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PROGNOSTIC SIGNIFICANCE OF ESTROGEN RECEPTOR, PROGESTERONE RECEPTOR AND HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2 IN PATIENTS WITH BREAST CANCER

PROGNOSTIČKI ZNAČAJ ESTROGENIH, PROGESTERONSKIH I RECEPTORA FAKTORA RASTA 2 KOD BOLESNICA SA KARCINOMOM DOJKE

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Summary
Introduction. The importance of understanding the biology of breast cancer is increasing and the determination of certain phenotypic characteristics of malignant cells, especially estrogen, progesterone and human epidermal growth factor receptor 2 expression, became a standard evaluation procedure in breast cancer patients, in order to provide prognostic information and best therapeutic options. **Material and methods.** This study included a total of 206 patients, all treated and followed up in the *Daily Chemotherapy Hospital* of the *Health Center Vranje*. Estrogen, progesterone and human epidermal growth factor receptor 2 statuses were evaluated in all patients to assess their potential impact on the progression-free and overall survival. **Results.** Two-thirds of patients were diagnosed at the early stage of the disease. Ductal carcinoma was the most common histological type. Patients with early stage breast cancer, with hormone-receptor positive and human epidermal growth factor receptor 2 negative tumors, had a significantly longer progression-free survival. **Conclusion.** Hormone-receptor and human epidermal growth factor receptor 2 status evaluation is still of great clinical importance with a reliable prognostic value in breast cancer patients.
Key words: Breast Neoplasms; Receptors, Estrogen; Receptors, Progesterone; Receptor, Epidermal Growth Factor; Prognosis; Immunohistochemistry; Biomarkers, Tumor

Sažetak
Uvod. Značaj razumevanja biologije raka dojke sve je veći i određivanje pojedinih fenotipskih odlika malignih ćelija, pre svega ekspresije receptora za estrogen, progesteron i humani epidermalni faktor rasta 2, postali su standard celokupnog sagledavanja stanja obolelih, a sa ciljem postavljanja što preciznije prognoze, uspešnijeg lečenja i odgovora na sprovedeni tretman. **Materijal i metode.** Ukupno je retrospektivno analizirano 206 pacijenata sa rakom dojke, lečenih u Dnevnoj bolnici za hemioterapiju, Zdravstvenog centra u Vranju. Svima je u okviru histopatološke dijagnoze određivan status receptora za estrogen, progesteron i humani epidermalni faktor rasta 2, a nakon toga analiziran uticaj njihove ekspresije na dužinu perioda bez progresije bolesti i ukupnog preživljavanja obolelih. **Rezultati.** Kod dve trećine pacijenata dijagnostika je urađena u ranom kliničkom stadijumu. Duktalni karcinom je bio najčešće zastupljen histopatološki tip. Pacijenti kojima je karcinom dijagnostikovan u ranoj fazi sa tumorom pozitivnim na hormonski receptor i tumorom negativnim na humani epidermalni hornom rasta receptor 2, imali su statistički značajno duži period preživljavanja bez progresije bolesti. **Zaključak.** Određivanje ekspresije hormonskog receptora i receptora za humani epidermalni faktor rasta 2 na malignim ćelijama raka dojke sa pravom je i dalje standard u onkološkoj kliničkoj praksi jer pouzdano ukazuje na prognozu obolelih.
Ključne reči: karcinom dojke; estrogenski receptori; progesteronski receptori; receptori epidermalnog faktora rasta; prognoza; imunohistohemija; tumorski biomarkeri

Introduction

According to the *National Cancer Registry of Serbia*, breast cancer accounts for 20.2% of all diagnosed and additionally for 18.2% of all registered cancer deaths in women of central Serbia [1]. Factors that are the cornerstone for the tumor, nodes, and metastasis (TNM) staging system (size of primary tumor, involvement of regional lymph nodes, and presence of distant metastases) are prognostic factors used to describe the anatomic extent of the disease and stratify all patients with breast cancer with comparable outcomes [2]. Over the past half century, our understanding of breast cancer has improved and the focus has shifted from radical mastec-

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Abbreviations

ER – estrogen receptor
PR – progesterone receptor
HER2 – human epidermal growth factor receptor 2
PFS – progression-free survival
OS – overall survival
TNM – tumor, nodes and metastasis

tomy to breast-conserving surgery and personalized systemic therapy, based mainly on prognostic and predictive biological factors of the primary tumor. The relevance of the TNM staging system in the era of biomarkers, genomic analysis, and personalized medicine is becoming limited, but at the same time, parameters of tumor biology represent essential factors for therapeutic decision making. That is also the reason why patients staged similarly using the TNM staging system, have significantly different outcomes [2, 3]. Factors, such as histological grade, invasion of lymphovascular elements and biomarker status, have become very important in identifying patients with biologically aggressive cancers who may not show a good response to adj- uvant systemic regimens. Breast cancer is a biologically heterogeneous disease with many different subtypes, which are differentiated using immuno-histochemical and molecular analyses [4].

Molecular classification has become a standard procedure in the diagnosis of breast cancer, based on gene profiling, with the aim to predict the disease outcome [5, 6]. Despite all the remarkable achievements of molecular biology, clinicians still rely on traditional clinical pathological features and rapid test markers such as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). The determination of these markers is routinely done by immuno-histochemistry in breast cancer tissue samples and the results have not only a prognostic value, but also play a crucial role in therapeutic decision making. This method is still a reasonable substitute for expensive molecular technologies [7].

The aim of this study was to evaluate the prognostic significance of the estrogen and progesterone receptors and human epidermal growth factor receptor 2 status in patients with breast cancer.

Material and Methods

This study included a total of 206 patients, all treated and followed up in the *Daily Chemotherapy Hospital* of the *Health Center Vranje*, in the period 2009–2014. The retrospective analysis included parameters obtained from patients' medical records. We evaluated the impact of primarily phenotypic

Table 1. Clinical stage and tumor characteristics
Tabela 1. *Klinički stadijum bolesti i karakteristike tumora*

Clinical stage/*Klinički stadijum*	N	%
Early breast cancer/*Rani karcinom dojke*	140	68
Advanced breast cancer/*Uznapredovali karcinom dojke*	66	32
Total/*Ukupno*	206	100
Histological types/*Histološki tipovi*		
Ductal carcinoma/*Duktalni karcinom*	162	78.6
Lobular carcinoma/*Lobularni karcinom*	38	18.4
Other subtypes/*Ostali suptipovi*	6	3
Total/*Ukupno*	206	100
Estrogen receptor/*Estrogenski receptor*		
ER- + status/*ER pozitivni status*	158	76.7
ER- – status/*ER negativni status*	48	23.3
Total/*Ukupno*	206	100
Progesterone receptor/*Progesteronski receptor*		
PR + status/*PR pozitivni status*	126	61.2
PR – status/*PR negativni status*	80	38.8
Total/*Ukupno*	206	100
Hormone receptor status/*Hormon-receptorski status*		
Hormone receptor-positive tumor/*Tumor pozitivan na hormonski receptor*	122	59.2
Partially hormone receptor-positive tumor*/*Tumor parcijalno pozitivan na hormonski receptor**	40	19.4
Hormone receptor-negative tumor/*Tumor negativan na hormonski receptor*	44	21.4
Total/*Ukupno*	206	100
HER2 status/*HER2 status*		
HER2- + status/*HER2 pozitivni status*	36	17.5
HER2- – status/*HER2 negativni status*	170	82.5
Total/*Ukupno*	206	100

ER – *estrogeni receptor,* PR – *progesteronski receptor,* HER2 – *receptor za humani epidermalni faktor rasta 2;* *ER+ or PR+/*ER+ ili PR+

tumor characteristics on the progression-free survival (PFS) and overall survival (OS) of patients.

Statistical analysis was performed using SPSS 17.0 statistical package. All results were expressed as mean values ± standard deviation (SD). The statistical significance of differences between mean values was analyzed using the t-test. Correlations between the investigated variables were studied by a Pearson's test. A p-value < 0.05 was considered statistically significant.

Results

This retrospective study included a total of 206 patients. Early stage breast cancer was diagnosed in 140 patients, while one-third of patients (32%) presented with an advanced stage **(Table 1).** Histopathological analysis confirmed ductal carcinoma in 162 patients (78.6% of the total number of patients), and lobular carcinoma in 18.4% **(Table 1)**. Out of 206 patients, only 6 had a diagnosis of another histological type of breast cancer (papillary, tubulopapillary medullary, all in two patients), which makes a total of 3%.

Immunohistochemical phenotype expression of the steroid receptor as well as the HER2 protein status were determined in all breast cancer tissue samples. The estrogen receptor expression was confirmed in 158 (76.7%) patients, i.e. in the majority of the investigated group. The progesterone receptor positivity was found in 126 patients or 61.2% **(Table 1)**. The assessment of steroid receptor status showed that 59.2% (122) of patients had both estrogen and progesterone receptor positive phenotypes, and additionally 19.4% (40) of patients had ER or PR positive steroid receptor status. Therefore, hormone-sensitive tumors accounted for 78.6% of patients **(Tables 1 and 2)**. Overexpression of HER2 protein was detected in 17.5% of cases (36 patients).

The main objective of this study was to analyze the impact of the clinical stage of the disease, as well as of some characteristics of the tumor tissue (histopat-

Table 2. Hormone receptor and HER2 status
Tabela 2. *Hormon-receptorski i HER2 status tumora*

Parameters *Parametri*	Hormone receptor positive tumor† *Hormon receptor pozitivni tumor†*	Hormone receptor negative tumor *Hormon receptor negativni tumor*	Total *Ukupno*
HER2- – status *HER2 negativni status*	140 (68%)	30 (14.5%)	170 (82.5%)
HER2- + status *HER2 pozitivni status*	22 (10.6%)	14 (6.9%)	36 (17.5%)
Total/*Ukupno*	162 (78.6%)	44 (21.4%)	206 (100%)

HER2 - receptor za humani epidermalni faktor rasta 2; † ER+ and/or PR+/† ER+ i/ili PR+

Table 3. Differences in progression-free survival related to clinical parameters and different receptor status
Tabela 3. *Razlike u dužini preživljavanja do progresije bolesti u odnosu na kliničke parametre i različiti status receptora*

Histological types *Histološki tipovi*	N	%	PFS (mean value in months) *PFS (srednja vrednost u mesecima)*	SD	p value *p vrednost*
Ductal carcinoma/*Duktalni karcinom*	162	78.6	49,62	21,14	p = 0,104
Lobular carcinoma/*Lobularni karcinom*	38	18.4	43,16	25,18	
Clinical stage of disease/*Klinički stadijum bolesti*					
Early breast cancer/ *Rani karcinom dojke*	140	68	56,99	14,88	p = ,001*
Advanced breast cancer/*Uznapredovali karcinom dojke*	66	32	30,36	23,39	
Estrogen receptor/*Estrogeni receptor*					
ER- + status/*ER pozitivni status*	158	76.7	49,73	21,0	p = 0,129
ER- – status/*ER negativni status*	48	23.3	44,25	24,29	
Progesterone receptor/*Progesteronski receptor*					
PR- + status/*PR pozitivni status*	126	61.2	53,62	19,1	p = 0,001*
PR- – status/*PR negativni status*	80	38.8	40,33	23,56	
Hormone receptor status/*Hormon-receptorski status*					
Receptor positive tumor †/*Receptor pozivni status†*	162	78.6	50,11	20,89	p = 0,03*
Receptor negative tumor/*Receptor negativni status*	44	21.4	42,36	24,48	
HER2 status/*HER2 status*					
HER2- + status/*HER2 pozitivni status*	36	17.5	40,11	25,43	p = 0,011*
HER2- – status/*HER2 negativni status*	170	82.5	50,22	20,7	

*HER2 - receptor za humani epidermalni faktor rasta 2; * p<0,05*

Table 4. Correlation analysis of the examined parameters
Tabela 4. *Korelacijska analiza ispitivanih parametara*

Parameters *Parametri*		Clinical stage *Klinički stadijum*	Histological types *Histološki tipovi*	ER status *ER status*	PR status *PR status*	HR status *HR status*	HER2 status *HER2 status*
Clinical stage *Klinički stadijum*	Cor.coefficient	1	0,184	-0,34	0,179	0,089	-0,287
	p value		0,008*	0,628	0,01*	0,202	0,000*
	N	206	200	206	206	166	206
Histological types *Histološki tipovi*	Cor.coefficient	0,184	1	-0,166	0,093	-0,013	-0,033
	p value	0,008*	200	0,017*	0,185	0,853	0,640
	N	200		200	200	166	200
ER status *ER status*	Cor.coefficient	-0,34	-0,166 0,017*	1	0,597	0,877	-0,170
	p value	0,628	200	206	0,000*	0,001*	0,015*
	N	206			206	166	206
PR status *PR status*	Cor.coefficient	0,179	0,093	0,597	1	0,909	-0,368
	p value	0,01	0,185	0,000*	206	0,000*	0,001*
	N	206	200	206		166	206
HR status *HR status*	Cor.coefficient	0,089	-0,013	0,877	0,909	1	-0,308
	p value	0,202	0,853	0,001*	0,000*		0,000*
	N	166	166	166	166	166	166
HER2 status *HER2 status*	Cor.coefficient	-0,287	-0,033	-0,170	-0,368	-0,308	1
	p value	0,000*	0,640	0,015*	0,001*	0,000*	
	N	206	200	206	206	166	206

* p<0,05; *HER2 - receptor za humani epidermalni faktor rasta 2; ER status – estrogen-receptorski status; PR status – progesteron-receptorski status; HR status – hormon-receptorski status*

hological type, ER, PR, HER2 status) on the PFS and OS.

The majority of studied patients, nearly 70%, diagnosed with early breast cancer, had a clinically and statistically longer PFS compared with patients with advanced breast cancer, 26 months on average (56.99 vs. 30.36 months, respectively) **(Table 3)**. This indicates and confirms that lower stage of disease at the time of diagnosis, is a very important factor for a longer PFS. **Table 3** shows no evidence of a statistically significant

Table 5. Differences in OS related to clinical parameters and different receptor status
Tabela 5. *Razlike u dužini ukupnog preživljavanja (UP) u odnosu na kliničke parametre i različiti status receptora*

Histological types/*Histološki tipovi*	N	%	OS (mean value in months) *UP (srednja vrednost u mesecima)*	SD	p value *p vrednost*
Ductal carcinoma/*Duktalni karcinom*	42	77.78	31,38	16,53	p=0,624
Lobular carcinoma/*Lobularni karcinom*	12	22.22	33,83	8,6	
Clinical stage of disease/*Klinički stadijum bolesti*					
Early breast cancer/*Rani karcinom dojke*	18	32.14	42	9,22	p=0,000*
Advanced breast cancer/ *Uznapredovali karcinom dojke*	38	67.86	27	14,61	
Estrogen receptor/*Estrogenski receptor*					
ER- + status/*ER pozitivni status*	40	71.42	32,85	14	p=0,417
ER- – status/*ER negativni status*	16	28.58	29,25	16,91	
Progesterone receptor/*Progesteronski receptor*					
PR- + status/*PR pozitivni status*	22	39.28	30,63	15,72	p=0,635
PR- – status/*PR negativni status*	34	60.72	32,58	14,41	
Hormon receptor status/*Hormon-receptorski status*					
Receptor positive tumor/*Receptor pozitivni status*†	40	71.42	32,85	14	p=0,417
Receptor negative tumor/*Receptor negativni status*	16	28.58	29,25	16,91	
HER2 status/HER2 status					
HER2-+ status/*HER2 pozitivni status*	14	25	27,43	18,18	p=0,203
HER2-– status/*HER2 negativni status*	42	75	33,29	13,46	

HER2 - receptor za humani epidermalni faktor rasta 2; * p<0,05; † ER+ and/or PR+/† *ER+ i/ili PR+*

prolongation of PFS in the group of patients with ductal breast cancer compared to patients with lobular carcinoma, although the first group had a longer PFS, 6 months on average (49.62 vs. 43.16 months, respectively).

Patients with steroid receptor-positive tumors had almost 8 months longer PFS compared to patients with hormone-independent tumors (50.11 vs. 42.36 months) presenting a statistically significant difference **(Table 3)**. This difference in duration of PFS cannot be associated with the fact that the group of patients with hormone receptor-positive tumors were diagnosed at an earlier stage of the disease, because a statistically significant correlation between these two parameters was not observed **(Table 4)**. A highly statistically significant difference in PFS was found in the group of patients with PR positive status in comparison with the PR negative group, over a year longer in favor of patients from the first group (53.62 vs. 40.33 months) **(Table 3)**. A statistically significantly longer PFS was also recorded in the group of patients whith HER2-negative tumor status. On average, they had 10 months longer PFS compared with the group of patients with overexpression of HER2 protein (50.22 vs. 40.11 months).

Just like in case of PFS, a statistically signifi- cantly longer OS was found in patients with early breast cancer (15 months longer OS) compared with patients with advanced breast cancer (42 vs. 27 months, respectively) **(Table 5)**. In contrast to the stage of the disease at the time of diagnosis, histopathological type had no significant impact on the OS. There was no statistically significant difference in OS between patients with ductal and lobular breast cancer (31.38 vs. 33.83 months). The impact of steroid-receptor and HER2 tumor phenotype on overall survival is also shown in **Table 5.** In evaluating the impact of all receptor parameters on OS, a statistically significant difference within individual groups was not observed. There was a difference between OS of patients with HER2-negative status compared to the group with HER2 overexpression (almost 6 month longer survival), but probably due to high standard deviation, because statistical significance was not achieved.

The above analysis examined individual groups to establish differences in PFS and OS in relation to the clinical stage of breast cancer and specific phenotypic characteristics of tumors. We also performed a correlation analysis of all the parameters **(Table 6)** in regard to living status (alive vs deceased) at the last follow-up examination. The correlation between the clinical stage of the disease at the time of diagnosis with actual life status, showed that there was a statistically significant positive correlation between advanced stage and lethal outcome. Out of 56 deceased patients, 38 had advanced, and 18 early breast cancer. There was also a statistically significant positive correlation between steroid receptor-positive breast cancer and longer survival, while in the group of patients with hormone-independent disease, at the time of analysis 36% were deceased (16 of 44 patients). The correlation anal- ysis between expression of HER2 protein and actu- al li-

Table 6. Correlation between the life status (alive/deceased) with clinical stage of the disease and some phenotypic tumor features
Tabela 6. *Korelacija životnog statusa pacijenata (živi/umrli) sa kliničkim stadijumom bolesti i pojedinim fenotipskim karakteristikama tumora*

Parameters *Parametri*		Life status/*Životni status*		Total	p value
		alive/*živi*	deceased/*umrli*	*Ukupno*	*p vrednost*
Clinical stage *Klinički stadijum*	Early breast cancer/*Rani klinički stadijum*	122	18	140	p=0,001*
	Advanced breast cancer *Uznapredovali klinički stadijum*	28	38	66	
	Total/*Ukupno*	150	56	206	
Histological types *Histološki tipovi*	Ductal carcinoma/*Duktalni karcinom*	120	42	162	p=0,735
	Lobular carcinoma/*Lobularni karcinom*	26	12	38	
	Total/*Ukupno*	146	54	200	
Estrogen receptor *Estrogeni receptor*	ER-+ status/*ER pozitivni status*	118	40	158	p=0,274
	ER- – status/*ER negativni status*	32	16	48	
	Total/*Ukupno*	150	56	206	
Progesterone receptor *Progesteronski receptor*	PR-+ status/*PR pozitivni status*	104	22	126	p=0,002*
	PR- – status/*PR negativni status*	46	34	80	
	Total/*Ukupno*	150	56	206	
Hormon receptor status *Hormon receptorski status*	Receptor positive tumor † *Hormon pozitivni tumor†*	122	40	162	p=0,001*
	Receptor negative tumor *Receptor negativni tumor*	28	16	44	
	Total/*Ukupno*	150	56	166	
HER2 status/*HER2 status*	HER2-+ status/*HER2 pozitivni status*	22	14	36	p=0,082
	HER2- – status/*HER2 negativni status*	128	42	170	
	Total/*Ukupno*	150	56	206	

HER2 - receptor za humani epidermalni faktor rasta 2; * p<0,05; † ER+ and/or PR+/† *ER+ i/iliPR+*

ving status showed a statistically significant association, although 24.7% of patients with HER2 negative status (42 of 170) and 38.8% with HER2 positive status (14 of 36) were deceased **(Table 6)**.

All the analyzed parameters were correlated with each other, and the results are shown in **Table 4.** A statistically significant positive correlation was established between early breast cancer and ductal histological type, while lobular carcinoma was more often diagnosed at an advanced stage. Patients with early stage breast cancer were mostly PR-positive and at the same time had a HER2-negative receptor status. An interesting fact is that the ductal carcinoma, which is commonly diagnosed at an early stage, was ER-negative in most cases. There was a statistically significant correlation between positive ER status and simultaneous positive PR, but a negative correlation with HER2 overexpression; so, ER positive tumors were mostly HER2 negative. If HER2 status is analyzed as a variable, we can conclude that HER2 overexpression was statistically significantly positively correlated with advanced stage and steroid receptor-negative breast cancer.

Discussion

In the examined patients, almost two thirds were diagnosed with an early stage, and one third with advanced breast cancer. Despite improved survival for all stages of the disease, survival remains poor in patients with a widespread disease. The group of patients with early breast cancer had 26 months longer PFS, and 15 months longer OS than the group of patients with advanced cancer **(Tables 3 and 5)**. Improvments in survival for specific stages of breast cancer in the last 30 years are considered to be the result of better treatment regimens (neoand adjuvant chemotherapy, radiotherapy, hormone and target therapy), followed by a better characterization of prognostic factors, as well as the progress in terms of increasing the number of diagnosed patients with small size tumors [8, 9].

For a long time it has been known that without adjuvant systemic therapy ER-negative breast cancer is associated with poor outcome compared with ER-positive breast cancer. Estrogen receptor is also a very reliable factor in predicting response to endocrine therapy; higher expression in tumor cells is associated with a higher response rate to hormone therapy [10]. In our group, two-thirds of patients (76.7%) were with ER-positive status, which is in agreement with a series of patients that have already been tested in our country and in the region [11, 12]. The rate of PR-positive status was slightly lower (61.2% of patients), which is also in accordance with literature data.

In general, all phenotypic features of tumor tissues determined by immunohistochemical analysis are very important and necessary in order to obtain significant prognostic and predictive information for better classification and differentiation of various types of breast cancer. In patients included in this study, 40 patients (almost 20%), had a positive ER or PR status. If we add 122 patients who had both ER positive and PR positive status, we can see that the majority of patients (78.6%) had a steroid-receptor positive breast cancer. Concordant expression of steroid-receptor phenotypes (PR and ER positive) with a negative HER2 protein is a characteristic of tumors mainly found in elderly/postmenopausal women [13]. In our study, 140 patients had this type of phenotype, which makes 68% of the total number of examined patients. According to clinical and epidemiological studies, this immunohistochemical phenotype of steroid receptors in breast cancer is the most favorable in terms of prognosis and therapy [14]. Therefore, the presence of ER in the primary tumor remains the most important predictive factor predicting response to hormonal therapy. Positive estrogen and progesterone receptors can determine even with greater accuracy the probability of response to hormone therapy, so that patients with ER and PR positive tumors respond to this therapy in 75% of cases, while less than 10% of patients with negative steroid-receptor phenotype benefit from hormone therapy [15, 16].

Steroid receptors are not strong independent prognostic factors, but in combination with other factors and HER2 status, they are used for classification of patients into prognostic subgroups.

The triple positive subtype, namely: ER+ and/or PR+ with HER2+ status was found in 10.6% of cases, while triple negative (TN) breast cancer was found in 14.5%; it means that 30 patients were in the phenotypic group, which, according to the literature data, is most common in younger/premenopausal women. Recent studies reported that the incidence of triple negative breast cancer accounts for 12 - 25% of all invasive carcinomas [17, 18]. This entity, triple negative breast cancer is characterized by aggressive biological behavior and lack of response to the currently available systemic therapy.

In the literature, the percentage of HER2- overexpressed/amplified breast carcinomas range from 3% to 30% [19, 20]. In our group of patients, HER2 status was determined in all patients, and overexpression was found in 36 (17.5%), which is in agreement with data published in extensive breast cancer histologic assessment in our country, and in our region as well [11, 12]. It is well known that anthracyclines have inhibitory effects on topoisomerase II alpha and a few recent studies have shown that amplification of the gene for this enzyme may be a better predictor of response to anthracycline drugs than the gene encoding the HER2 protein (c-erbB-2) gene [15]. Although these two genes are close together, the amplification of c-erbB-2 gene is not always associated with an amplification of the gene for the enzyme to- poisomerase II alpha. In this case the status of HER2 protein in breast cancer tissue would not be needed for a therapeutic decision with anthracycline containing chemotherapy, but should be decided on the basis of the status of gene for topoisomerase II alpha enzyme. However, HER2 status assessment remains a mandatory parameter in order to assess the best further treatment, in terms of using target therapies directed at this protein receptor.

The proliferation marker Ki67 has become a standard predictive factor in histopathological assessments

of breast cancer. This antigen is expressed in the nucleus of neoplastic cells through all phases of the cell cycle and for that reason it is a useful marker for assessing the proliferative potential of malignant cells. Detection of changes in the expression of Ki-67 after neoadjuvant, chemo- and endocrine treatment is a useful predictor of long term outcome in some cases. So, if "something" inhibits new cell growth, Ki67 expression decreases, which is also a good predictor of response to applied therapy [21, 22]. Considering the fact that in the group of patients included in this study, Ki-67 was determined just sporadically (our Center had not established a routine testing of Ki67 in these patients at that time), so it could not have been systematically analyzed as a predictor of prognosis.

Conclusion

The results of this study suggest that determination of estrogen receptor, progesteron receptor, and human epidermal growth factor receptor 2 status is of great clinical importance for all breast cancer patients, not only to predict prognosis, but also for the prediction of response to therapy, which is applied after complete insight into the status of the tumor phenotype. This study has confirmed the previosly reported facts that patients with a hormone-dependent and human epidermal growth factor receptor 2 negative breast cancer have a better prognosis and therapeutic options than patients with other breast cancer immunophenotypes.

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