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Immunohistochemical subtyping and evaluation of the prognosis of basal-like triple-negative breast cancer based on IDO1 protein expression

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Abstract

Background. Known subtypes of triple-negative breast cancer, despite the common basal-like profile, have certain features that differ in terms of the course and prognosis of the disease. Therefore, the study of molecular markers that make it possible to identify subtypes of basal-like carcinomas and evaluate a probable prognosis based on their expression indicators is relevant today.

Aim. Conduct immunohistochemical subtyping of basal-like triple-negative breast cancer based on the assessment of IDO1 protein expression, compare the obtained data with clinical and morphological characteristics, as well as indicators of tumor sensitivity to neoadjuvant therapy.

Material and methods. The study was retrospective and included 42 patients diagnosed with triple negative breast cancer (mean age 50.5 ± 12.7 years), disease stage T1–4dN0–3M0. The patients were treated in the Departments of Chemotherapy and General Oncology of the Research Institute of Oncology of the Tomsk National Research Medical Center from 2016 to 2021. A morphological study of tumor tissue samples, breast core biopsies, and surgical material was performed. Immunohistochemical study was carried out on sections of core biopsies. The expression of markers was evaluated, the obtained data were compared with clinical and morphological characteristics, as well as with indicators of tumor sensitivity to neoadjuvant therapy. Statistical analysis was carried out using the Statistica 10.0 program, data comparison was performed using the methods of descriptive statistics and nonparametric method of Pearson's χ^2 test.

Results. The immunoactivated subtype was determined with the status of CK5/6⁺/IDO1⁺ carcinomas, such tumors were dominant and accounted for 81.25% (n=26/32). In these cases (CK5/6⁺/IDO1⁺), the presence of metastatic lesions in the axillary lymph nodes was statistically significantly less common, and the frequency of complete pathomorphological regression was higher than in carcinomas, the molecular profile of which corresponds to the immunosuppressive subtype (CK5/6⁺/IDO1⁻).

Conclusion. Expression of the IDO1 protein may serve as a molecular and biological marker allowing immunohistochemical identification of subtypes of basal-like triple-negative breast cancer.

Keywords: basal-like triple-negative breast cancer, IDO1 expression, prognosis, Residual Cancer Burden (RCB) system.

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Background

Basal-like breast cancer is diagnosed based on the positive expression of one or more basal markers, such as high-molecular-weight cytokeratins (CK5/6, CK14, or CK17) or epidermal growth factor receptor, in the tumor. It accounts for up to 25%–30% of all cases of malignant epithelial neoplasms in this site [1–3].

Basal-like carcinomas are a predominant subtype of triple-negative breast cancer (TNBC), with incidence varying from 47%–50% to 70%–88% [4–8]. In 2018, S. Kim et al. noted that the basal-like phenotype is more common among young patients (aged <50 years) with TNBC. Tumors often contain necrotic foci and have a high grade of malignancy (Grade 3). In addition, the Ki-67 proliferative

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activity index was $\geq 50\%$. Studies have indicated that neoadjuvant therapy, among other options, results in a significantly higher rate of complete tumor response in breast neoplasms [9, 10].

According to the TNBC classification proposed by B.D. Lehmann et al. (2016), basal-like carcinomas are currently divided into two subtypes: immune-activated and immunosuppressive subtypes, also known as basal-like type 1 (BL1) and basal-like type 2 (BL2), respectively [11].

M. Burstein et al. reported that immune-activated TNBC (BL1) is a more favorable variant with a better prognosis even if these tumors have higher Ki-67 index and high frequency of DNA damage and mutations in genes involved in cell cycle regulation, such as *TP53*, *BRCA*, *PTEN*, and *MDM2* [6]. Immunosuppressive TNBC (BL2) has a significantly worse prognosis because of its high proliferative phenotype and low numbers of T, B, and NK cells in the cytokine and immune response pathways. This subtype is also characterized by high indices of growth factor signaling activity and metabolic pathways [6, 12, 13].

S. Kim et al. analyzed literatures on prognostic markers for basal-like TNBC and revealed significant results [9]. In their work, they attempted to identify breast carcinoma subtypes with a molecular-genetic profile corresponding to TNBC ($n = 200$) using a panel of immunohistochemical markers proposed by the authors.

The study identified carcinomas positive for CK5/6 and/or epidermal growth factor receptor as tumors of the basal-like subtype ($n = 85$; 42.5%). Tumors of immune-activated (BL1) or immunosuppressive (BL2) subtype were identified based on the positive/negative expression status of the markers indoleamine 2,3-dioxygenases 1 (IDO1) and forkhead box transcription factor C1 (FOXC1). Accordingly, 27 of 85 IDO1⁺/FOXC1⁻ basal-like TNBCs were categorized as having an immune-activated subtype. In basal-like TNBC of immunosuppressive subtype ($n = 11/85$), IDO1⁻/FOXC1⁺ tumors were identified. IDO1⁻/FOXC1⁺ carcinomas were frequently associated with larger primary tumor node size and significantly lower density of tumor-associated lymphocytes [9].

S. Kim et al. found that carcinomas with IDO1⁺ status are often associated with smaller neoplasms and denser infiltrations of the stroma by tumor-associated lymphocytes. IDO1⁺ expression was more frequently observed in TNBCs of basal-like subtype than in other variants of carcinomas with a TN profile. The combination of IDO1⁺ expression, high stromal infiltration with lymphoid cells, and basal-like phenotype in TNBCs is associated with immunogenic properties of such carcinomas

and reflects the immune response to tumor proteins [14].

IDO1 is an intracellular protein enzyme encoded by *IDO1* and is the most studied among other enzymes in the kynurenine pathway of tryptophan metabolism. Its role in inflammatory response mechanisms in bacterial infections has been detailed, making IDO1 an important component of the immune system. IDO1-expressing cells can exert a dual effect on the microenvironment by implementing tryptophan catabolism, resulting in the formation of toxic kynurenine metabolites. These processes activate regulatory T cells and suppressor cells of myeloid origin while suppressing the function of effector T and NK cells. IDO1 induces tryptophan catabolism, leading to immunosuppression and essentially helping tumor cells evade destruction by the immune system [15–19].

Recent studies have comprehensively examined the significance and role of the IDO1 enzyme in various malignant neoplasms, including esophageal, gastric, pancreatic, colorectal, cervical, ovarian, and renal cancers [19–25]. The study of IDO1 marker expression is relevant, including in breast cancer, because the effect of this enzyme is actively considered a possible and significant point in the immunotherapy of breast carcinomas. However, the criteria for evaluating and determining the aforementioned protein may vary among studies [9, 26–29].

This study aimed to conduct subtyping of basal-like TNBCs through immunohistochemical evaluation of IDO1 protein expression. The obtained data were compared with clinical and morphological characteristics and tumor sensitivity to neoadjuvant therapy.

Materials and methods

The study retrospectively analyzed 42 patients with histologically confirmed invasive breast cancer at disease stages T1–4dN0–3M0 who were treated at the chemotherapy and general oncology departments of the Research Institute of Oncology of the Tomsk National Research Medical Center between 2016 and 2021. The mean patient age was 50.5 ± 12.7 years.

The inclusion criteria require a TN molecular profile of invasive breast carcinoma and the presence of neoadjuvant chemotherapy on a regimen of four courses of AC + 2–3–4 courses of Taxotere.

In the initial stage, tumor tissue samples obtained through core biopsy of breast neoplasms were examined. No treatment was administered at the time of biopsy sampling in any of the patients. According to the World Health Organization classification (2019), the tumor histotype was determined through morphological examination at

the light-optical level using a Zeiss Axio Scope A1 microscope (Germany) [3]. In all cases, the histological variant corresponded to an unspecified invasive ductal carcinoma.

The molecular types of neoplasms were evaluated based on the immunohistochemical expression of estrogen receptor (clone 6F11, RTU, Leica Biosystems, Australia), progesterone receptor (clone 16, RTU, Leica Biosystems), Ki-67 marker (clone MM1, RTU, Leica Biosystems), and Her2/neu tumor status (Bond Oracle HER2 IHC System TA9145 kit) using the Bond-maX immunohisto-stainer (Leica Biosystems). The molecular profile of carcinomas was analyzed based on the criteria for evaluating negative/positive expression of sex hormone receptors presented in the guidelines of the American College of Pathologists and the American Society of Clinical Oncologists (2010).

TNBC was registered as having a negative nuclear expression of estrogen and progesterone receptors in the tumor, with <1% of tumor cells showing positive nuclear expression of different staining intensities in the presence of the correct reaction in positive controls. In addition, the negative Her2/neu status of the tumor was evaluated according to the criteria of the American Society of Clinical Oncologists and the American College of Pathologists (2018). The Ki-67 proliferative activity index was calculated according to the recommendations of the International Working Group for its evaluation in breast cancer [29].

After TNBC diagnosis, all patients received the specified courses of chemotherapy. Subsequently, based on the efficacy control of neoadjuvant chemotherapy, the patients received operative treatment, which involved radical mastectomy or sectoral resection of the breast with axillary lymph node dissection.

The surgical material was used in the morphological examination of the residual tumor in the breast tissue. Therapeutic pathomorphosis was assessed according to the residual cancer burden (RCB) system proposed by W.F. Symmans et al. in 2007 [30]. In addition, the presence of metastases in the axillary lymph nodes was examined, including the total number of examined lymph nodes and nodes with metastases. In this study, core biopsy specimens of breast carcinomas (n = 42) were selected based on their representativeness and tumor tissue volume.

Additional slices from selected paraffin blocks were subjected to a second-step immunohistochemical study using Monoclonal Antibody Cytokeratin 5/6 (RTU, Clone D5/16 B4, Diagnostic BioSystems) and Polyclonal Antibody IDO1 (dilution 1:80, PA5-107329, Invitrogen Thermo Fisher) on a Bond-maX immunohistostainer (Leica Bio-

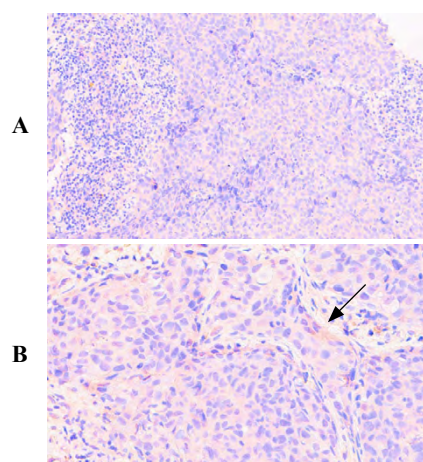


Fig. 1. IDO1 protein expression in the tumor tissue of triple-negative breast cancer (TNBC). A, absence of IDO1 protein expression in the tumor in basal-like TNBC (negative marker expression). Immunohistochemical study, $\times 200$; B, presence of cytoplasmic expression of the IDO1 protein in the tumor in basal-like TNBC (positive expression of the marker). Immunohistochemical study, $\times 400$

systems) under a modified standard protocol F. Bond Polymer Refine Detection was used as the imaging system.

The evaluation of CK5/6 expression in tumor samples followed the criteria proposed by T.O. Nielsen et al. [4]. Positive CK5/6 marker expression was defined as the presence of >1% of positively stained cells in the tumor.

IDO1 marker expression was positive if the proportion of tumor cells with focal positive expression (cytoplasmic expression) or the proportion of stromal mononuclear cells with positive expression (nuclear expression) in the core biopsy sample was >0.5% [31]. IDO1 marker expression was negative if it was not expressed in both the tumor parenchyma and stromal cellular composition, without considering the intensity of expression (Fig. 1).

The study group was evaluated based on clinical and morphologic parameters, including age (up to 49 years/ ≥ 50 years), menstruation status (preserved or menopause), localization (right or left gland), size of the primary tumor (<2, 2–5, or >5 cm), degree of malignancy (grade), Ki-67 index (<50% and $\geq 50\%$), presence of metastatic lesions in axillary lymph nodes, and degree of therapeutic pathomorphosis of the tumor according to the RCB system.

Statistical analysis was performed using the Statistica 10.0 program. Nominal data were described using absolute values and percentages. Data were compared using descriptive statistics and Pearson's nonparametric criterion χ^2 . Statistically significant differences were observed at the $p < 0.05$ level of significance.

Table 1. Clinical parameters of the patients and morphological parameters of the primary tumors in the triple-negative breast cancer group

Clinical parameters of patients and morphological parameters of the primary tumor		Incidence (n = 42)
Age, years	<35	14,3% (n=6)
	35–50	38,1% (n=16)
	>50	47,6% (n=20)
Menstrual status	Menstrual function is preserved	57,1% (n=24)
	Menopause	42,9% (n=18)
Disease stage	I	9,5% (n=4)
	IIA	64,3% (n=27)
	IIB	—
	IIIA	—
	IIIB	2,4% (n=1)
	IIIC	23,8% (n=10)
Localization of the breast tumor	Left breast	57,1% (n=24)
	Right breast	42,9% (n=18)
Primary tumor size, cm	<2 (T1)	4,76% (n=2)
	2–5 (T2)	90,48% (n=38)
	>5 (T3)	4,76% (n=2)
	T4	—
Localization of the primary tumor	Upper outer quadrant	66,7% (n=28)
	Upper inner quadrant	9,5% (n=4)
	Lower outer quadrant	11,9% (n=5)
	Lower inner quadrant	2,4% (n=1)
	Central chest	9,5% (n=4)
Ki-67 index, %	<50	33,33% (n=14)
	>50	66,67% (n=28)
Grade of malignancy	Grade 1	—
	Grade 2	47,62% (n=20)
	Grade 3	52,38% (n=22)

Results and discussion

Table 1 presents the main clinical parameters of the patients and the morphological characteristics of the primary tumor. The majority of the patients in the study group were women aged >50 years, and the left breast was more frequently affected than the right. In addition, more than half of the patients had preserved menstrual function. Tumors were predominantly located in the upper outer quadrant and measured 2–5 cm in size. The degree of malignancy was Grade 3, and no patients had Grade 1 tumors.

The RCB system is currently the only recommended method for assessing the therapeutic pathomorphosis of tumors. This system is presented in the clinical guidelines of the Association of Russian Oncologists, Russian Clinical Society of Oncologists, and World Health Organization clas-

sification of breast cancer (2019) [3]. The severity of the response of breast tumors to neoadjuvant chemotherapy was evaluated in the surgical material according to accepted criteria to determine disease prognosis.

Out of 42 patients, 24 (57.14%) achieved complete pathomorphologic regression (pCR, RCB 0), whereas 2 (4.76%) had minimal residual tumor (RCB I). Moderate residual tumor (RCB II) was detected in 23.81% (n = 10) of the cases, and 14.29% (n = 6) of the patients had a pronounced residual tumor (RCB III) as determined by the RCB system. In the examination of axillary lymph nodes, metastatic lesions were present in 23.81% (n = 10) of the cases.

An immunohistochemical study was conducted on core biopsy tissue samples of breast tumors

using the cytokeratin 5/6 antibody. The study revealed positive expression of this marker in 76.19% of the cases ($n = 32/42$). The detected CK5/6⁺ profile allowed the classification of these TNBC carcinomas as basal-like. The selected CK5/6⁺ tumors ($n = 32$) were further subtyped into basal-like TNBCs (BL1 and BL2) based on the expression characteristics of the IDO1 protein.

Based on the evaluation criteria outlined by Jacquemier et al. in 2012 [31], 81.25% of breast tumor tissue samples ($n = 26/32$) exhibited positive expression of the IDO1 marker. Therefore, carcinomas with a CK5/6⁺/IDO1⁺ immunohistochemical profile ($n = 26$) were classified as having a basal-like immune-activated subtype (BL1).

Moreover, we assessed whether statistically significant differences exist between clinical and morphologic characteristics in groups with neoplasm status corresponding to CK5/6⁺/IDO1⁺ and CK5/6⁺/IDO1⁻. When assessing the age ($\chi^2 = 0.82$; $p = 0.365$), menstrual function status ($\chi^2 = 1.57$; $p = 0.209$), localization ($\chi^2 = 0.33$; $p = 0.568$), and size ($\chi^2 = 0.49$; $p = 0.482$) of the primary tumor, grade of malignancy ($\chi^2 = 1.57$; $p = 0.209$), Ki-67 index ($\chi^2 = 0.054$; $p = 0.815$) in cases of basal-like carcinomas ($n = 32$) in CK5/6⁺/IDO1⁺ ($n = 26$) and CK5/6⁺/IDO1⁻ ($n = 6$), no statistically significant differences were noted among the tumors.

Analysis of basal-like TNBCs with IDO1⁺ and IDO1⁻ expression revealed statistically significant differences in lymphogenic metastasis frequency and RCB evaluation of therapeutic pathomorphosis degree.

In this study, patients with basal-like immune-activated TNBC (BL1, CK5/6⁺/IDO1⁺ profile) had a statistically significantly lower incidence of lymph node metastases ($n = 22/26$; 84.6%) than patients with CK5/6⁺/IDO1⁻ tumor status ($n = 2/6$; 33.3%; $\chi^2 = 6.83$, $p = 0.009$).

The distribution of patients in the CK5/6⁺/IDO1⁺ TNBC group according to the degree of curative pathomorphosis of the tumor (RCB system) ($n = 26$) was obtained. Among the designated cases, 18 (69.24%) showed complete pathomorphological regression of RCB 0, 2 (7.69%) showed minimal residual tumor with RCB I, 4 (15.38%) showed moderate residual tumor with RCB II, and 2 (7.69%) showed severe residual tumor with RCB III.

Among patients with TNBC having a CK5/6⁺/IDO1⁻ immunohistochemical profile ($n = 6$), the RCB evaluation data were as follows: 33.33% ($n = 2$) showed complete tumor regression upon morphological examination of the surgical material, whereas the remaining 66.67% ($n = 4$) had a significant residual tumor of RCB III.

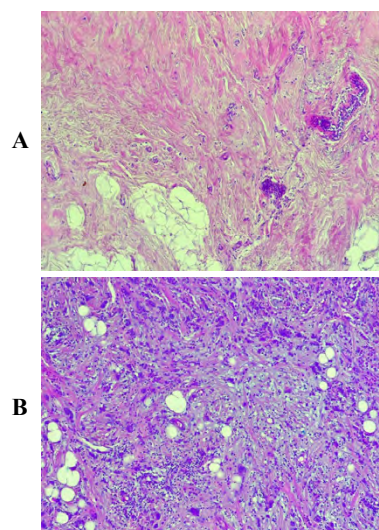


Fig. 2. Treatment pathomorphosis of CK5/6⁺/IDO1⁺ (BL1) and CK5/6⁺/IDO1⁻ (BL2) tumors in the basal-like carcinoma, triple-negative breast cancer (TNBC) group (RCB system). A, complete pathomorphologic tumor regression (pCR) of CK5/6⁺/IDO1⁺ TNBC (BL1). Hematoxylin and eosin staining, $\times 100$; B, exposed residual tumor (RCB III) in the case of CK5/6⁺/IDO1⁻ TNBC (BL2). Hematoxylin and eosin staining, $\times 100$

The comparison of the incidence of complete tumor response to treatment (pCR, RCB 0) in basal-like CK5/6⁺ TNBCs with positive and negative IDO1 expressions showed that the frequency was statistically significantly higher in CK5/6⁺/IDO1⁺ TNBC (presumably immune-activated type) than in CK5/6⁺/IDO1⁻ TNBC (immunosuppressive subtype profile) (69.24% and 33.33%; $\chi^2 = 11.43$, $p = 0.009$).

The microphotographs display the morphological changes in the tumor and breast tissue that reflect the treatment pathomorphosis of CK5/6⁺/IDO1⁺ (BL1) and CK5/6⁺/IDO1⁻ (BL2) tumors in the TNBC basal-like carcinoma group (Fig. 2).

This study demonstrates the possibility of immunohistochemical subtyping of basal-like TNBCs into immunoactivated and immunosuppressive subtypes. The expression profile of the IDO1 protein in breast malignant neoplasm tissue may be a basis for this division of tumors. The study found that 81.25% ($n = 26/32$) of TNBC cases had a presumably immune-activated (BL1) subtype, as determined by the CK5/6⁺/IDO1⁺ status of the carcinomas. These tumors were dominant.

This study compared the results of clinical and morphological characteristics and tumor sensitivity to neoadjuvant therapy in CK5/6⁺/IDO1⁺ and CK5/6⁺/IDO1⁻ TNBC groups. The findings showed that metastatic lesions in axillary lymph nodes were significantly less frequent among patients with basal-like TNBC with a CK5/6⁺/IDO1⁺ profile.

In the analysis of breast tissue surgical material, the frequency of complete pathomorphologic regression (RCB 0) was significantly higher than that in carcinomas. The molecular profile of the carcinomas reflects the immunosuppressive subtype of TNBC. Thus, complete pathomorphologic regression is a favorable characteristic that determines a better prognosis for the oncological process.

The results are consistent with the data presented in similar studies, including the study by M.D. Burstein (2015) [6]. The lower frequency of lymphogenic metastasis found in patients with basal-like CK5/6⁺/ID01⁺ TNBC is an important factor to consider when assessing disease prognosis.

Conclusions

1. ID01 protein expression may be used as a potential marker for the immunohistochemical identification of basal-like TNBC subtypes.

2. In the study group, a positive expression of the CK5/6 marker was detected in 76.19% of TNBCs, which allowed us to consider these tumors as having a basal-like subtype. Further study of isolated cases of basal-like TNBCs revealed positive expression of the ID01 protein in 81.25% of the cases. The profile of CK5/6⁺/ID01⁺ carcinomas in the TNBC group suggests the possibility of categorizing them as immune-activated basal-like subtypes.

3. In CK5/6⁺/ID01⁺ TNBCs, the frequency of cases of complete pathomorphological tumor regression (RCB 0) was significantly higher than that CK5/6⁺/ID01⁻ TNBCs. In addition, the absence of metastases in axillary lymph nodes was significantly higher in CK5/6⁺/ID01⁺ TNBCs.

Authors' contribution. N.V.K., collection and processing of clinical data, morphological study of the material, immunohistochemical study, statistical processing of the material, analysis of the obtained data, and drafting of the manuscript; V.O.T., collection and processing of clinical data and analysis of the obtained data; S.V.V., scientific supervision of the work, analysis of the scientific work, analyzing the obtained data, and critical revision with the introduction of valuable intellectual content.

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