The Role of the Thyroid Disease and Other Biomarkers in Invasive Breast Cancer. 3-Year Retrospective Comparative Study

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Article

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Abstract: Objective: Breast cancer (BC) is the most common cause of death among females. There are many prognostic systems, the most recent of which are the molecular subtypes. Recently, it was included that thyroid disease is a favorable factor for the patients with BC and that the thyroid hormones have an effect on the disease. Thyroid stimulating hormone (TSH), however, is thought to be unrelated to the cancer aggressiveness. In the current study we aimed to examine the status of the postoperative TSH levels, as well as the impact of the thyroid disease on the cancer aggressiveness. Methods: A retrospective comparative analysis of invasive BC patients was performed between 2017 and 2019 year. 111 patients were selected. They were divided into group A (n=62) and group B (n=49), based on the presence or absence of thyroid disease, respectively. The data was processed with SPSS version 25. Results: There is a significant difference between group A and group B concerning the molecular subtypes, ki-67, and estrogen receptor. The current study supports the fact that TSH is responsible for the aggressiveness of the invasive BC and not the thyroid hormones themselves. Moreover, based on the correlation analyses calcium levels preoperatively are linked to cancer aggressiveness and could be used as future prognostic factors. Conclusion: Indeed, the thyroid disease appears to have more favorable prognosis based on the molecular subtype frequency but more emphasis in future studies should be given to the TSH and its relation to the overall survival of the patients.

Keywords: thyroid disease; breast cancer; thyroid hormones; TNM; Calcium

1. Introduction

Worldwide, there are about 2.1 million people affected by breast cancer [1]. It is one of the major causes of disability and deterioration and fifth major cause of death among females [2]. Recently, to the histological and immunochemistry classification of breast tumors were also added molecular subtypes [4,5]. The latter have the main goal of prognosis and improved treatment planning of the patients suffering from breast cancer. According to the molecular classification of breast tumors by Perou et.al. [5] there are four molecular subtypes (Luminal A, Luminal B, HER2 Overexpression and triple negative breast cancer (TNBC)) , each having different prognosis and different treatment results. Based on the latest studies, Luminal A has the greatest rates of overall survival, while TNBC has the poorest prognosis [6,7,8].

Furthermore, current studies link thyroidopathy to breast disease. According to the latest researches, hypothyroidism is a favorable prognostic factor among breast cancer patients. It is believed that the thyroid hormones play significant role for the cell proliferation, therefore increased levels of Thyroxin (T4) and triiodothyonine (T3) are linked to increased incidence of mutations [9].

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On the other hand, the thyroid stimulating hormone (TSH) appears to be unrelated factor to the cancer aggressiveness [10].

The purpose of the study is to assess the status of the postoperative TSH levels, as well as the impact of the thyroid disease on the cancer aggressiveness.

2. Methods

Data Collection and Data Selection

A protocol of the study was submitted to the ethical committee of the "Metaxa" Anti-cancer hospital. After its approval, the study was conducted as a retrospective analysis of patients with invasive breast cancer, operated by the second department of surgery between 01.01.2017 and 31.12. 2019.

Thereafter, a filtration of the histological reports, archived in the department, was obtained, where only patients suffering from invasive breast cancer were included. The identification (ID) number of each patient, written on the histological report, was written down, in order to find each patient's folder from the general archive of the hospital .The same ID number was used for access in the electronic system of the hospital to obtain the results of the blood tests. As protocol of the department, the patients arrive a few days before operation for preoperative check – up. Blood samples are collected at the first day of hospitalization from each patient. Patients suffering from thyroidopathy are also tested for the levels of their hormones at the first day of hospitalization. Thus, data for the analysis was collected from both electronic system and patients' folder.

The entire sample of breast disease for 3-year period included 183 patients. 53 patients were excluded cause of benign disease, 4 for in – situ breast cancer, 15 had cancer but either the immochemistry reports were missing from the archive or the patients were refereed for chemotherapy without surgery, while 111 were included for analysis. From those 111 patients were collected data for the analysis from the histological reposts, including primary tumor, molecular subtype and ki-67 biomarker if available and from the electronic system of the hospital - age, levels of thyroid hormones (free T3 and freeT4) , lactate dehydrogonase (LDH), calcium and TSH preoperatively and TSH levels post- operatively.

Afterwards, the study sample was divided into two categories: patients with invasive breast cancer and thyroidopathy or group A and patients with breast cancer without thyroidopathy or group B. (Table 1)

Table 1. Patients' characteristics.

	NON Thyroidopathy group (Group B)		Thyroidopathy group (Group A)	
Number of patients	**49**		62	
Age (Yrs)	64,27		65,67	
Estrogen receptor (ER) (number of patients) (n)	36 +	13 -	53 +	9 -
Progesterone receptor (PR) (number of patients) (n)	29 +	20 -	41 +	21 -
HER 2 receptor (Number of patients) (n)	7 +	42-	13 +	49 -
LDH	196,82		210,38	
Calcium (Ca) (mg/dL)	9,66		9,63	
Ki-67 (%)*	38,42		31,03	
Luminal A (Number of patients) (n)	31		43	

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Luminal B (Number of patients)(n)	5	10
HER 2 Overexpression (Number of patients) (n)	2	3
Basal or triple negative (Number of patients)(n)	11	6

* many missing values.

Thereafter each group was divided based on the molecular subtype into 4 subgroups based on the status of the estrogen receptor(ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2 or c-erb/B2):

1. Luminal A or ER/PR + and HER2-
2. Luminal B or ER/PR +/- and HER 2+
3. Her 2 overexpression or ER/PR – and HER2 +
4. And triple negative or ER/PR- and HER2 –

Moreover, the patients were divided based on the age into premenopausal and postmenopausal groups. All the patients who were 50 years of age or less at the time of surgery were included as premenopausal and the rest (over <50 years of age) as postmenopausal. After that, the groups were subdivided into molecular subtype and thyroidopathy subgroups.

3. Data Analysis

The aforementioned groups were statistically processed with SPSS version 25. Independent T-test was performed to compare the equality of the groups. Correlation analysis and logistic regression analysis were used to explain the relationship between the factors for each group. The α value was set up at 0,05. P-value was considered statistically important if $p \leq 0,05$.

4. Results

Independent T-tests

Independent sample T-test comparing group A and B was performed, where the results showed that there is a difference between the molecular subtypes, as well as between the rates of ki-67 and ER receptors (Table 2). C-erb/B2 or HER2 receptor had the tendency but the result was not statistically significant.

Second independent T-test was performed for the premenopausal group, without any statistically significant results.

Finally, the third independent t-test showed that postmenopausal group A and B differ in the molecular subtypes, ER, HER2 receptors and ki-67 analog (Table 3).

Table 2. Independent sample T-test. Group A vs. group B.

	Levene's Test for Equality of Variances		**t-test for Equality of Means**						
	F	**P-value**	**t**	**df**	**P-value (2-tailed)**	**Mean Difference**	**Std. Error Difference**	**95% Confidence Interval of the Difference**	
								Lower	**Upper**
Age	**1,327**	**,252**	**,377**	**109**	**,707**	**,99276**	2,63207	-4,22392	6,20944
			,371	96,025	,711	,99276	2,67359	-4,31426	6,29978
ER	10,012	,002**	-1,580	109	,117	-,12014	,07603	-,27083	,03054
			-1,539	90,304	,127	-,12014	,07807	-,27524	,03495
PR	2,000	,160	-,748	109	,456	-,06945	,09286	-,25350	,11459
			-,744	101,203	,458	-,06945	,09330	-,25453	,11562

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HER2	3,423	,067*	-,905	109	,368	-,06682	,07386	-,21321	,07957
			-,921	108,154	,359	-,06682	,07258	-,21068	,07704
LDH	,061	,805	1,279	108	,204	13,56072	10,60006	-7,45044	34,57189
			1,280	103,112	,203	13,56072	10,59556	-7,45279	34,57424
Ki-67	11,628	,001**	-1,040	60	,303	-7,391	7,108	-21,610	6,827
			-,982	41,776	,332	-7,391	7,530	-22,590	7,807
Calcium levels	,115	,735	-,301	108	,764	-,03185	,10596	-,24188	,17817
			-,287	79,990	,775	-,03185	,11084	-,25243	,18872
T (TNM)	1,752	,191	-,210	54	,834	-,046	,219	-,486	,394
			-,207	46,760	,837	-,046	,223	-,496	,403
Molecular Subtype	7,854	,006**	-1,460	109	,147	-,30876	,21143	-,72780	,11028
			-1,417	88,320	,160	-,30876	,21789	-,74175	,12424

**Significant results; *Tendency of significance. T- Tumor from the TNM. LDH - lactate dehydrogenase; PR- Progesterone Receptors; ER – estrogen receptors; HER2 - human epidermal growth factor receptor 2.

Table 3. Independent T-test. Postmenopausal group A vs. group B.

.	**Levene's Test for Equality of Variances**		**t-test for Equality of Means**						
	F	**P-value (one-tail)**	**t**	**df**	**P-value (2-tailed)**	**Mean Difference**	**Std. Error Difference**	95% Confidence Interval of the Difference	
								Lower	Upper
Age	**,320**	**,573**	**-,587**	**84**	**,559**	**-1,25444**	2,13872	-5,50753	2,99864
			-,584	74,509	,561	-1,25444	2,14689	-5,53173	3,02284
LDH Level	,332	,566	1,504	84	,136	18,86667	12,54688	-6,08417	43,81750
			1,486	72,218	,142	18,86667	12,69554	-6,44012	44,17345
Ki-67 (%)	14,331	,000**	-,822	47	,415	-6,119	7,441	-21,088	8,850
			-,772	31,596	,446	-6,119	7,926	-22,273	10,034
Calcium Levels	,261	,611	-,324	83	,746	-,04171	,12859	-,29747	,21404
			-,300	52,359	,765	-,04171	,13912	-,32083	,23740
ER	6,585	,012**	-1,291	84	,200	-,11000	,08523	-,27948	,05948
			-1,244	64,741	,218	-,11000	,08840	-,28656	,06656
PR	2,757	,101	-,915	84	,363	-,09667	,10567	-,30679	,11346
			-,906	72,809	,368	-,09667	,10670	-,30933	,11600
HER2	7,706	,007**	-1,311	84	,194	-,10889	,08308	-,27411	,05633
			-1,369	83,699	,175	-,10889	,07952	-,26704	,04926
Molecular Subtype	7,801	,006**	-1,342	84	,183	-,32111	,23936	-,79710	,15487
			-1,273	60,083	,208	-,32111	,25217	-,82551	,18328

**Significant result; LDH - lactate dehydrogenase; PR- Progesterone Receptors; ER – estrogen receptors; HER2 - human epidermal growth factor receptor 2.

5. Correlations

A correlation analysis was performed for group A, illustrated in table 4a and 4b. The results show significant results, important also for future treatment planning. The same analysis was done for group B. In contrast to group A, group B presents only positive correlations and none negative ones. (Table 5). In order to show the role of the calcium and ki-67 in relation to the cancer aggressiveness, we performed also a correlation analysis on the entire sample. The positive correlations are illustrated in Table 6, while there was only one negative correlation between HER2 and Molecular subgroup (Pearson's r=-0.252; p-value = 0.004).

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Table 4. (**a**) Positive correlations for group A. (**b**) Negative correlations for group A.

(**a**)

		Calcium	fT4	fT3	ki-67	LDH	TSH PostOp	TSH PreOP	Age
fT4	Pearson's r	-0.019	—						
	p-value	0.558	—						
fT3	Pearson's r	-0.023	0.044	—					
	p-value	0.570	0.371	—					
Ki-67	Pearson's r	0.187	-0.193	-0.065	—				
	p-value	0.141	0.863	0.644	—				
LDH	Pearson's r	0.068	0.047	-0.049	-0.235	—			
	p-value	0.305	0.362	0.643	0.913	—			
TSH PostOp	Pearson's r	-0.367	-0.084	0.243	-0.537	-0.231	—		
	p-value	0.968	0.658	0.115	0.971	0.866	—		
TSH PreOp	Pearson's r	0.119	-0.464	-0.090	0.285*	-0.012	0.314	—	
	p-value	0.183	1.000	0.754	0.049	0.535	0.059	—	
Age	Pearson's r	0.073	0.193	-0.016	-0.099	0.089	-0.044	0.042	—
	p-value	0.289	0.072	0.548	0.714	0.251	0.584	0.375	—
Her2	Pearson's r	0.165	0.233	0.072	-0.228	0.127	-0.118	-0.135	-0.164
	p-value	0.104	0.038	0.293	0.906	0.169	0.718	0.848	0.895
PR	Pearson's r	0.339*	-0.220	-0.089	0.402*	0.059	-0.342	0.275*	-0.032
	p-value	0.004	0.953	0.751	0.008	0.328	0.956	0.017	0.597
ER	Pearson's r	0.109	-0.085	-0.055	0.439*	-0.110	-0.174	0.080	0.017
	p-value	0.203	0.738	0.663	0.004	0.797	0.802	0.273	0.449
Molecular subgroup	Pearson's r	0.059	-0.176	-0.082	0.554*	-0.190	-0.113	0.238*	0.006
	p-value	0.326	0.909	0.732	< .001	0.925	0.708	0.034	0.483

(**b**)

		Calcium	fT4	fT3	ki-67	LDH	TSH postOp	TSH Pre Op	Age
fT4	Pearson's r	-0.019	—						
	p-value	0.442	—						
fT3	Pearson's r	-0.023	0.044	—					
	p-value	0.430	0.629	—					
Ki-67	Pearson's r	0.187	-0.193	-0.065	—				
	p-value	0.859	0.137	0.356	—				
LDH	Pearson's r	0.068	0.047	-0.049	-0.235	—			
	p-value	0.695	0.638	0.357	0.087	—			
TSH PostOp	Pearson's r	-0.367*	-0.084	0.243	-0.537*	-0.231	—		
	p-value	0.032	0.342	0.885	0.029	0.134	—		
TSH PreOp	Pearson's r	0.119	-0.464*	-0.090	0.285	-0.012	0.314	—	
	p-value	0.817	< .001	0.246	0.951	0.465	0.941	—	
Age	Pearson's r	0.073	0.193	-0.016	-0.099	0.089	-0.044	0.042	—
	p-value	0.711	0.928	0.452	0.286	0.749	0.416	0.625	—
HER2	Pearson's r	0.165	0.233	0.072	-0.228	0.127	-0.118	-0.135	-0.164
	p-value	0.896	0.962	0.707	0.094	0.831	0.282	0.152	0.105
PR	Pearson's r	0.339	-0.220	-0.089	0.402	0.059	-0.342	0.275	-0.032
	p-value	0.996	0.047	0.249	0.992	0.672	0.044	0.983	0.403*
ER	Pearson's r	0.109	-0.085	-0.055	0.439	-0.110	-0.174	0.080	0.017
	p-value	0.797	0.262	0.337	0.996	0.203	0.198	0.727	0.551
Molecular subgroup	Pearson's r	0.059	-0.176	-0.082	0.554	-0.190	-0.113	0.238	0.006
	p-value	0.674	0.091	0.268	1.000	0.075	0.292	0.966	0.517

*significant result.

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Table 5. Positive correlations. Group B.

		ki-67	LDH	Calcium	Age	ER	PR	HER2	Molecular subtype
LDH	Pearson's r	**0.336**	—						
	p-value	**0.050**	—						
Calcium	Pearson's r	0.338*	-0.135	—					
	p-value	0.049	0.814	—					
Age	Pearson's r	-0.081	0.112	0.103	—				
	p-value	0.650	0.226	0.248	—				
ER	Pearson's r	0.431*	0.014	0.115	-0.123	—			
	p-value	0.016	0.464	0.223	0.795	—			
PR	Pearson's r	0.261	-0.091	0.113	-0.119	0.711*	—		
	p-value	0.104	0.728	0.228	0.788	< .001	—		
HER2	Pearson's r	0.197	0.062	0.121	0.154	-0.029	-0.021	—	
	p-value	0.173	0.340	0.212	0.150	0.577	0.555	—	
Molecular subtype	Pearson's r	0.398*	-0.008	0.111	-0.068	0.957*	0.680*	-0.156	—
	p-value	0.024	0.522	0.231	0.675	< .001	< .001	0.852	—
TNM (T)	Pearson's r	0.490*	0.016	0.149	-0.026	0.573*	0.268	0.269	0.531*
	p-value	0.017	0.468	0.233	0.550	0.001	0.093	0.092	0.003

Note . all tests one-tailed, for positive correlation. * p < .05, ** p < .01, *** p < .001, one-tailed.

Table 6. Positive Correlation analysis for the entire sample.

		Age	LDH	ki-67 (%)	Calcium Levels	Subgroup all	T (TNM)
LDH	Pearson's r	0.098	—				
	p-value	0.158	—				
ki-67 (%)	Pearson's r	-0.072	-0.006	—			
	p-value	0.710	0.518	—			
Calcium levels	Pearson's r	0.088	-0.062	0.301*	—		
	p-value	0.182	0.737	0.009	—		
Molecular subtype	Pearson's r	-0.042	-0.123	0.471*	0.089	—	
	p-value	0.669	0.898	< .001	0.181	—	
T (TNM)	Pearson's r	0.138	-0.012	0.272*	0.245*	0.467*	—
	p-value	0.158	0.534	0.045	0.036	< .001	—
HER2	Pearson's r	-0.029	0.101	-0.036	0.135	-0.252	0.143
	p-value	0.616	0.150	0.610	0.082	0.996	0.148
PR	Pearson's r	-0.079	-0.002	0.313*	0.214	0.611*	0.399*
	p-value	0.794	0.508	0.007	0.013	< .001	0.001
ER	Pearson's r	-0.059	-0.078	0.453*	0.121	0.935*	0.489*
	p-value	0.730	0.790	< .001	0.107	< .001	< .001

*Significant result.

Paired T-test

T-paired test was obtained for the preoperative TSH levels versus postoperative TSH levels and the results showed that they are equal (Table 7).

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Table 7. Paired T-test preoperative TSH vs. postoperative TSH.

Paired Samples T-Test					
			t	**df**	**p**
TSH PRE OP	-	tsh post OP	-1.219	26	0.234

Note. Student's t-test.

6. Discussion

Worldwide, breast cancer is the most common type of cancer and 5th major cause of death among females [2]. In 2019 are estimated 271.000 new cases in the USA and about 42.260 of the cases are expected to have fatal outcome. The risk of invasive breast cancer diagnosis increases with age [11]. Thus, 1 per 25 in the seventh decade might have invasive breast cancer, while the same number in the second decade (twenties) is only 1 per 567 people [11]. According to the American Cancer Society (ACS), lifetime risk of invasive breast cancer diagnosis is about 12,8% and about 2,6% for breast cancer mortality.

The incidence of breast cancer is also variable among different races or ethnicities. According to the ACS's statistics for 2011-2015, Non-Hispanic white race has the highest incidence rate, while Asian/Passific Islander race has the lowest incidence rates [11].

The factors predisposing to an increased incidence of breast cancer are divided into genetic factors, and non-genetic factors such as everyday life, exposure to radiation, hormone therapy and hormone imbalance (early menopause, miscarriages etc.) [12].

Family history, however, is the most important of those factors. Inherited mutation in the genes BRCA1 and BRCA2 are responsible for about 3% to 10% of the female breast cancers, and for about 30% of the early onset breast cancer, worldwide [12,13,14,15]. BRCA1 and BRCA2 are both acting as suppressors of cell growth by repairing DNA or inducing apoptosis, and produce an anti-oncogene protein called tumor suppressor gene (TSG) protein. BRCA1 and BRCA2 are located in chromosome 17 and 13, respectively, so any mutation in those genes increases the risk of breast prostate and ovarian cancer. BRCA1 mutation causes from 60% to 80% breast cancer, while 35% from the breast cancers are caused by Germ-line mutation in BRCA2 [12,16]. About half of the families with breast cancer are caused by BRCA1 mutations and 30% by BRCA2 mutations [12,17]. It is observed that BRCA1 mutations not only are more common, but also they are related to poorer prognosis, evaluated by the higher rate of mitosis, higher frequency of lymph node invasion, lack of ER, PR and/or Her2 receptors expression, as well as p53 gene mutation [18,19,20,21,22]. Moreover, patients with BRCA1 and/or BRCA2 mutations have an increased risk of developing peptic system cancer or melanoma [23,24].

Breast cancer is categorized in three main groups: histological, molecular and functional. Histologically, the World Health Organization (WHO) classified in 2019 the subtypes of breast cancer in the following categories: epithelial tumors, invasive carcinoma, rare and salivary gland type neoplasms, neuroendocrine tumors, epithelial – myoepithelial tumors (in- situ ductal carcinoma, non invasive lobular neoplasms), benign epithelial proliferations and precursors papillary tumors and adenomas, mesenchymal tumors, tumors of the nipple, malignang lymphoma, fibroepithelial neoplasms, metastatic tumors and male breast neoplasms[4,5]. In this term, breast adenocarcinoma is the most common form, accounting for about 95% of all types of breast cancer [25]. Invasive ductal carcinoma is consisting 55% of all breast carcinomas, arising from the same final segments of the lobular duct unit [25,26]. On the other hand, lobular carcinoma stem from the terminal duct lobular unit. According to Toikkanen et.al. [27] invasive lobular carcinoma has better prognosis, including overall survival (OS). In his study, patients with ILC compared to those with IDC had 5-year survival rater of 78% and 50%, respectively.

Recently, the efforts to improve the diagnostic and prognostic accuracy led to the involvement of molecular parameters in the treatment planning [28,29,30]. Perou et.al. [5] classified breast cancer subtypes with the help of microarray technology as follows: 1) Luminal A – ER + and/or PR + and

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HER2 - ; 2) Luminal B – ER+ and/or PR +, HER2 +; 3) ER- and PR -, HER2 + and 4) Basal like – ER-, PR- and HER2 -.

According to Fallahpour et.al. [7] and Fragomeni et.al. [6], luminal A subtype has the greatest survival, as it is the most common type, followed by luminal B, HER2 overexpression, while the triple negative molecular subtype had the lowest rates of survival and the greatest rates of recurrence. Moreover, He et.al. [31] supports the fact that the molecular subtype plays a significant role for the patients, receiving radiation therapy. According to his study, luminal A subtype has the greatest benefit of radiation, while triple negative and HER2 overexpression types , independently of the type of radiation therapy, have less benefit.

HER2 receptor is also known as proto-oncogene Neu, encoded by the ERBB2 gene (erythroblastic oncogene 2). HER2 receptor is a part of the epidermal growth factor (EGF) family but in contrast to the other receptors, the extracellular domain has no particular ligand [32]. It is presented in an active state and might undergo "ligand- independent" dimerization. The main function of the HER2 receptor is transmission of signals, targeting endorsement of cell proliferation, while opposing apoptosis [33]. HER2 is believed to induce carcinogenesis in vivo and in vitro [33]. From 15% to 30% of the patients with breast cancer have increased expression of c-erb/B2. There might be approximately 25-50 copies of the HER2 gene in breast carcinomas, resulting in the extensive release of HER2 protein and HER2 receptors (HER2 overexpression). In such cases, the estrogen, normally stimulating ER receptors, could act by stimulating HER2 receptors. This overexpression of c-erb/B2 gene is linked to poor OS and progression free survival (PFS) [33]. According to Slamon et.al. [8] HER2 overexpression is a prognostic factor for both overall survival and "time to relapse".

Estrogen receptors (α and β)are intracellular proteins, activated by the steroid hormone estrogen, which are able to control gene expression. There are genomic and non-genomic signaling pathways through the ER receptors, both of which could influence gene expression [34]. Through the first signaling pathway, after binding to the ER receptor, the latter dimerizes and binds to a specific compounds named estrogen response elements (EREs), located in the gene promoters. The second and not so common signaling pathway is performed without direct bind to the DNA, but through protein-protein signaling [34]. The non – genomic pathway launches mobilization of intracellular calcium (Ca^{+2}), motion of adenalyte cyclase, creation of cyclic adenosine monophosphate (cAMP), and synthesis of endothelial nitric oxide (eNOS) [34, 35]. It is thought that by binding to ER receptor, estrogen also stimulates cell proliferation, thus increasing the rate of mutations [34,36]. In about 70% of the breast carcinomas there is present ER receptor. According to the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) [37], the ER positive breast cancer had better prognosis and OS, independently of the PR receptor. Despite that, ER positive breast cancer is a heterogeneous entity, and a personalized approach is always needed [38].

Progesterone receptor is also an intracellular protein, known also as nuclear receptor subfamily 3, group C, number 3 (NR3C3) and it is being activated by the steroid hormone progesterone. Similar to the ER, positive PR receptors in breast cancer have better prognostic value [39,40]. In a prospective cohort study by Dunnwald et.al. [40], patients with ER/PR positive receptors had improved mortality rates, compared to patients who had negative PR either ER receptors, or both. Moreover, patients with positive PR receptors had enhanced PFS.

Ki-67 is a nuclear protein and biomarker of cell proliferation [41]. In breast cancer patients ki-67 is thought to have predictive role [42.43]. Athough the topic of its effectiveness remains vague, it is believed that it could have additionally purpose in the prediction of responsiveness, resistance to therapy, residual risk in patients on standard therapy, and as a active biomarker in cases of its continuous evaluation during therapy [44].

Not once, it has been reported that breast cancer is associated with thyroidopathy [9]. Moreover, thyroidopathy and more concrete hypothyroidism is linked to favorable progress of the breast cancer [45,46]. Normally, the thyroid hormone activates mitogen-activated protein kinase (MAPK) transmission, which results in phosphorylation of nuclear transactivator proteins, and possibly plasma membrane proteins [47]. 17β-Estradiol is also promoting phosphorylation of serine-118 of the ER receptor α and it is thought that the latter process is MAPK dependant. Cancer cells express

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thyroid receptors on the integrin - $\alpha v\beta 3$. Furthermore, it is believed that thyroid hormone has an estrogen-like effect in breast cancer, resulting in cell proliferation of the cancer cells with this receptor [47]. This means that thyroid hormone could support the breast cancer by two independent mechanisms in both ER negative and ER positive cases. In this term, Falstie-Jense et.al. [48] showed that patients with hypothyroidism and breast cancer had better prognosis. Ortega-Olvera et.al. [49] adds to the fact that thyroid hormones are strongly related to breast cancer, the role of the body mass index (BMI). On the other hand, TSH levels were not associated with breast cancer aggressiveness [10].

Calcium is a chemical element and very important compound of the in vivo metabolism and functions. Studies on animals suggest that it could inhibit also breast tumors [50]. In humans, because of its role in the vitamin D metabolism and parathyroid hormone cycle, it is believed that it could also play a role in breast cancer [51,52]. In the first prospective cohort study Almquist et.al. [53] proposed that calcium is related to an increased risk of breast tumors, while Sprague [54] et.al. reported no such associations.

In the current study we found that patients with thyroidopathy differ significantly from the patients without such, concerning the molecular subtypes, ER and ki-67 biomarker. Moreover, the correlation analysis showed that group A had an interesting pattern of positive correlations. Greater interest here represents the fact that, in contrast to the world data, TSH was mainly linked to poor prognostic values. fT4 hormone had a single association with HER2 receptors. In regard to the postoperative status of the TSH, the analysis showed no difference, concluding that breast cancer does not influence the thyroid disease, but the latter affects the breast cancer.

For group B, the same analysis showed only positive correlations between ki-67, ER,PR, LDH, molecular subtype and T from TNM. Increased ki-67 is linked definitely to worse prognosis. Interest here represents the fact that ki-67 is linked to increased preoperative calcium and LDH levels. The latter could be used also as future prognostic factors.

The entire sample showed significant positive correlations between ki-67 and preoperative calcium levels, ER, PR, Molecular subtype and T (TNM), indicating that preoperative calcium levels could be used for prognosis.

The limitation of the study is that it could not follow patients to calculate overall survival and progression free survival, as well as ki-67 factor was not available for all the patients. The latter was available only after the late 2018 year and later.

Concluding to this, thyroidopahty appears to be a favorable factor for the breast cancer patients and their prognosis, regarding the molecular subtype. In contrast to the world literature, the current study finds that TSH is mainly responsible for the cancer aggressiveness and not the thyroid hormones fT4 and fT3. Calcium levels are linked to the higher tumor stage in the thyroidopathy group and ki-67, suggesting the possibility of its additional use as prognostic factor. Because of the limitations of the current study, more prospective studies would be helpful to examine to what extend the latter plays role for the overall survival and progression free survival.

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References

1. World Health Organization. Breast Cancer. https://www.who.int/cancer/prevention/diagnosis-screening/breast-cancer/en/ Available on 24.03.2020
2. Ferlay, J., Soerjomataram, I., Dikshit, R. et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015; 136: E359–E386
3. Hoon Tan P[1], Ellis I[2], Allison K[3], Brogi E[4], Fox SB[5], Lakhani S[6], Lazar AJ[7], Morris EA[4], Sahin A[7], Salgado R[8], Sapino A[9,10], Sasano H[11], Schnitt S[12], Sotiriou C[13], van Diest P[14], White VA[15], Lokuhetty D[15], Cree IA[15]; WHO Classification of Tumours Editorial Board.The 2019 WHO classification of tumours of the breast. Histopathology. 2020

4. Agarwal I. Blanco L. WHO classification of breast tumors. 2020 https://www.pathologyoutlines.com/topic/breastmalignantwhoclassification.html
5. Perou CM, Sorlie T, Eisen MB, Rijn M van de, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-52.
6. Fragomeni SM, Sciallis A, Jeruss JS. Molecular Subtypes and Local-Regional Control of Breast Cancer. Surg Oncol Clin N Am. 2018;27(1):95–120.
7. Fallahpour S, Navaneelan T, De P, Borgo A. Breast cancer survival by molecular subtype: a population-based analysis of cancer registry data. CMAJ Open. 2017;5(3):E734–E739.
8. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235(4785):177–182
9. Hercbergs A, Mousa SA, Leinung M, Lin HY, Davis PJ. Thyroid Hormone in the Clinic and Breast Cancer. Horm Cancer. 2018;9(3):139–143. doi:10.1007/s12672-018-0326-9
10. Villa NM, Li N, Yeh MW, Hurvitz SA, Dawson NA, Leung AM. SERUM THYROTROPIN CONCENTRATIONS ARE NOT PREDICTIVE OF AGGRESSIVE BREAST CANCER BIOLOGY IN EUTHYROID INDIVIDUALS. Endocr Pract. 2015;21(9):1040–1045.
11. American Cancer Society. Breast Cancer Facts & Figures 2019-2020. Atlanta: American Cancer Society, Inc. 2019
12. Mehrgou A, Akouchekian M. The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. Med J Islam Repub Iran. 2016;30:369.
13. Armaou S, Pertesi M, Fostira F, Thodi G, Athanasopoulos PS, Kamakari S. et al. Contribution of BRCA1 germ-line mutations to breast cancer in Greece: a hospital-based study of 987 unselected breast cancer cases. Br J cancer. 2009;101(1):32–7
14. Calderon-Garciduenas AL, Ruiz-Flores P, Cerda-Flores RM, Barrera-Saldana HA. Clinical follow up of mexican women with early onset of breast cancer and mutations in the BRCA1 and BRCA2 genes. Salud publica de Mexico. 2005;47(2):110–5
15. Tereschenko IV, Basham VM, Ponder BA, Pharoah PD. BRCA1 and BRCA2 mutations in Russian familial breast cancer. Human mutation. 2002;19(2):184
16. Ayub SG, Rasool S, Ayub T, Khan SN, Wani KA, Andrabi KI. Mutational analysis of the BRCA2 gene in breast carcinoma patients of Kashmiri descent. Mol Med Reports. 2014;9(2):749–53.
17. Balraj P, Khoo AS, Volpi L, Tan JA, Nair S, Abdullah H. Mutation analysis of the BRCA1 gene in Malaysian breast cancer patients. Singapore Med J. 2002;43(4):194–7.
18. Chen W, Pan K, Ouyang T, Li J, Wang T, Fan Z. et al. BRCA1 germline mutations and tumor characteristics in Chinese women with familial or early-onset breast cancer. Breast cancer Res treat. 2009;117(1):55–60
19. Musolino A, Naldi N, Michiara M, Bella MA, Zanelli P, Bortesi B. et al. A breast cancer patient from Italy with germline mutations in both the BRCA1 and BRCA2 genes. Breast cancer res treat. 2005;91(2):203–5
20. Lakhani SR, Van De Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L. et al. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol. 2002;20(9):2310–8.
21. Phillips KA. Immunophenotypic and pathologic differences between BRCA1 and BRCA2 hereditary breast cancers. J Clin Oncol. 2000;18(21 Suppl):107s–12s.
22. Pylkas K, Erkko H, Nikkila J, Solyom S, Winqvist R. Analysis of large deletions in BRCA1, BRCA2 and PALB2 genes in Finnish breast and ovarian cancer families. BMC cancer. 2008;8:146.
23. Jancarkova N, Zikan M, Pohlreich P, Freitag P, Matous B, Zivny J. [Detection and occurrence of BRCA 1 gene mutation in patients with carcinoma of the breast and ovary] Ceska gynekologie / Ceska lekarska spolecnost J Ev Purkyne. 2003;68(1):11–6
24. Dutil J, Colon-Colon JL, Matta JL, Sutphen R, Echenique M. Identification of the prevalent BRCA1 and BRCA2 mutations in the female population of Puerto Rico. Cancer genetics. 2012;205(5):242–8.
25. Makki J. Diversity of Breast Carcinoma: Histological Subtypes and Clinical Relevance. Clin Med Insights Pathol. 2015;8:23–31
26. Eheman CR, Shaw KM, Ryerson AB, Miller JW, Ajani UA, White MC. The changing incidence of in situ and invasive ductal and lobular breast carcinomas. Cancer Epidemiol Biomarkers Prev. 2009;18(6):1763–9.
27. Toikkanen S, Pylkkänen L, Joensuu H. Invasive lobular carcinoma of the breast has better short- and long-term survival than invasive ductal carcinoma. Br J Cancer. 1997;76(9):1234–1240.
28. Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol. 2011;22(8):1736–1747. doi:10.1093/annonc/mdr304
29. Harbeck N, Thomssen C, Gnant M. St. Gallen 2013: brief preliminary summary of the consensus discussion. Breast Care (Basel). 2013;8(2):102–109.
30. Balic M, Thomssen Ch, Wuerstlein R, Gnant M, Harbeck N. St. Gallen/Vienna 2019: A Brief Summary of the Consensus Discussion on the Optimal Primary Breast Cancer Treatment. Breast care. 2019; 14: 103-110

31. He L, Lv Y, Song Y, Zhang B. The prognosis comparison of different molecular subtypes of breast tumors after radiotherapy and the intrinsic reasons for their distinct radiosensitivity. Cancer Manag Res. 2019;11:5765–5775
32. Mitri Z, Constantine T, O'Regan R. The HER2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy. Chemother Res Pract. 2012;2012:743193.
33. Iqbal N, Iqbal N. Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. Mol Biol Int. 2014;2014:852748.
34. Björnström L[1], Sjöberg M.Mechanisms of Estrogen Receptor Signaling: Convergence of Genomic and Nongenomic Actions on Target Genes. Mol Endocrinol. 2005 Apr;19(4):833-42
35. Lu Q[1], Pallas DC, Surks HK, Baur WE, Mendelsohn ME, Karas RH.Striatin assembles a membrane signaling complex necessary for rapid, nongenomic activation of endothelial NO synthase by estrogen receptor α. Proc Natl Acad Sci U S A. 2004 Dec 7;101(49):17126-31
36. Paruthiyil S[1], Parmar H, Kerekatte V, Cunha GR, Firestone GL, Leitman DC.Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. Cancer Res. 2004 Jan 1;64(1):423-8.
37. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Davies C, Godwin J, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378(9793):771–784.
38. Song F, Zhang J, Li S, et al. ER-positive breast cancer patients with more than three positive nodes or grade 3 tumors are at high risk of late recurrence after 5-year adjuvant endocrine therapy. Onco Targets Ther. 2017;10:4859–4867.
39. Yao N, Song Z, Wang X, Yang S, Song H. Prognostic Impact of Progesterone Receptor Status in Chinese Estrogen Receptor Positive Invasive Breast Cancer Patients. J Breast Cancer. 2017;20(2):160–169.
40. Dunnwald LK, Rossing MA, Li CI. Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. Breast Cancer Res. 2007;9(1):R6. doi:10.1186/bcr1639
41. Scholzen T, Gerdes J (March 2000). "The Ki-67 protein: from the known and the unknown". J Cell Phys. 182 (3): 311–22
42. Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer Biol Med. 2016;13(4):496–504.
43. Inwald EC, Klinkhammer-Schalke M, Hofstädter F, et al. Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat. 2013;139(2):539–552.
44. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the international Ki67 in Breast Cancer working group. J Natl Cancer Inst. 2011;103:1656–64.
45. Cristofanilli M, Yamamura Y, Kau SW, Bevers T, Strom S, Patangan M, Hsu L, Krishnamurthy S, Theriault RL, Hortobagyi GN. Thyroid hormone and breast carcinoma. Primary hypothyroidism is associated with a reduced incidence of primary breast carcinoma. Cancer. 2005;103(6):1122–1128.
46. Sogaard M, Farkas DK, Ehrenstein V, Jorgensen JO, Dekkers OM, Sorensen HT. Hypothyroidism and hyperthyroidism and breast cancer risk: a nationwide cohort study. Eur J Endocrinol. 2016;174(4):409–414.
47. Tang HY, Lin HY, Zhang S, Davis FB, Davis PJ. Thyroid Hormone Causes Mitogen-Activated Protein Kinase-Dependent Phosphorylation of the Nuclear Estrogen Receptor. Endocrinology 145(7):3265–3272
48. Falstie-Jensen, A.M., Kjærsgaard, A., Lorenzen, E.L. et al. Hypothyroidism and the risk of breast cancer recurrence and all-cause mortality - a Danish population-based study. Breast Cancer Res 21, 44 (2019).
49. Ortega-Olvera, C., Ulloa-Aguirre, A., Ángeles-Llerenas, A. et al. Thyroid hormones and breast cancer association according to menopausal status and body mass index. Breast Cancer Res 20, 94 (2018).
50. Jacobson EA, James KA, Newmark HL, Carroll KK. Effects of dietary fat, calcium, and vitamin D on growth and mammary tumorigenesis induced by 7,12-dimethylbenz(a)anthracene in female Sprague-Dawley rats. Cancer Res. 1989;49:6300–3
51. Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States) Cancer Causes Control. 2005;16:83–95
52. McCarty MF. Parathyroid hormone may be a cancer promoter - an explanation for the decrease in cancer risk associated with ultraviolet light, calcium, and vitamin D. Med Hypotheses. 2000;54:475–82.
53. Almquist M, Manjer J, Bondeson L, Bondeson AG. Serum calcium and breast cancer risk: results from a prospective cohort study of 7,847 women. Cancer Causes Control. 2007;18:595–602
54. Sprague BL, Skinner HG, Trentham-Dietz A, Lee KE, Klein BE, Klein R. Serum calcium and breast cancer risk in a prospective cohort study. Ann Epidemiol. 2010;20(1):82–85.

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