. The gold standard for breast cancer diagnostic management is based upon three diagnostic methods, including clinical examination, imaging, and percutaneous biopsy. The main percutaneous biopsy method is an ultrasound-guided core-needle biopsy. It is sufficiently representative of the composition of the tumor although it represents a limited part of it, and some cellular subpopulations are often scantly represented or completely absent. We present a case of a 41-year-old breast cancer patient with primary intratumoral morphological heterogeneity diagnosed through core-needle biopsy and with primary resistance to neoadjuvant targeted therapy.

Key words: breast cancer, core-needle biopsy, intratumoral heterogeneity, non-adjuvant chemotherapy

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Introduction

Breast cancer is the most common cancer in women. In Bulgaria, it ranks first in mortality among cancers in women. In 2020, the number of new cases in the 27 countries of the European Union (EU) was 355 457, with an estimated annual frequency of 142.8/100,000 population. For Bulgaria, the frequency is 100/100 000, i.e. 26.7% of all newly registered oncological diseases in women. The mortality in Bulgaria is higher than the average for the European Union, 36.3/100,000 compared to 34.1/100,000 population [1].

In recent years, studies have confirmed that breast cancer is highly heterogeneous at the morphological, genomic, and transcriptomic levels, manifesting clinically with different behavior and different responses to therapy. Many of the therapeutic solutions, and neoadjuvant chemotherapy (NACT) in particular, are based on the possibility of a complete pathological response. Most often, it is achieved with targeted therapy, based on the molecular subtype of cancer, i.e. molecular markers expressed from the cancer cells found in the biopsy specimen. The samples taken by core-needle percutaneous biopsy (CNB) represent a limited part of the

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tumor, where different cellular subpopulations are often scantly represented or completely absent [2]. However, a biopsy under ultrasound control is considered the gold standard in breast cancer diagnosis. The obtained biopsy samples are processed for histomorphological evaluation, including the morphological variant, degree of differentiation, invasiveness, expression of biomarkers, including steroid receptors (ER and PgR), HER 2 status, and proliferative index Ki-67. There is a statistically significant correlation between the results obtained in the pathological examination of tumor tissue taken by CNB and tissue taken by surgical excision, in which the available volume of pathological tissue is larger. Multiple authors have confirmed this view in their studies [3, 4]. The morphological heterogeneity is often accompanied by a heterogeneous expression of biomarkers, a fact that further complicates the choice of therapy. It calls into question the effect of NACT, which in some cases delays surgical intervention. Tumor heterogeneity has been associated with poorer prognosis and survival [5]. It is also the leading cause of therapeutic resistance [6].

A clinical case

We present a case of a 41-year-old breast cancer patient with core-needle biopsy-proven primary intratumoral morphological heterogeneity and primary resistance to chemotherapy.

The patient was admitted to the Surgical Oncology Clinic at Dr. Georgi Stranski University Hospital in Pleven, with complaints of a palpable mass in the left mammary gland dating back six months. The patient reported arterial hypertension and hypothyroidism as concomitant diseases treated with L-thyroxine and antihypertensive drugs. The clinical examination revealed a formation in the upper lateral quadrant of the left mammary gland near the nipple-areolar complex. It was a solid mass about 30 mm in diameter, painless, fused with the surrounding tissues, with no changes involving the skin. Enlarged solid lymph nodes of about 20 mm in diameter were also painless, palpated in the homolateral axilla. The mammography examination classified the finding as 4C according to the BI-RADS system with a recommendation for subsequent histological verification. Following a lidocaine susceptibility test, an ultrasound-guided core-needle biopsy (CNB) with local anesthesia and a fine-needle aspiration biopsy (FNA) of an enlarged homolateral axillary lymph node were performed. The samples were sent for histopathological and cytological examination. Findings from cytological examination demonstrated ductal carcinoma tumor cells arranged individually and in small groups, and the presence of lymphocytes and erythrocytes.

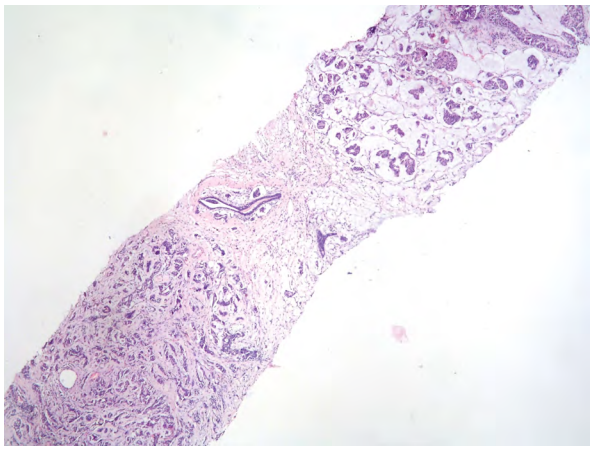

Figure 1. A heterogeneous tumor composed of two components mucinous (hypocellular variant) carcinoma and NST G2 carcinoma. HE 40×

The processing and immunohistochemical staining of the preparation was made according to the current standard laboratory protocols.

The histological evaluation of the core needle biopsy samples demonstrated 5 tissue cylinders containing mammary gland parenchyma infiltrated by tumor cells, composed of two morphologically distinct components (Fig.1).

Immunohistochemistry of the core needle biopsy demonstrated positivity for steroid receptors, HER2 was interpreted as equivocal (2+), and in situ hybridization was advised. In situ hybridization for HER2 demonstrated the presence of amplification. The proliferation index estimated by Ki-67 was about 35% (Fig. 2).

The patient was evaluated clinically as cT2N1M0, stage IIB, and the Medical Oncology Committee referred the patient for NACT. Four courses with docetaxel, trastuzumab, and pertuzumab at intervals of 21 days were applied. After the last course, a restaging was performed and showed no response to therapy. The physical exam revealed enlargement of the tumor lesion to 35 mm in diameter. The lymph nodes persisted up to 20 mm in diameter. CT results confirmed progression for the soft tissue lesion (26 × 24 mm) in the left mammary gland, which did not increase its density in post-contrast enhancement, and the pathologically enlarged lymph nodes (21 × 10 mm) to the left side. There was no CT data for dissemination to the internal organs and bone structures. The patient was referred for radical surgical treatment. A mastectomy with axillary lymph node dissection was performed.

After the breast was surgically removed, the breast specimen was cut in a standard manner and fixed in 10% NBF. A round, gray-white nonhomogeneous, infiltrative tumor with a cartilaginous density, and partly soft consistency measuring 50 × 45 × 30 mm was found. The axillary lymph nodes harvested from the axillary dissection were enlarged up to 25 mm.

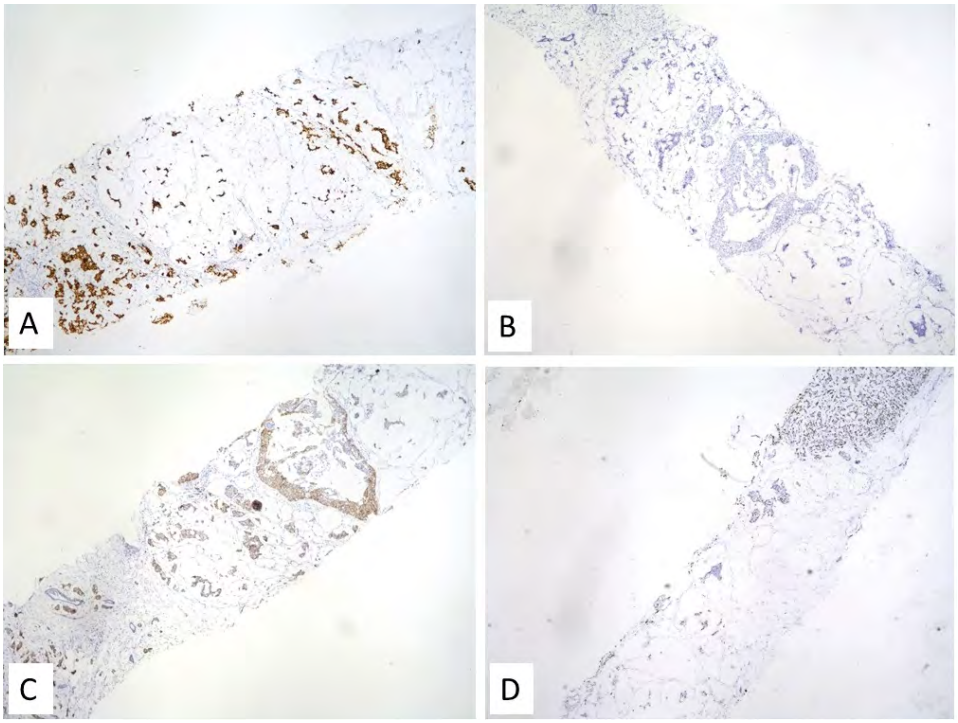

Figure 2. A. ER staining in the two components ER, 40×; **B.** PgR staining in the two components PgR, 40×; **C.** HER2 staining in the two components HER2, 40×; **D.** Ki-67 staining in the two components Ki-67, 40×

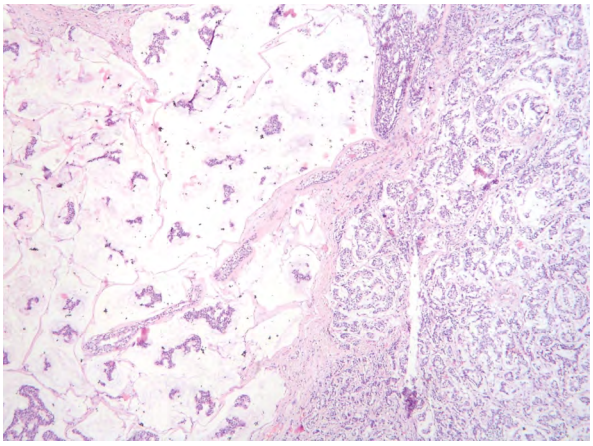

Figure 3. Heterogeneous tumor, composed of two components mucinous (hypocellular variant) carcinoma and NST G2 carcinoma. HE 40×

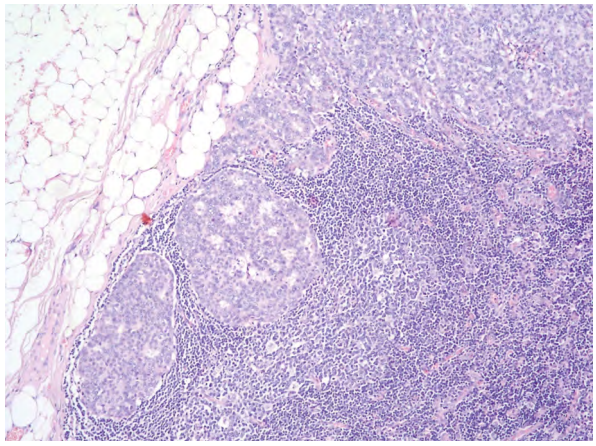

Figure 4. Lymph node metastasis HE 100×

A histopathological examination demonstrated the presence of a heterogeneous carcinoma, composed of mucinous (hypocellular variant) and NST G2 component with moderately desmoplastic stroma, vascular invasion, presence of DCIS-G2, usual ductal hyperplasia, columnar cell changes, and fibroadenomatoid hyperplasia (Fig. 3).

Metastases were obtained in 5 of the 18 evaluated lymph nodes. Additionally, focal necrosis cholesterol crystals and hemorrhages were found focally in some lymph nodes (Fig. 4).

Upon IHC retesting, the NST component demonstrated positivity for steroid receptors, equivocal (2+) result for HER2 (with amplification after in situ hybridization testing), and Ki-67 proliferation index of about 75%. The mucinous component demonstrated positivity for steroid receptors, negative (1+) result for HER2, and low Ki-67 proliferation index (Fig. 5).

The tumor response to therapy was limited. According to Sataloff criteria, it was estimated as T-D and N-D, respectively. The pathology report confirmed progression with pT3N2M0, stage IIIA.

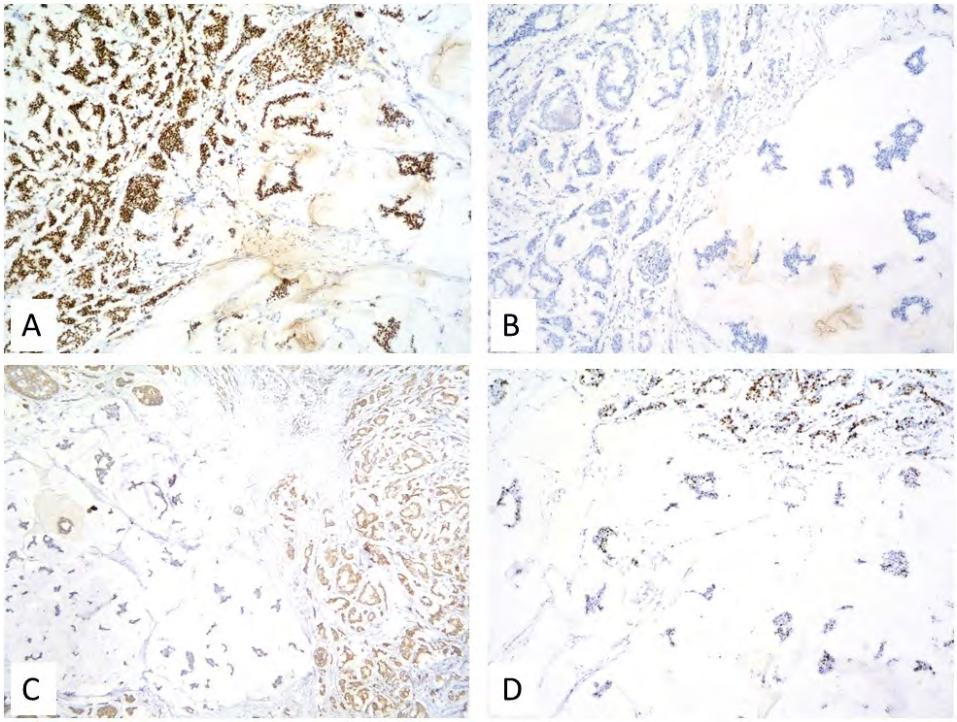

Figure 5. A. ER staining in the two components ER, 40×; **B.** PgR staining in the two components PgR, 40×; **C.** HER2 staining in the two components HER2, 40×; **D.** Ki-67 staining in the two components Ki-67, 40×

Discussion

Breast cancer is a heterogeneous disease involving many tumor subtypes characterized by different morphology, behavior, and clinical consequences [7]. Preoperative assessment of breast lesions and their histological verification are crucial for an accurate diagnosis, determining the appropriate therapeutic treatment plan and prognosis. According to the European Society for Medical Oncology (ESMO) Guidelines, the pathological diagnosis should be made after CNB under ultrasound control before starting any treatment. If preoperative systemic therapy (NACT) is required, an invasive process must be identified, and molecular biomarkers tested [8]. According to the recommendations of the American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP), estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2) should be routinely, immunohistochemically tested biomarkers in all primary histologically proven breast tumors [9] They are the basis of the molecular classification of breast tumors presented in 2000 by Perou et al. in an attempt to include the manifestation of genetic tumor heterogeneity in clinical practice [10].

In recent years, the proliferative index Ki-67, an element of the same classification, has also been studied. It has a predictive and prognostic value in clinical practice. It is a factor that can predict a complete pathological response in NACT [11]. Chemotherapy significantly improves survival in patients with breast cancer, and NACT has become an established first choice in the treatment of locally advanced large tumors, enhancing surgical success [12]. It is also increasingly used in patients in the early stages of the disease, with an unfavorable prognosis, mostly HER 2 positive and triple-negative breast cancers.

Neoadjuvant chemotherapy (NACT) allows evaluation of the therapy outcome and subsequent optimization of systemic therapy in the absence of response [13]. Preoperative therapy has been shown to lead to changes in tumor biomarkers, which is relevant to crucial for patients' subsequent prognosis and survival [12]. Excessively aggressive therapies select tumor cells and cell clones with a resistant phenotype. This leads to a rapid progression of the disease, making it virtually unresponsive to subsequent treatment [14].

The morphological heterogeneity is accompanied by molecular heterogeneity (heterogeneous immunomarker expression). Morphological heterogeneity is presented as different subpopulations within a single tumor and was described as early as the 1950s [15]. The existence of components with unclear morphological features or foci with different differentiation can also be attributed to morphological tumor heterogeneity and reflect different genetic aberrations [2]. They further

complicate the choice of therapy and question the effect of NACT, which can delay surgical treatment. In our case, the patient was in an advanced stage of the disease and was suitable for neoadjuvant targeted therapy with an expected complete pathological response. However, morphological heterogeneity together with the presence of heterogeneous molecular subtypes (marker expression) within the tumor mass resulted in a lack of therapeutic effect of the applied therapy, leading to prolongation of the time to surgical intervention and causing cancer progression. To optimize therapeutic effect in patients with morphological heterogeneity, additional research is required.

Conclusion

Tumor heterogeneity in breast cancer may be manifested in every characteristic of the disease, including histopathological, molecular, and functional. Additional genetic and epigenetic changes and various adaptive responses during the disease generate different cell populations that exacerbate tumor heterogeneity and lead to disease progression and drug resistance. Morphologically heterogeneous tumors and tumors demonstrating molecular heterogeneity cannot be classified and treated with established therapeutic standards. They require personalized therapy as they are often associated with therapeutic resistance and poor prognosis.

Conflict of interest

Authors declare no conflict of interest.

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