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EXPRESSION OF p53 PROTEIN PRODUCT IN TRIPLE NEGATIVE BREAST CANCERS AND RELATION WITH CLINICAL AND HISTOPATHOLOGICAL PARAMETERS

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Abstract

Introduction: "Triple" negative breast cancer is a subgroup of so-called basal-like breast cancer. They are represented with 15% of all breast cancers, characterized with lack of hormone receptor as well as with negative expression of HER2 test. These tumors are more frequent in Afro-Americans and Latin-Americans, in patients with BRCA1 mutations and in patients with recent delivery. The aim of this study is to present the immunohistochemical and clinico-pathological characteristics of the triple negative breast cancer and their correlation with expression of the protein product of the tumor suppressor gene p53.

Methods: A retrospective analyses of 24 patients with triple negative breast cancer was performed. All of the patients were evaluated in the Histopathological Laboratory of the Clinical Hospital Sistina, during the period from June 2009, until June 2011. The standard immunohistochemical procedures, including the hormone receptor status, HER2 status, proliferative index – Ki67 and p53 gene protein product were performed, as well as additional immunohistochemical staining for so-called basal keratins (Cytokeratin 5/6 and high molecular weight cytokeratin 34BE12).

Results: The age of the patients ranged from 29–77 years. Positive lymph nodes were found in 14 (59%) patients. The tumor was poorly differentiated in 19 patients (79%). Overexpression of the p53 protein product was evaluated in 19 (79%) of the cases. All p53 negative patients (5/5) had poorly differentiated tumors (G3), associated with positive regional lymph nodes. The p53 positive group expressed quite opposite correlation, only 9/19 (47%) were with positive lymph nodes ($p = 0.03$). The expression of p53 protein product was also associated with the nuclear grade ($p = 0.005$), the mitotic index ($p = 0.001$), lymph-vascular invasion ($p = 0.005$) and with the proliferation index Ki67 ($p = 0.003$). There was a trend for association with the tumor size – pT ($p = 0.05$).

Conclusion: According to the results, the triple negative breast cancers are subgroup of the poorly differentiated neoplasms frequently associated in the younger age groups. The majority of these have overexpression of the p53 protein product, which in other hand, are inversely correlated with lymph nodes metastases. Hence, the necessity of enriching the immunohistochemical protocol of these patients with new antibodies, in order to evaluate their expression, which would be helpful for prediction the outcome of different therapeutical modalities.

Key words: breast cancer, triple negative, p53 protein product.

Introduction

Breast cancer is one of the most investtigated neoplasms in human population. Alttough the breast is an organ that is available for all types of conventional or clinical, radiological and laboratory tests, the high percentage

of representation of this type of cancer in the body leads to the conclusion that this disease has an unpredictable course [1–4]. Conventional clinicopathological parameters such as age of the patient, the degree of histological differentiation, nuclear grade, the size of the primary tumor, the status of axillary lymph nodes and in the recent era the immunohistochemistry, determining hormone status, proliferative index, severity or amplification the HER2 gene and p53 gene mutations have been shown as important prognostic parameters regarding the course and outcome of the disease [5–8]. However, these parameters still do not provide sufficient information, particularly with regard to determining the groups of patients who would benefit from adjuvant chemotherapy compared with those who do not need additional treatment [9].

The heterogeneity of this disease at the molecular level can be explained by the existence of numerous genes involved in the cell proliferation, differentiation and apoptosis [10], and genetic changes result in different phenotypic breast cancers [11]. In 2000, Perou, Sorlie and colleagues published the groundbreaking work that probably started the era of molecular testing in the field of breast cancer [12]. Appealing to the phenotypic diversity of breast cancer through divergency of gene expression, and appropriate immunohistochemical profile, manage to form a so-called "Molecular portrait". It comprises four groups of breast cancer on molecular basis that refers to the type of malignant epithelial cells and their immunohistochemical expression concerning the hormone receptor status, the degree of biological aggressiveness determined by expression of proliferative index Ki67 and in relation to the expression of HER2 gene [13].

According to this study, as well as in most subsequent studies [14–25] there are essentially four main molecular subtypes of breast cancer, including: Luminal type A, Luminal type B, Triple negative/basal-like and HER2 positive.

The complex profile of each subtype is determined by the molecular – biological and genetic testing of each tumor cell type [14, 17]. However, some features such as hormone receptor status, HER2 testing, proliferative index and the expression of appropriate antibodies give a clearer picture of the four major categories that are shown in Table 1.

Table 1

Immunohistohemical expression and representation of the four molecular subtypes of breast cancer (adapted from Voduc KD, et al. [15])

Subtype	Immunohistochemical expression	Frequency
Luminal type A	ER+ and/or PR+, HER2-, low % of Ki67	40%
Luminal type B	ER+ and/or PR+, HER2+ (or HER2- with high % of Ki67)	20%
Triple negative	ER-, PR-, HER2-, CK 5/6 + and/or HER2 = 0 or 1+	15–20%
HER2 +	ER-, PR-, HER2 = 3+	10–15%

Legend: ER – Estrogen receptor, PR – Progesterone receptor, HER2 – Human epidermal growth factor receptor 2, CK – cytokeratin

Triple negative/basal-like breast cancer, according to data from Table 1, represents a subtype of breast cancer characterized by negative expression of hormone receptors as well as the HER2 receptor [22]. Frequency of this subtype is less than previous two subgroups (15–20%). These tumors originate from primary or basal cells of the outer layer of the mammary ducts. It is characterized by expression of intermediate filaments of so called basal class cytokeratins (CK5/6) and has frequent p53 mutations [23, 26, 27, 28]. Usually occurs in younger African – American women and nearly all of them possess BRCA1 gene mutation [19–24]. Triple negative breast cancer is aggressive neoplasm and has a poor prognosis

compared to estrogen positive subgroups such as Luminal A and Luminal B types of breast cancer [26, 29]. Genes related to this type of tumor are still insufficiently studied, hence the fact of the absence of so-called "target" gene therapy which include modifications of the expression of Epidermal growth factor receptor – EGFR, aB-crystallin and Cyclin E [24].

According to the aforementioned features of triple negative breast cancer, this study established the following objectives: to determine the prevalence of patients operated in Clinical Hospital Sistina from June 2009 to June 2011 with histopathological diagnosis of triple negative breast cancer and their age distribution; to define immunohistochemical profile of neoplastic cells, to determine the expression of the protein product of the tumor suppressor gene p53 in this subtype of breast cancer and to correlate the p53 expression with certain clinical and histological parameters (age, tumor size, lymph node status, degree of histological differentiation, nuclear grade, mitotic index, lymphvascular invasion, the stage of disease and the proliferative index).

Materials and Methods

The object of analyzes in this study were 24 patients with triple negative breast cancer, according to their immunohistochemical features. They are separated from the group of 220 breast cancers patients diagnosed in the histopathological laboratory at the Clinical Hospital Sistina, from June 2009 to June 2011, to whom a retrospective analysis of the histopathological findings was performed.

All the patients underwent a radical mastectomy with lymphadenectomy and routine processing of the specimen was carried out according to breast cancer macroscopic examination protocol described in the literature [30]. Routine sections were first analyzed with standard staining for haematoxylin and eosin. Postoperative histopathological classification and staging of the disease in all patients was uniformed (refer to patients in 2009 and part of 2010) and adjusted (re-staged) according to the rules of the American Cancer Society (AJCC 2010) [31]. The determination of histological parameters was done based on modified protocols by Nottingham's histological scoring system of Bloom-Richardson [32–36].

Representative samples of the tumor were selected and given to further processing for immunohistochemical analysis. Freshly trimmed sections with thickness up to 2.5 microns were set on Poly-L-lysine pretreated slides and left for 30 minutes drying at a temperature of 60^0C. The slide deparaffinization was performed in xylene and the consequent dehydration in different descending alcohol concentrations (100%, 96%, 80%). Immunohistochemical analysis was performed in all patients in order to determine the hormone receptor and HER2 status as well as the expression of p53 protein product and proliferative index Ki67.

The patients with triple negative breast cancer, have been additionally treated with immunohistochemical analyzes involving basal markers (cytokeratin CK5/6 and high molecular weight cytokeratin, 34βE12).

Heat induced epitope retrieval with citrate buffer in a microwave oven from 700W was used in the pretreatment of the hormone receptors (ER, PR), Ki67, p53 and cytokeratins CK5/6 and 34BE12. For the detection of HER2 receptor epitopes, a water bath at a temperature of 96ºC was used.

The technique of immunohistochemical analysis was performed on a biotin-avidin complex. Immunohistochemical analysis of the estrogen receptor and progesterone, proliferative index Ki67, a tumor suppressor gene p53 protein product, and cytokeratins CK5/6 and 34βE12, a mouse monoclonal antibodies (DAKO, Glostrup, Denmark) were used as well as visualization system (Dako REAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse) with a dilution of 1:100. For determination of HER2 receptor ready Hercep Test kit from the same manufacturer (DAKO, Glostrup, Denmark) was used. To the slides of hormone receptors and HER2 evaluation positive and negative controls were added for accurate evaluation and control of the staining. After the performed steps of immunohistochemical staining, a hematoxylin was used as a counter staining.

Immunohistochemical evaluation of all markers was performed on semi quantitative fashion according to the rules of Rakha [37, 38] and Pathmanathan [39, 40] as follows: intensity of nuclear positivity with the percentage of positive cells for hormone receptors, the prolifera-

tive index Ki67 and the protein product of tumor suppressor gene p53. Evaluation of HER2 and cytokeratins was performed by counting the percentage and severity of cytoplasmic membrane signal [41, 42]. The type of antibody, clone, working dilution, the manufacturer and the cut-off point for a positive or negative signal is represented at Table 2.

Table 2

Type of the antibody, clone, dilution, manufacturer and cut-off point of positive or negative signal

Antibody	Clone	Dilution	Manufacturer	Cut-off point*
Estrogen receptor (ER)	1D5	1:50	DAKO, Glostrup, Denmark	< 5% (negative)
Progesterone receptor (PR)	PgR636	1:50	DAKO, Glostrup, Denmark	< 5% (negative)
Ki67	MIB1	1:50	DAKO, Glostrup, Denmark	> 15% (positive)
p53	DO7	1:50	DAKO, Glostrup, Denmark	> 10% (positive)
HER2	-		DAKO, Glostrup, Denmark	0 и 1+ (negative)
Cytokeratin 34βE12	K903	1:100	DAKO, Glostrup, Denmark	> 10% (positive)
Cytokeratin CK5/6	D5/16B4	1:100	DAKO, Glostrup, Denmark	> 10% (positive)

* According to the proposals of Rakha et al. [37], evaluation of HER2 test was performed upon the criteria of the American Society of Clinical Oncology (ASCO). For other antibodies cut-off points are modified according to the proposals of Pathmanathan et al. [39]. Finding with the HER2 = 2+ was not present in this patient group.

After the primary staining, immunohistochemical analysis showed that 24 (11%) of the patients belong to the category of "triple" negative cancers (estrogen and progesterone negative, HER2 negative). Histologically, all 24 cancers were of ductal type.

The categorization of the analyzed parameters was represented by numbers (percentages). The correlation of each parameter separately in relation to the expression of a tumor suppressor gene p53 protein product is expressed by χ^2-test, and Fisher's exact test. For the statistical significance, the values of $p < 0.05$, were accepted.

Results

The age of the patients ranged from 29–77 years (mean 37.8 years). Only two patients from the group of triple negative breast cancer were older than 50 years (51 and 77 years old). Fourteen (59%) of these 24 patients at the time of diagnosis had a tumor diameter greater than 2 cm and positive lymph node status. Only 19 (79%) had poorly differentiated tumors (G3) and in 17 (71%) patients accordingly, the cells revealed high nuclear pleomorphism (nuclear grade 3), with the finding of numerous pathological mitoses present in 18 patients (75%). The lymph-vascular invasion was present in 17 patients (71%), and according to the histological parameters, 19 patients (79%) are in advanced stages of the disease. In 20 patients (83%), the tumor represented a high level of proliferative activity (Ki67 > 15%). All "triple" negative tumors showed a positive signal for basal cytokeratins CK5/6 and 34βE12.

Expression of tumor suppressor gene p53 protein product was identified in 19 patients (79%). The remaining 5 patients who were p53 negative breast cancers no correlation was found with age, degree of histological differrenttiation (G3) and the stage of the disease. There is a trend of correlation between the expression of the protein product of the tumor suppressor gene p53 and the size of the primary tumor ($p = 0.05$), and there is a reverse correlation with lymph node metastases, whereas in the p53 positive group, in 9 out of 19 patients (47%) lymph node metastases were found ($p = 0.03$).

It was also found that nuclear grade and the number of pathological mitoses are associated with expression of p53 protein product ($p = 0.005$ and $p = 0.001$). There is a positive correlation between the expression of p53 protein product with the appearance of lymph-vascular invasion, observed in 16 out of 19 patients with positive p53 ($p = 0.005$) There was also a correlation with the expression of proliferative index determined by the monoclonal antibody Ki67 ($p = 0.04$).

The analyzed parameters and their ratios are presented in Table 3, and the microscopic appearance of immunohistochemical expression of antibodies is presented in Panel 1.

Table 3

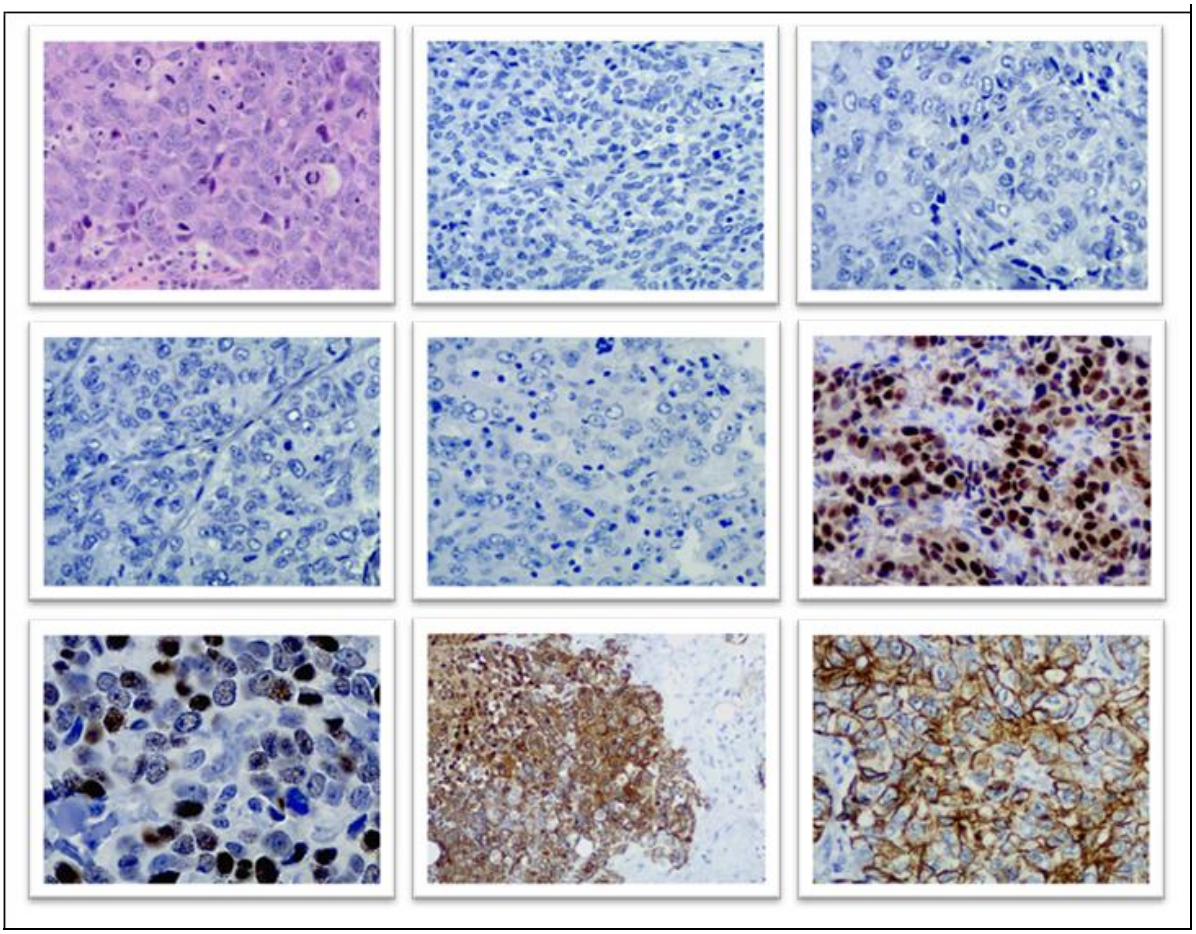
The clinical and histological parameter of these 24 patients with "triple" negative breast cancer in relation to the expression of p53 protein was the product of the tumor suppressor gene

Parameters	No. of patients (%)	p53+	p53-	p-value*
Age				
21–30	1 (5)	1		
31–40	10 (41)	8		
41–50	11 (46)	9		
< 50 yrs.	**22 (92)**	**1**		
> 50 yrs.	**2 (8)**	**8**		0.3
		1		
Tumor size (pT)				
< 2 см (pT1)	**10 (41)**	**6**		
pT2	8 (34)	7		
pT3	6 (25)	6		
pT4	0 (0)	0		
> 2 см	**14 (59)**	**1**		**0.05**
		3		
Lymph node status (pN)				
pN1	3 (13)	1		
pN2	5 (21)	2		
pN3	6 (25)	6		
positive lymph nodes (LN+)	**14 (59)**	**9**		
negative lymph nodes – pN0 (LN-)	**10 (41)**	**1**		**0.03**
		0		
Histological differentiation (G)				
G1	0 (0)	0		
G2	**5 (21)**	**5**		
G3	**19 (79)**	**1**		0.2
		4		
Nuclear grade (NG)				
NG 1	0 (0)	0		
NG 2	**7 (29)**	**3**		
NG 3	**17 (71)**	**1**		**0.005**
		6		
Mitotic index				
< 10 mitoses	**6 (25)**	**2**		
> 10 mitoses	**18 (75)**	**1**		**0.001**
		7		
Lymph-vascular invasion				
Present	**17 (71)**	**1**		
Absent	**7 (29)**	**6**		**0.005**
		3		
Stage of the disease				
I	5 (21)	5		
II	8 (34)	5		
I+II	**13 (55)**	**1**		
III	11 (45)	**0**		
IV	0 (0)	9		
III+IV	**11 (45)**	0		0.8
		9		
Proliferative Index				
Ki67 (> 15%)	**20 (83)**	**1**		
Ki67 (< 15%)	**4 (17)**	**8**		**0.003**
		1		
Total (%)	24 (100)	1		

		9 (79)	(21)	

- p-value refers to the parameters categorized as follows: age group of patients up to 50 years in terms of the group over 50 years, tumor status (primary tumor size) up to 2 cm (pT1) and over 2 cm (pT2, pT3, pT4), patients with present (LN+) and absent lymphonodal metastases (LN-), well- and moderately differentiated tumors (G1/G2) compared to poorly differentiated (G3), low and moderate nuclear grade (NG1/NG2) compared with the high nuclear grade (NG3) and stages I and II compared to the more advanced stages III and IV.

Panel 1



Evaluated immunohistochemical expression of antibodies. A. Routine stained section of breast cancer with haematoxylin and eosin (H&E, x20). B. Immunohistochemical expression of estrogen receptor (ER, x40) with negative nuclear signal. C. Immunohistochemical expression of progesterone receptor (PR, x40), with negative nuclear signal. D. Negative membrane expression of a HER2 staining (HER2, x40). E. Negative nuclear signal in breast cancer with immunohistochemical staining for p53 tumor suppressor gene protein product (p53, x40). F. Immunohistochemical staining for p53 tumor suppressor gene protein product with positive nuclear signal in 80% of malignant cells (p53, x40) G. Immunohistochemical expression of proliferative index determined with monoclonal antibody Ki67 with positive nuclear signal in 35% of malignant cells (Ki67, x60) H. Immunohistochemical expression of intermediate filament cytokeratin 5/6 (CK5/6, x10) with positive membrane staining of malignant cells I. Immunohistochemical expression of intermediate filament cytokeratin 34BE12 (34BE12, x40) with positive membrane staining of malignant cells

Discussion

Breast cancer is heterogeneous in terms of its morphology, flow and clinical response to the treatment. This heterogeneity is probably due to the diversity of the neoplasm cell population arising as the result of different combinations of mutations of normal cells from the mammary gland epithelium [12, 13, 42]. Mole-

cular classification based on the genetic profile of malignant cells somewhat gives a clear picture regarding the clinical, biological and therapeutic implications. Triple negative breast cancers are distinguished because their sign is similar to the myoepithelial "stem" cells [43, 44]. Therefore, the inclusion of positive markers such as basal cytokeratins CK5/6, high molecular weight cytokeratin 34BE12, and in some studies the Epidermal growth factor receptor-EGFR, allowing accurate diagnosis of basal phenotype correlated with the gene profile [44, 45].

In the study of Carey et al. [14] out of 657 analyzed cases of breast cancer, 26% were triple negative, and the great series of Rakha and colleagues [38] from 1944 patients triple negative were 16.3%. In our series of 220 patients, triple negative are 11% and this number is smaller than the published series probably due to the relatively small number of cases analyzed and evaluated immunohistochemical parameters in comparison with the above series range, which is used by a larger number of antibodies (cytokeratin CK14, C-kit, Epidermal growth factor – EGFR, Androgen receptor – AR etc.).

Concerning the age of the patients, as in our study and the aforementioned studies [14, 23, 27, 38, 43, 45, 46], suggest that triple negative breast cancers are more common in younger women and are generally poorly differentiated [14, 35, 36, 38]. In all studies analyzed the common denominator of triple negative breast cancer is low grade histological differentiation as shown in our study (79%). Also, in some of these studies [45, 47, 48, 49] and in our study there was not any correlation between the age of the patients, the degree of histological differentiation and the stage of disease expression with the expression of p53 protein product.

Umemura and coworkers [50] reported that 19% of breast cancers are hormone nonresponsive and HER2 negative and they show strong expression of the p53 protein product, as it is in our study. According to him, as well as by other authors [4, 44, 51] high expression of p53 in triple negative breast cancers are associated with marked expression of proliferative index Ki67 and Cyclin D1, especially compared with other subgroups of breast cancer. In our study there was a correlation between the expression of p53 protein product and proliferative index Ki67.

Concerning the size of the primary tumor, only 59% of primary breast cancers in our series are over 2 cm in their greatest diameter. Similar results in triple negative breast cancer were published by Foulkes [52], Lerma [53] and Kreike [54] with its associates, and Rakha [38] and Salman [46] emphasizing that the size of the tumor is due to the rapid growth and expressed proliferation of malignant cells. Therefore this is the significant correlation of nuclear grade and mitotic index that were published by those authors [38, 42, 46] in terms of p53 expression that was also observed in our study.

In studies of Tischkowitz [45], Kreike [54] and Bhargava et al. [55], the triple negative breast tumors with positive expression of tumor suppressor gene p53 protein product revealed a tendency toward the occurrence of lymph nodal metastases as in our study. This inverse correlation is due to their relation to mutation of the BRCA1 gene expressed in basal breast cancers published by Oldenburg et al [56]. On the other hand, by some authors the presence of lymphvascular invasion and emergence of lymph nodal metastases compared with other molecular groups, is somewhat more pronounced and is positively correlated with the expression of p53 protein product [14, 15, 29, 49]. The variations in the results are probably due to the characteristics of the analyzed population and percentage of medullary and adenoid cystic carcinomas that fall into this group of breast cancer, which as a rule, have indolent course of the disease [46]. In our series all 24 patients had ductal type of breast cancer.

Conclusion

"Triple" negative breast cancers represent a distinct subtype of neoplasms that are clinically aggressive tumors and are associated with poor prognosis. The identification of this immunophenotype is important because except the negative expression of hormone receptors and HER2 test, additional role in the diagnostic profiling of this subtype belongs to so called "Basal markers" as well as the clinical parameters such as patients' age and familiar history. Other histopathological and immunohistochemical parameters highlight the importance in determining the limited treatment modalities

in this group of patients. In this regard, the introduction of some immunohistochemical markers in the evaluation panel of this subtype of breast cancer is important not only in terms of diagnostic confirmation of the change but also in terms of design and identification of a new chemotherapeutic agent that will have main role in individual treatment of this group of patients.

REFERENCES

1. Tavassoli FA. Pathology of the Breast, 2nd ed. Stamford, Connecticut; Appleton & Lange; 1999.
2. Moinfar F. ed. Essentials of Diagnostic Breast Pathology, Berlin: Heidelberg: Springer; 2007.
3 Soerjomataram I, Louwman MW, Ribot JG, Roukema JA, Coebergh JW. An overview of prognostic factors for long-term survivors of breast cancer. Breast Cancer Res Treat. 2008; 107: 309–330.
4. Borresen-Dale AL. TP53 and breast cancer. Hum Mutat. 2003; 21: 292–300.
5. Lacroix M, Toillon RA, Leclercq G. p53 and breast cancer, an update. Endocr Relat Cancer. 2006; 13(2): 293–325.
6. Park D, Karesen R, Noren T, Sauer T. Ki-67 expression in primary breast carcinomas and their axillary lymph node metastases: clinical implications. Virchows Arch. 2007; 451: 11–18.
7. De Azambuja E, Cardoso F, De Castro G, Colozzo M, Mano MS, Durbecq V, Sotiriou C, Larsimont D, Piccart-Bebhart MJ, Paesmans M. Ki67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12.155 patients. Br J Cancer. 2007; 96: 1504–1513.
8. Eden P, Ritz C, Rose C, Ferno M, Peterson C."Good Old" clinical markers have similar power in breast cancer prognosis as microarray gene expression profilers. Eur J Cancer. 2004; 40: 1837–1841.
9. Riley RD, Abrams KR, Sutton AJ, Lambert PC, Jones DR, Heney D, Burchill SA. Reporting of prognostic markers: current problems and development of guidelines for evidence-based practice in the future. Br J Cancer. 2003; 88: 1191–1198.
10. Pease AC, Solas D, Sullivan EJ, Cronin MT, C. Holmes CP, Fodor SP. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. Proc Natl Acad Sci USA. 1994; 91: 5022–5026.
11. Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science. 1995; 270: 467–470.
12. Perou CM, Sorlie T, Eisen MB, van de Rijin M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikv A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D, Molecular portraits of human breast tumours. Nature. 2000; 406: 747–752.
13. Sorlie T, Perou CM, Tibshiranin R, Aas T, Geisler S, Johnsen H, Hasti T, Eisen MB, van de Rijn M, Jeffrey S, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lonning PE, Borresen- Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001; 98: 10869–10874.
14. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J,. Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC. Race, breast cancer subtypes, and survival in the Carolina Breast cancer Study. JAMA. 2006; 295(21): 2492–2502.
15. Voduc KD, Cheang MC, Tyldesley S, Gelmon K, Nielsen TO, Kennecke H. Breast cancer subtypes and the risk of local and regional relapse. J Clin Oncol. 2010; 28(10): 1684–1691.
16. Carey LA. Molecular intrinsic subtypes of breast cancer. In: UpToDate. Hayes DF, Dizon DS, eds. Waltham, MA: UpToDate; 2012. p. 77–84.
17. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012; 490(7418): 61–70.
18. Dawood S, Hu R, Homes MD, Collins LC, Schnitt SJ, Connolly J, Colditz GA, Tamimi RM. Defining breast cancer prognosis based on molecular phenotypes: results from a large cohort study. Breast Cancer Res Treat. 2011; 126(1): 185–192.
19. Arvold ND, Taghian AG, Niemierko A, Abi Raad RF, Sreedhara M, Nguyen PL, Bellon JR, Wong JS, Smith BL, Harris JR. Age, breast cancer subtype approximation, and local recurrence after breast-conserving therapy. J Clin Oncol. 2011; 29(29): 3885–3891.
20. Lund MJ, Butler EN, Hair BY, Ward KC, Andrews JH, Oprea Ilies G, Bayakly AR, O'Regan RM, Vertino PM, Eley JW. Age/race differences in HER2 testing and in incidence rates for breast cancer triple subtypes: a population-based study and first report. Cancer. 2010; 116(11): 2549–2559.
21. Turner NC, Reis-Filho JS. Basal-like breast cancer and the BRCA1 phenotype. Oncogene. 2006; 25(43): 5846–5853.
22. Millikan RC, Newman B, Tse CK, Moorman PG, Conway K,. Smith LV, Labbok MH, Geradts J, Bensen JT, Jackson S, Nyante S, Livasy C, Earp HS, Perou CM. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat. 2008; 109(1):123-139.
23. Stead LA, Lash TL, Sobieraj JE, Chi DD, Westrup JL, Charlot M, Banchard RA, Lee JC, King TC, Rosenberg CL. Triple-negative breast cancers are increased in black women regardless of age or body mass index. Breast Cancer Res. 2009; 11(2): R18.
24. Amirikia KC, Mills P, Bush J, Newman LA. Higher population-based incidence rates of triple-negative breast cancer among young African-American women: Implications for breast cancer screening recommendations. Cancer. 2011; 117(12): 2747–2753.

25. Atchley DP, Albarracin CT, Lopez A, Valero V, Amos CI, Gonzalez-Angulo AM, Hortobagyi GN, Arun BK. Clinical and pathologic characteristics of patients with BRCA-positive and BRCA-negative breast cancer. J Clin Oncol. 2008; 26(26): 4282–4288.
26. Millar EK, Graham PH, O'Toole SA, McNeil CM, Browne L, Morey AL, Eggleton S, Beretoy J, Theocharus C, Capp A, Nasser E, Kearsley JH, Delaney G, Papadatos G, Fox C, Sutherland RL. Prediction of local recurrence, distant metastases, and death after breast-conserving therapy in early-stage invasive breast cancer using a five-biomarker panel. J Clin Oncol. 2009; 27(28): 4701–4708.
27. Kim MJ, Ro JY, Ahn SH, Kim HH, Kim SB, Gong G. Clinicopathologic significance of the basal-like subtype of breast cancer: a comparison with hormone receptor and Her2/neu-overexpressing phenotypes. Hum Pathol. 2006; 37(9): 1217–1226.
28. Yang XR, Chang-Claude J, Goode EL, Couch FJ, Nevanlinna H, Milne RL, et al. Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies. J Natl Cancer Inst. 2011; 103(3): 250–263.
29. Tamimi RM, Colditz GA, Hazra A, Baer HJ, Hankinson SE, Rosner B, Marotti J, Connolly JL, Schnitt SJ, Collins LC. Traditional breast cancer risk factors in relation to molecular subtypes of breast cancer. Breast Cancer Res Treat. 2012; 131(1): 159–167.
30. Rosai J. Rosai and Ackerman's Surgical Pathology, 9th ed. London; Mosby; 2004.
31. American Joint Committee on Cancer – AJCC Cancer Staging Manual, 7th ed. New York: Springer-Verlag; 2010.
32. Bloom HJ, Richardson WW. Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. Br J Cancer. 1957; 11 (3): 359–377.
33. Genestie C, Zafrani B, Asselain B, Fourquet A, Rozan S, Validire P, Vincent-Salomon A, Sastre-Garau, X. Comparison of the prognostic value of Scarff-Bloom-Richardson and Nottingham histological grades in a series of 825 cases of breast cancer: Major importance of the mitotic count as a component of both grading systems. Anticancer Res. 1998;18(1B): 571–576.
34. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histologic grade in breast cancer: Experience from a large study with long-term follow-up. Histopathology. 1991; 19: 403–410.
35. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham Prognostic Index in primary breast cancer. Breast Cancer Res Treat. 1992; 22(3): 207–219.
36. Albergaria A, Ricardo S, Milanezi F, Carneiro V, Amendoeira I, Vieira D, Cameselle-Teijeiro J, Schmitt F. Nottingham prognostic index in triple negative breast cancer: a reliable prognostic tool? BMC Cancer. 2011; 11: 299–308.
37. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, Fox BS, Ichihara S, Jacquemier J, Lakhani SR, Palacios J, Richardson AL, Schnitt Sj, Scmitt FC, Tan PH, Tse GM, Badve S, Ellis IO. Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res. 2010; 12: 207–219.
38. Rakha EA, El-Sayed ME, Green AR, Lee AHS, Robertson JF, Ellis IO. Prognostic markers in triple negative breast cancer. Cancer. 2007; 109: 25–32.
39. Pathmanathan N, Balleine RL. Ki67 and proliferation in breast cancer. J Clin Pathol. 2013; 66(6): 512–6.
40. Pathmanathan N, Bilous AM. HER2 testing in breast cancer: an overview of current techniques and recent developments. Pathology. 2012; 44(7): 587–95.
41. Mehta R, Jain RK, Sneige N, Badve S, Resetkova E. Expression of high-molecular-weight cytokeratin (34bE12) is an independent predictor of disease-free survival in patients with triple-negative tumors of the breast. J Clin Pathol. 2010; 63: 744–747.
42. Gusterson B, Ross D, Heath V, Stein T. Basal cytokeratins and their relationship to the cellular origin and functional classification of breast cancer. Breast Cancer Res. 2005; 7: 143–148.
43 Rakha EA, Reis-Filho JS, Ellis IO.Basal-like breast cancer: a critical review. J Clin Oncol. 2007; 26: 2568–2581.
44. Cheang M, Voduc D, Bajdik C, Leung S, Mckinney S, Chia SK. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res. 2008;14: 1368–1376.
45. Tischkowitz M, Brunet JS, Bégin LR, Huntsman DG, Cheang MC, Akslen LA, Nielsen TO, Foulkes WD. Use of immunohistochemical markers can refine prognosis in triple negative breast cancer. BMC Cancer. 7.1 2007: 134.
46. Salman MI, Elhefnawy NG, Shash LS. Morphological and immunohistochemical characteristics of triple negative and basal breast carcinoma. Egypt J Path. 2012; 32: 6–13.
47. Reed W, Hannisdal E, Boehler PJ, Gundersen S, Host H, Marthin J. The prognostic value of p53 and c-erb B-2 immunostaining is overrated for patients with lymph node negative breast carcinoma: a multivariate analysis of prognostic factors in 613 patients with a follow-up of 14–30 years. Cancer. 2000; 88: 804–813.
48. Carter CL, Allen C, Henderson DE. Relation of tumour size, lymph node status and survival in 24,740 breast cancer cases. Cancer. 1989; 63: 181–187.
49. Korkolis DP, Tsoli E, Fouskakis D, Yiotis G, Koullias J, Giannopoulos D, Papalambros E, Nikiteas NI, Spiliopoulou CA, Patsouris E, Asimacopoulos P, Gorgoulis VG. Tumor histology and stage but not p53, Her2-neu or cathepsin-D expression are inde-

pendent prognostic factors in breast cancer patients. Anticancer Res. 2004; 24: 2061–2068.
50. Umemura S, Takekoshi S, Suzuki Y, Saitoh Y, Tokuda Y, Osamura RY. Estrogen receptor-negative and human epidermal growth factor receptor 2-negative breast cancer tissue have the highest Ki67 labeling index and EGFR expression: gene amplification does not contribute to EGFR expression. Oncol Rep. 2005; 14: 337–343.
51. Nakagawa M, Bando Y, Nagao T, Morimoto M, Takai C, Ohnishi T, Honda J, Moriya T, Izumi K, Takahashi M, Sasa M, Tangoku A. Expression of p53, Ki67, E-cadherin, N-cadherin and Topoisomerase 2A (TOP-2A) in triple negative breast cancer. Anticancer Res. 2011; 31: 2389–2393.
52. Foulkes WD, Brunet JS, Stefansson IM, Straume O, Chappuis PO, Begin LR, Hammel N, Goffin JR, Wong N, Trudel M, Kapusta L Porter P, Akslen LA. Advances in brief the prognostic implication of the basal-like phenotype of BRCA1-related breast cancer (cyclin/high/p27low/p53/glomeruloid-microvascular-proliferation). Cancer Res. 2004; 64: 830–835.
53. Lerma E, Peiro G, Ramon T, Fernandez S, Martinez D, Pons C, Munoz F, Sabate JM, Alonso C, Ojeda B, Prat J, Barnadas A. Immunohistochemical heterogeneity of breast carcinomas negative for estrogen receptors, progesterone receptors and Her2/neu (basal-like breast carcinomas). Mod Pathol. 2007; 20: 1200–1207.
54. Kreike B, van Kouwenhove M, Horlings H,Weigelt B, Peterse H, Bartelink H, van der Vijver M. Gene expression profiling and histopathological characterization of triple- negative/basal-like breast carcinomas. Breast Cancer Res. 2007; 9: R65.
55. Bhargava R, Striebel J, Beriwal S, Flickinger JC, Onisko A, Ahrendt G, Dabbs DJ. Prevalence, morphologic features and proliferation indices of breast carcinoma molecular classes using immunohistochemical surrogate markers. Int J Clin Exp Pathol. 2009; 2: 444–455.
56. Oldenburg RA, Kroeze-Jansema K, Meijers-Heijboer H, van Asperen CJ, Hoogerbrugge N, van Leeuwen I, Vasen HF, Cleton-Jansen AM, Kraan J, Houwing-Duistermaat JJ, Morreau H, Cornelisse CJ, Devilee P. Characterization of familial non-BRCA1/2 breast tumours by loss of heterozygosity and immunophenotyping. Clin Cancer Res. 2006; 12: 1693–1700.

Резиме

ЕКСПРЕСИЈА НА p53 ПРОТЕИНСКИОТ ПРОДУКТ КАЈ ТРОЈНО НЕГАТИВНИТЕ КАРЦИНОМИ НА ДОЈКА ВО СООДНОС СО КЛИНИЧКИТЕ И ХИСТОПАТОЛОШКИТЕ ПАРАМЕТРИ

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Вовед: „Тројно“ негативните карциноми на дојка претставуваат подгрупа на т.н. basal-type карциноми на дојка. Опфаќаат околу 15% од сите карциноми на дојка и се карактеризираат со отсуство на експресија на хормонските рецептори и на HER2/neu рецепторот. Почести се кај Афроамериканците и кај Латиноамериканците, кај оние со мутации на BRCA1 генот и кај скоро породени. Целта на оваа студија е да ги прикаже имунохистохемиските карактеристики на „тројно“ негативните карциноми на дојка во корелација со протеинскиот продукт на тумор супресорскиот ген p53 и одредени клинички и хистолошки параметри.

Методи: Направена е ретроспективна анализа на 24 случаи на „тројно“ негативни карциноми на дојка обработени во нашата лабораторија во периодот од јуни 2009 до јуни 2011 година. Покрај стандардните имунохистохемиски испитувања за хормонските рецептори (естроген и прогестерон рецептор), HER2 генот, пролиферативниот индекс Ki67 и протеинскиот продукт на тумор супресорскиот ген p53, направени се и дополнителни испитувања за т.н базални маркери (цитокератини CK5/6 и 34BE12).

Резултати: Пациентите беа на возраст од 29 до 77 години и кај 14 (59%) од нив беа најдени лимфонодални метастази. Туморот беше лошо диференциран кај 19 пациенти (79%). Прекумерна експресија на протеинскиот продукт на тумор супресорскиот ген p53 беше идентификирана кај 19 (79%) случаи. Сите p53 негативни пациенти (5/5) имаа лошо диференцирани тумори (G3), асоцирани со лимфонодални метастази. Кај p53 позитивната група само 9/19 (47%) беше асоцирана со присуство на регионални метастатски депозити ($p = 0,03$). Освен со лимфонодалните метастази, експресијата на p53 е асоцирана со нуклеарниот градус ($p = 0,005$), митотскиот индекс ($p = 0,001$), инвазијата во лимфоваскуларните простори ($p = 0,005$) и со пролиферативниот индекс Ki67 ($p = 0,003$), а постои тренд на асоцијација со големината на примарниот тумор ($p = 0,05$)

Заклучок: Врз основа на резултатите, може да се заклучи дека „тројно негативните“ карциноми на дојка претставуваат поттип на мамарни карциноми кои се полошо диференцирани и се јавуваат кај помладата возрасна група. Најголем дел од нив пројавуваат прекумерна експресија на тумор супресорскиот ген p53, која е со инверзна асоцијација во однос на појавата на лимфонодалните метастази. Согласно со овие параметри, се наметнува потребата од проширување на имунохистохемиски протокол што би се применил кај овие пациенти со цел да се евалуира и предвиди одговорот на различните терапевски модалитети.

Клучни зборови: карцином на дојка, тројно негативен тумор супресорски ген p53.