

IMMUNOHISTOCHEMICAL STUDY OF FEMALE BREAST CANCER IN VARNA, BULGARIA

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Abstract

An immunohistochemical study of 128 female breast cancer patients in Varna is performed. The expression of estrogen and progesterone receptors, human epidermal growth factor receptor 2 (HER2) and proliferation marker protein Ki-67 in terms of histological and molecular types and differentiation grade is assessed. Ductal carcinoma and luminal B HER2(+) prevail. The discrepancies between immunohistochemical positivity and negativity of single biomarkers are outlined.

Key words: female breast cancer, immunohistochemistry, molecular types

Introduction. Female breast cancer (BC) remains the most common cancer worldwide and in Bulgaria. BC molecular hallmarks include the immunohistochemical markers estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2), proliferation marker protein Ki-67, the genomic markers BRCA1, BRCA2 and PIK3CA, as well as the immunomarkers tumour-infiltrating lymphocytes and programmed death-ligand-1 [1].

The concept of intralaboratory and external quality control in ER immunohistochemical detection is considered and the problems of harmonization in immunohistochemical analysis of BC with these nuclear biomarkers are discussed [2].

Computational platform assessments of 17 immunohistochemical markers (ER, PR, Ki-67 and others) measured in BC tissue microarrays reveal variable

correlations with interpretations by expert pathologists and should be used in large-scale epidemiologic studies [3]. By means of a multiplexed microfluidic immunohistochemistry technology, distinctive expression patterns of the biomarkers ER, PR, HER2 and Ki-67 are identified on cell microarray consisting of six different BC cell lines and these bar-like signals are a biomarker barcode for the tissue microarray core [4].

In a comprehensive review of the available classification strategies for the triple-negative breast cancer (TNBC), the overlap between the molecular, immunohistochemical and clinical characteristics between these approaches is evaluated and the perspective about the increasing applications of artificial intelligence to identify definitive and clinically relevant TNBC subtypes is discussed [5].

The purpose of this study was to share our experience with female BC immunohistochemistry.

Materials and methods. Our investigation covered a total of 128 randomly selected female BC patients at a mean age of 59.48 ± 11.99 years (range, 30–84 years) operated on in Marko Markov Specialized Hospital for Active Treatment of Oncological Diseases in Varna, Bulgaria. It was carried out between December 1, 2017 and November 30, 2020.

The expression of ER and PR was examined in mammary gland biopsies and surgical specimens by using the indirect immunoperoxidase method with En-Vision™ FLEX MiniKit, (HighpH, DAKO Denmark A/S), that of HER2 with HercepTest™ (DAKO Denmark A/S) and that of Ki-67 with Leica Aperio Scan Scope AT2 device (AperioTechnologies, Vista, CA, USA).

We analyzed BC histology, molecular types, differentiation grades and frequency of positive and negative immunohistochemical markers.

Results. Patients' distribution according to age groups shows that a half of them are aged between 51 and 70 years (Fig. 1).

Ductal invasive carcinoma is the most common histological type (in 41 patients or in 32.03%) followed by ductal carcinoma (in 38 patients or in 29.69%),

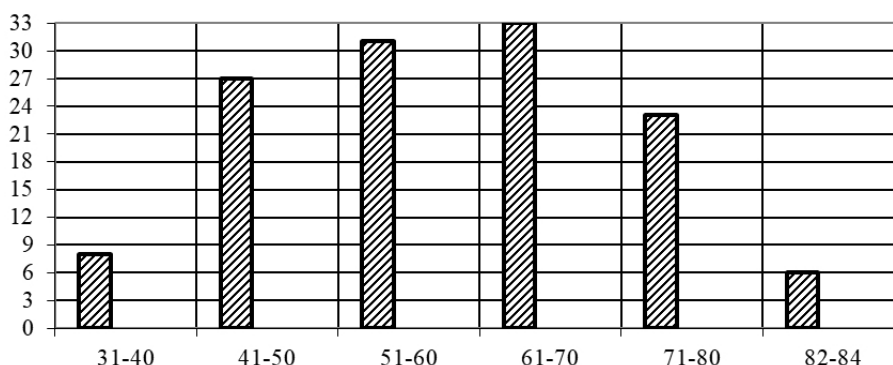


Fig. 1. Patients' distribution according to age groups

non-specific invasive carcinoma (in 26 patients or in 20.31% of the cases), etc.

Forty-seven patients are with luminal B HER2(+), 45 – with luminal B HER2(–), 15 – with basocellular (TNBC), 11 – with luminal A, and 10 – with non-luminal HER2(+) BC molecular type. Patients' distribution according to BC molecular type and differentiation grade (G) is shown in Table 1.

T a b l e 1

Patients' distribution according to BC molecular type and differentiation grade

Molecular type	G1	G2	G3	G4	total
luminal B HER2(+)	0	33	13	1	47
luminal B HER2(–)	3	33	9	0	45
basocellular (TNBC)	0	7	7	1	15
luminal A	0	9	2	0	11
non-luminal HER2(+)	0	6	4	0	10
total n	3	88	35	2	128
total %	2.35	68.75	27.34	1.56	100.00

There are 31 patients with positive values of ER, PR and HER2. Among them, Ki-67 values are $> 14\%$ in eight cases and $< 14\%$ in one case. Among TNBC patients, there are seven cases with Ki-67 values $> 14\%$.

Five combinations of positive and negative receptors in one and the same patient according to differentiation grade among 60 patients are displayed in Table 2. There are 19 patients with Ki-67 values $> 14\%$ and 10 patients with Ki-67 values $< 14\%$.

T a b l e 2

Combinations of positive and negative receptors according to differentiation grade

Receptor values	G1	G2	G3	G4	total
ER(+) PR(+) HER2(–)	1	23	1	0	25
ER(+) PR(–) HER2(+)	0	11	5	2	18
ER(–) PR(–) HER2(+)	0	6	5	0	11
ER(+) PR(–) HER2(–)	1	1	3	0	5
ER(–) PR(+) HER2(–)	0	1	0	0	1
total n	2	42	14	2	60
total %	3.33	70.00	23.34	3.33	100.00

Patients' distribution according to favourable (≥ 5) and unfavourable (< 5) hormone receptor expression values among 113 BC patients is presented in Table 3. The number of ER with values ≥ 5 is 5.65 times greater than that of ER with values < 5 while the number of PR with values ≥ 5 is only 1.63 times greater than

that of PR with values < 5 . The number of the HER2 with values of 2 and 3 is almost equal to that of the HER2 with values of 0 and 1.

T a b l e 3

Distribution of favourable and unfavourable receptor expression values

Receptor values	ER		PR		HER2	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
favourable	96	84.96	70	61.95	56	49.56
unfavourable	17	15.04	43	38.05	57	50.44

Discussion. Our results are similar to those of several recent publications by foreign authors. The discrepancy of receptor expression values in some patients necessitates additional examinations, e.g. of cisplatin, for the proper chemotherapy regime selection.

The retrospective study of 708 BC patients diagnosed in Saudi Arabia shows a significant difference in tumour grade ($p < 0.0001$) between younger patients aged ≤ 40 years and older ones [6]. Most younger patients have TNBC ($p = 0.008$). They are less likely to be with luminal type A ($p = 0.002$) and to be ER(+) ($p = 0.0001$) and PR(+) ($p < 0.0001$) than older patients.

The most common molecular subtypes among 222 BC patients examined by immunohistochemistry of core needle biopsies and/or surgical specimens are luminal A (in 43.2%) and luminal B HER2(−) (in 29.7% of the cases) [7]. There is a good statistical agreement between core needle biopsies and surgical specimens (concordance rate = 85.29%, kappa = 0.771 and $p < 0.001$).

The results from a retrospective cross-sectional study of 379 BC patients at a mean age of 54.63 years (range, 23–89 years) in Mexico indicate luminal B subtype in 143 (in 37.73%), luminal A subtype in 139 (in 36.67%), TNBC in 65 (in 17.15%) and HER2(+) in 32 patients (in 8.44% of the cases) [8]. The retrospective analysis of paraffin-embedded BC tissue microarray slides from 164 BC patients at a mean age of 51.35 years displays a significant association between substance P expression level and BC molecular subtype ($p = 0.002$), ER and PR statuses ($p < 0.001$) [9]. The average percentage of Ki-67 expression is 27.05%. There are statistically significant differences between the mean Ki-67 scores and molecular subtype ($p = 0.001$), differentiation grade ($p = 0.003$), and ER and PR statuses ($p < 0.001$).

There is positive expression rate of ER, PR and HER2 as well as high expression of Ki-67 in 73.36%, 59.85%, 24.09% and 66.06% out of a total of 274 immunohistochemically examined BC patients, respectively [10]. Ki-67 high expression is a risk factors for overall and disease-free survival while PR positivity is a protective factor for overall survival.

Among 417 ductal BC patients at a mean age of 33.5 ± 6.4 years in Southeastern Nigeria, both ER and PR are immunohistochemically positive in 217 patients

(in 61.63% of the cases) [11]. Positive ER are established in 70.3% of the patients in the age groups of 41–50, 20–30 and > 70 years and in 51.4% in the age group of 31–40 years.

In a total of 129 BC patients at a mean age of 47.41 years with core needle biopsy and mastectomy specimens, differentiation grade 2 is established in half of the cases [12]. ER, PR and Her2neu are positive in 65, 61 and 59 cases, respectively. There is a significant correlation of ER expression ($p = 0.035$) and Her2neu overexpression ($p = 0.035$) with Ki-67.

The discordance in ER, PR, HER2 and Ki-67 expression between primary and recurrent/metastatic lesions in 75 primary early-stage BC patients in Beijing, China, is 9.3%, 14.7%, 14% and 21.5%, respectively [13]. Some 66.7%, 11.8%, 14.3% and 0% of the patients with luminal A, luminal B, HER2, and TNBC present with a different subtype for the recurrent/metastatic BCs, respectively.

Immunohistochemically, low HER2 expression levels in advanced TNBC patients do not influence progression-free survival as overall survival is poorer in patients with HER2(+) than in those with HER2(–) expression [14].

Among 919 early HER2(+) BC patients, there are 442 hormone receptor(+)/HER2(+) and 477 hormone receptor(–)/HER2(+) cases [15]. There is a potential correlation between ER/PR levels and disease-free survival in hormone receptor(+)/HER2(+) BCs ($p = 0.074$). A higher ER/PR level is associated with better disease-free survival in early HER2(+) BC.

Among 2660 consecutive female BC patients at a mean age of 59.6 ± 13.3 years (range 21–99 years), there are 2208 PR(+) and 452 PR(–) cases [16]. Both ductal subtype ($p = 0.002$) and differentiation grade 3 ($p < 0.001$) are associated with PR negativity. This negative expression independently predicts a worse disease-free ($p = 0.001$) and overall survival ($p < 0.001$) as well as a worse overall survival in ER(+)/HER2(–) disease ($p = 0.004$).

Mitochondrial calcium uniporter regulator 1 expression in BC patients is immunohistochemically examined [17]. It is the highest in TNBC. The overexpression is significantly related to the clinical characteristics of the disease as well as to poor overall survival, distant metastasis-free survival, and recurrence-free survival.

There is cluster of differentiation 155 expression in 25 out of 61 TNBC patients (in 40.98% of the cases) [18]. The immunohistochemistry does not show any correlation between this expression and differentiation grade, Ki-67 labelling index and pathological stage.

The immunohistochemical analysis of TNBC patients reveals that Ki-67 labelling index as a biomarker of aggressive metastatic disease is significantly expressed in most cases [19]. This expression significantly correlates with high tumour nuclear grade, clinical stage and failure to achieve complete pathological response.

A total of 203 406 BC patients from the SEER programme in 2010–2016 are divided into ER(+), PR(–), ER(–), PR(+), ER(+), PR(+), and ER(–), PR(–)

molecular subtypes stratified by HER2 status [20]. For HER2(−) subtype, 16 906 pairs of patients with ER(+)PR(−) and 1395 pairs of patients with ER(−)PR(+) have a worse prognosis than those with ER(+)PR(+). Some 1394 pairs of patients with ER(+)PR(−) show a better prognosis than 9626 pairs of patients with ER(−)PR(+).

Conclusion. Based on our own results and convincing literature data available we could conclude that modern immunohistochemistry plays a decisive role in BC patients.

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