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Molecular subtype shift in breast cancer upon trastuzumab treatment: a case report

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Breast cancer is the most common cancer in women. The mortality remains significant despite advanced treatment possibilities. The management of breast cancer is guided by immunohistochemical data that are summarized into molecular subtypes, namely, luminal A, luminal B, HER2 positive and triple negative. HER2 positive and triple negative subtypes of breast cancer are considered to be biologically distinct. We present a case of clinically aggressive breast cancer in a 58-year-old female. Along the course of the disease, the molecular type switched from HER2 positive to triple negative. The patient deteriorated despite combined therapy. We recommend making a possible change of the molecular subtype and employing repeated immunohistochemical investigation in case of relapse.

Key words: breast cancer, immunohistochemistry, molecular subtype.

Introduction

Breast cancer is the most common cancer among women in the European Union. Despite the advances in early diagnostics, surgical and radiation treatment as well as chemotherapy, the mortality remains significant [1]. The established prognostic molecular factors in breast cancer include immunohistochemical expression of estrogen receptors (ER) and progesterone receptors (PR) as well as proliferation activity by Ki-67 protein expression [2] and evaluation of human epidermal growth factor receptor 2 (HER2) status at the protein and gene level. These parameters result in the molecular classification of breast cancer [3]. The breast tumours can pursue different clinical course in accordance with the molecular characteristics. Here, we demonstrate a molecular and clinical course of breast cancer subtype switch with an impact on the choice of treatment.

Material and methods

Records of the Clinic of Surgery, Pauls Stradiņš Clinical University Hospital were reviewed to identify the clinical and treatment data. The founder mutations characteristic of the Latvian population [4] in the *BRCA1* (4154delA, 5382insC and 300T/G) and *BRCA2* (873delG and 886del TG) genes were searched for. The morphological data were acquired at the Institute of Pathology, Pauls Stradiņš Clinical University Hospital. The gross and microscopic evaluation was performed on a breast cancer protocol basis, aiming at complete description of morphological prognostic factors. The tissues were fixed in neutral buffered formalin, processed in vacuum infiltration processor Tissue-Tek® VIP™ 5 (Sakura Seiki Co., Ltd., Nagano, Japan) and embedded in paraplast (Diapath S.r.l., Bergamo, Italy) using tissue embedding system TES 99 (Medite GmbH, Burgdorf, Germany). Four-micron-thick sections were stained with haematoxylin-eosin. The formalin-fixed, paraffin-embedded tissues, cut at 3-micron-thick sections on electrostatic slides (Histobond, Marienfeld, Germany) were investigated by immunohistochemistry (IHC), using heat-induced epitope retrieval in TEG buffer at pH 9.0 in microwave oven 3 × 5 min. A panel of primary antibodies against

estrogen receptor alpha (clone 1D5, dilution 1 : 1), progesterone receptors (clone PgR636, 1 : 1), mammaglobin (clone 304-1A5, 1 : 100), thyroid transcription factor-1 (clone 8G7G3/1, 1 : 100), CD56 (clone 123C3, 1 : 100), chromogranin A (polyclonal rabbit antibody, 1 : 1000), E-cadherin (clone NCH-38, 1 : 50), actin (clone HHF35, 1 : 400), p53 (DO-7, 1 : 400), cytokeratin 5/6 (D5/16B4, 1 : 100) and Ki-67 (clone MIB-1, 1 : 100) was employed. Peroxidase-conjugated labelled streptavidin-biotin visualisation system was applied for the detection of bound primary antibodies, followed by colour development by 3,3'-diaminobenzidine. All IHC reagents were produced by Dako, Glostrup, Denmark. HER2 protein overexpression was detected by HercepTest™ according to the manufacturer's (Dako, Glostrup, Denmark) instructions. Appropriate positive and negative controls were performed.

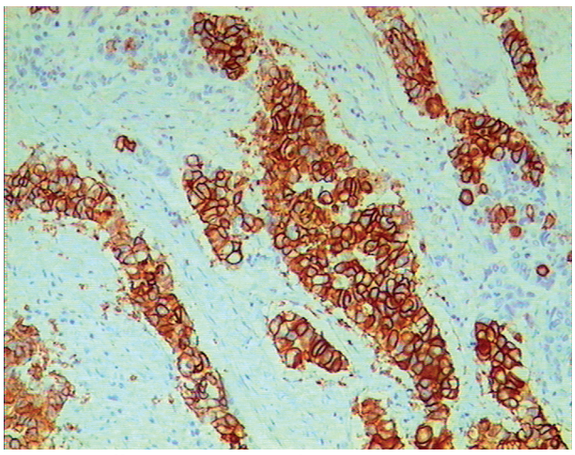

Fig. 1. Diffuse, intense membranous expression (3+) of HER2 protein in tumour cells by immunohistochemistry (Hercep Test™, original magnification 100×)

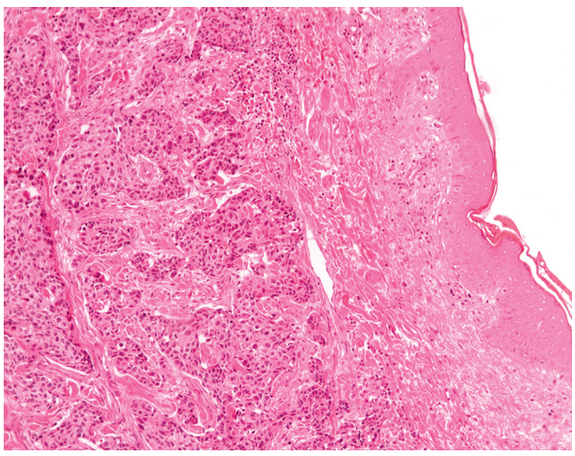

Fig. 2. Invasive, high-grade ductal breast carcinoma in dermis (haematoxylin-eosin, original magnification 50×)

Results

A 58-year-old female was complaining about a mass in the right breast. Mammography, ultrasonography, breast core biopsy with subsequent histological evaluation in conjunction with the general evaluation of the health status yielded the diagnosis of invasive ductal breast cancer, T4N2M0G3. No founder mutations in the *BRCA1* (4154delA, 5382insC and 300T/G) and *BRCA2* (873delG and 886del TG) genes were found. Four courses of chemotherapy including doxorubicine 100 mg and cyclophosphamide 1200 mg were applied, followed by right mastectomy with right axillary lymph node dissection. At gross pathologic evaluation, a solid, firm, greyish red mass, measuring 6 cm in diameter was found in the upper lateral quadrant of the right breast. Histologically, the tumour was high-grade ductal cancer. There were 9 lymph nodes in armpit fat, all affected by tumour metastasis. By IHC, neoplastic cells did not express ER and PR. There was strong complete membranous HER2 protein expression in 20% of neoplastic cells, corresponding to positive (3+) result (Fig. 1) in the primary tumour. However, sharp demarcation between HER2 positive and negative components was found. The metastasis in the axillary lymph node did not express HER2 protein. The mean proliferating cell fraction by nuclear Ki-67 expression was 47%. The proliferative activity was highly heterogeneous and reached high values (up to 89% per 200 consecutively counted cells) in triple negative component. The tumour cells uniformly intensively expressed p53 protein in nuclei and E-cadherin in cell membranes but were negative for actin and cytokeratin 5/6. The pathological diagnosis was invasive, high-grade ductal breast carcinoma, pyT3N2G3R0 with combined molecular immunophenotype. The immediate postoperative period was uneventful. The patient was discharged on the 10th postoperative day after evaluation by a council of oncologists. Chemotherapy with doxorubicin 100 mg and cyclophosphamide 1200 mg was continued 3 times on an out-patient basis. Radiotherapy to the supraclavicular region with 50 grays (Gy), axillary region with 44 Gy and post-operative scar with 50 Gy was performed after chemotherapy. Trastuzumab (300 mg, followed by 7 × 150 mg) was applied after radiotherapy on an out-patient basis.

Six months after the mastectomy, the patient found a node under skin on the right side of thorax. The node was excised on an out-patient basis. Grossly, white, solid mass, measuring 4 × 2.5 × 2 cm, was found. Microscopically, high-grade non-small cell cancer was found invading subcutaneous adipose tissue and dermis (Fig. 2). The neoplasm had solid and trabecular architecture, focal necrosis (7%) and

moderate stromal desmoplasia without reactive inflammation. By IHC, tumour cells focally (5%) intensively expressed mammaglobin (Fig. 3), but did not express TTF-1, CD56, chromogranin A, ER and PR (both 0%), HER2 protein (0, negative). Proliferating tumour cell fraction was 82%. Metastasis of triple negative, high-grade ductal breast cancer in the subcutaneous adipose tissue and dermis was diagnosed. Palliative chemotherapy with vinorelbine 50 mg was offered 3 times. The patient developed liver metastases and died 10 months after the excision of subcutaneous breast cancer metastasis.

Discussion

Despite the advanced breast cancer treatment strategies, 25-40% of patients with breast cancer still eventually develop largely incurable metastatic disease. Results of the treatment depend on many factors: patient's age as well as TNM stage, grade and molecular subtype of the tumour [5]. The molecular subtypes of breast cancer can be classified by IHC as luminal A (ER and PR positive, HER2 negative), luminal B (ER and PR+, HER2+), HER2 positive (ER and PR–, HER2+), or triple negative (ER, PR, HER2–) [3]. In this case, breast cancer in mastectomy was classified as HER2+ subtype due to intense appropriate immunoreactivity that is described to correlate with gene amplification located in the HER2 amplicon on 17q21 [6]. Subcutaneous metastasis did not express HER2 protein, thus, there has been a change in tumour molecular subtypes upon treatment within 6 months from HER2 positive to triple negative breast cancer. Hypothetically, the HER2 positive component was suppressed by Trastuzumab and the remaining part of tumour progressed. Alternatively, higher malignant and metastatic potential in the triple negative component could be suspected as the metastasis in lymph nodes was of a triple negative subtype and the proliferative activity was the highest in the triple negative component of the primary tumour.

Triple negative breast cancers are associated with aggressive behaviour and poor prognosis [7]. The risk of tumour recurrence increases from the date of diagnosis with sharp peak between 1 and 3 years. The median survival time from recurrence to death is 9 months, significantly less than in other types of breast cancer characterized by the median survival time of 20 months [8]. In our case, the time period between the primary operation and relapse as well as between relapse and death was similar to the described mean findings for triple negative breast cancer [8].

The case demonstrates very clear-cut evidence that the molecular subtype of tumour may change in the course of the disease with significant implications for the treatment. Although Trastuzumab treatment was justified by the primary histology and possibly had beneficial effects in terms of eradicating the HER2 positive component, the relapse occurred as pure triple negative breast cancer. Hypothetically, the cases of breast cancer showing a combination of sharply demarcated molecular subtypes might necessitate combined treatment aiming at both components if such treatment is available. If the HER2 positive and triple negative subtypes of molecular receptors are considered biologically distinct, in future, additional molecular markers should also be sought for in order to predict the need for combined treatment.

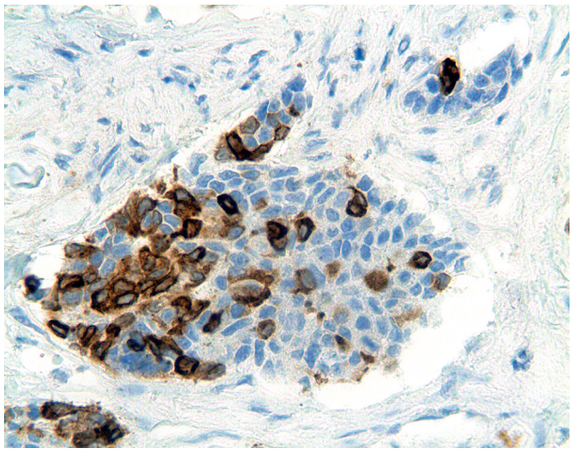

Fig. 3. Diffuse, intense cytoplasmic and membranous expression of mammaglobin in tumour cells by immunohistochemistry (immunoperoxidase, original magnification 400×)

In conclusion, the breast cancer case demonstrates molecular subtype switch with definite practical diagnostic implications. The findings also reveal an intimate relationship between different aggressive molecular subtypes that might highlight important aspects considering the molecular classification and carcinogenesis of breast tumours.

Acknowledgements

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