Evaluation of Biochemical Markers Correlated with Breast Cancer Young Patients

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Abstract. Breast cancer ranks first in women in terms incidence and mortality in the world. In Romania its frequency continues to increase and now is the most common cancer in the women. Despite therapeutic advances, there is still severe cancer with heavy sequelae both physical and mental. It's even harder to accept or even reject when it comes to a young women. For a long time, breast cancer was linked to a more or less old age. However, in the recent literature, breast cancer is on the rise more observed in a young population, its frequency is estimated at 7% all cases of breast cancer. Breast cancer diagnosed in young patients tends to be more aggressive than the rest of the cases. In this paper, we present the results of a study that included 59 women presenting primary invasive breast carcinomas selected from the database of the Institute of Oncology Bucharest in which clinical follow-up data cover long intervals of time (2014 - 2019) and whose histological pieces met technical conditions optimal for extended immunohistochemical processing. The available material resources allowed the completion of a complete analyzes in all cases in relation to the immunohistochemical examination . The correlation between the expression of each of the antibodies was calculated using Pearson correlation coefficient.

Keywords: Breast cancer, young age, grade tumors, prognosis, HER2

1.Introduction

Breast cancer ranks first in women in terms incidence and mortality in the world[1]. In Romania its frequency continues to increase and now is the most common cancer in the women, and is therefore a real public health problem [2]. Despite therapeutic advances, there is still severe cancer with heavy sequelae both physical and mental. It's even harder to accept or even reject when it comes to a young women. For a long time, breast cancer was linked to a more or less old age. However, in the recent literature, breast cancer is on the rise more observed in a young population, its frequency is estimated at 7% all cases of breast cancer [3,4].

The prognosis of patients under 35-40 years of age is often more reserved, with a tumor presentation that differs from that of older ones: larger size, worse tumor differentiation, higher proliferation index, lower expression of hormone receptors (close 50% versus 70% in older patients) and a higher rate of lymph node metastasis. Regardless of these usual risk factors, age 35 years remains a negative prognostic factor altering 4.4 times relapse-free survival, 3.2 times the risk of distant metastases, and 2.9 times overall survival [5] . The biology of breast cancer in young women is indeed different from that in older women and this is also evident from genomic analysis studies. [6-7]

The aim of the study was to evaluate the impact of age in breast cancer patients in relation to histological and immunohistochemical parameters, the degree of histological differentiation G, *estrogens receptor* ER, *progesterone receptor* PR, HER 2 status, and the proliferation index KI-67 to increase the impact on the prognosis and management of this patients.

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2. Materials and methods

The retrospective study was conducted on a group of 59 women presenting primary invasive breast carcinomas selected from the database of the Institute of Oncology Bucharest in which clinical follow-up data cover long intervals of time (2014 - 2019) and whose histological pieces met technical conditions optimal for extended immunohistochemical processing (variants morphopathological being ductal invasive, lobular or mixed, in the absence of chemotherapy neoadjuvant). The available material resources allowed the completion of a complete analyzes in 59 cases in relation to the immuno-histochemical evaluation .

This study was reviewed and approved by the Institute of Oncology Bucharest. The clinical characteristic of all the patients were obtained from their medical records.

The correlation between the expression of each of the antibodies was calculated using Pearson correlation coefficient. In all the analyses, the differences were considered significant at $P < 0.05$. Statistical analyses were carried out using SPSS.

3. Results and discussions

In this study, 59 women were diagnosed with invasive primary breast carcinoma with a mean age of 34.14 years (extremes: 24–40 years). Among 59 patients, 66.1% expressed ER, PR, and 33.9% not expressed ER/PR. The proportion of patients overexpressing HER2 was 13.6%. Grade 3 represented the majority, 66.1%, followed by the 2nd degree 28.1%. The mean value of the proliferation index Ki-67 in our study was 36.68 %, with values between 1% and 80%. The increased value of the proliferation index has been shown to be associated with a worse prognosis of the disease in young patients. [8,9]. St. Gallen criteria help in therapeutic decision making it possible to classify patients into risk groups and in 2019 there was no discussion about the Ki-67 values; the focus was on include the analysis of tumor infiltrating lymphocytes (TILS) for the purpose indication or de-escalation of the chemotherapy based on high TILS and establishing cut-off prescription of endocrine therapy for ER-positive tumors, but in 2011 and 2013, the use of proliferation index was recommended for the analysis of cellular proliferation, and for identifying the differentiation of luminal subtype of breast cancer [10].

Table 1. Independent prognostic factors associated with histologic grade

Grade		N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
G1	Ki67	3	74	1	75	**28.67**	40.377	1630.333
	Age	3	1	35	36	**35.33**	.577	.333
	Valid N (listwise)	3						
G2	Ki67	17	65	5	70	**33.41**	21.281	452.882
	Age	17	10	28	38	**34.53**	2.528	6.390
	Valid N (listwise)	17						
G3	Ki67	39	70	10	80	**38.72**	18.663	348.313
	Age	39	16	24	40	**33.87**	3.373	11.378
	Valid N (listwise)	39						

From the analysis of the Ki-67 proliferation index in correlation with age and histological grade resulted that the younger age of the patients was associated with an increased Ki-67 value as shown in Table 1.

Table 2 . Independent prognostic factors associated with her2 expression

| HER		N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
negative	Ki67	51	79	1	80	**36.75**	20.635	425.794
	Age	51	16	24	40	**34.24**	3.057	9.344
	Valid N (listwise)	51						
positive	Ki67	8	65	10	75	**36.25**	20.659	426.786
	Age	8	10	28	38	**33.50**	3.251	10.571
	Valid N (listwise)	8						

The comparisons between patients overexpressing HER2, Ki-67 and age, showed that KI-67 has the same value both in the HER2 positive patients' group and in the HER2 negative patients' group . Statistical analysis also showed that young patients with positive HER2 had higher Ki-67 proliferation rate. The mean value for Ki-67 is lower for the HER group (positive) = 36.25 than for the HER group (negative) = 36.75 (Table 2).

Although there is a difference, this is not statistically significant ($p > 0.05$)

Table 3. Independent prognostic factors associated with er expression

ER		N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
negative	Ki67	20	65	10	75	**39.15**	18.771	352.345
	Age	20	12	28	40	**34.40**	3.299	10.884
	Valid N (listwise)	20						
positive	Ki67	39	79	1	80	**35.41**	21.398	457.880
	Age	39	15	24	39	**34.00**	2.974	8.842
	Valid N (listwise)	39						

Among 59 patients, 66.1% expressed ER, PR, and 33.9% not expressed ER/PR. The age was the same in both groups, the difference was observed in the group of patients with the subtype triple negative where it was observed that the value of the proliferation index was higher than in the group of patients who had estrogen receptors (Table 3).
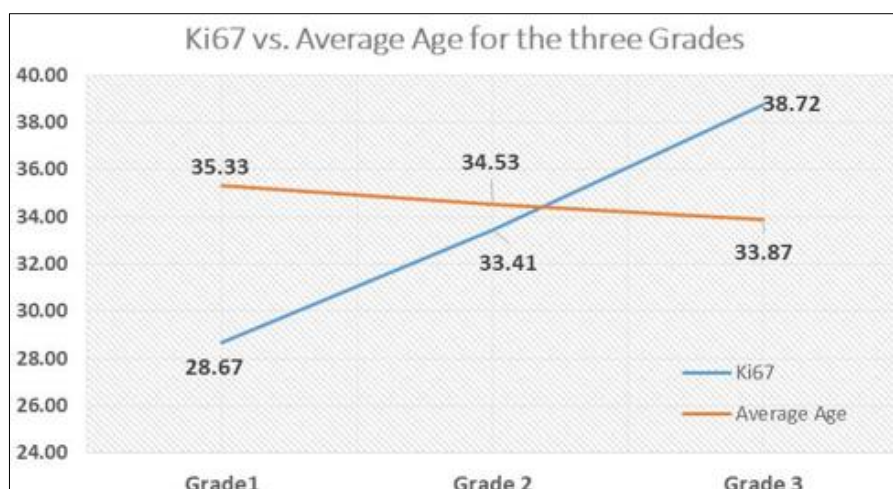
Figure 1. 1 Ki-67 vs. Average Age and histologic grade

From the evaluation of age in relation with the proliferation index Ki-67 and histological grade, it was found that the value of the index Ki-67 increases according to the histological grade.

The mean age of 33 years was associated with the degree of histological differentiation G3 and si represented in Figure 1 and Table 1.

The correlation between Ki-67 and Age is negative, -0.147 (Pearson correlation coefficient) but not statistically significant (has a p = 0.266> 0.05). The trends identified (these directions of correlation) although existing are very small and so it is recommended to continue the investigation on larger samples.

The IHC evaluation of HER2, estrogen receptors and progesterone having a direct impact on the therapeutic approach for young patients with breast cancer. HER2 is a membrane protein from the family of epidermal growth factor receptors (EGFR). This protein is overexpressed in 15 to 30% of breast cancers and is an unfavorable prognostic factor. Trastuzumab (Herceptin) is a humanized antibody targeting the HER2 protein. The benefit of this substance in the adjuvant treatment of HER2-positive breast cancers has been demonstrated in recent years by the preliminary results of four large international studies: a 35 to 50% reduction in the risk of relapse and a third in mortality. Determination of HER2 therefore has a significant impact on the prognosis and management of patients with breast cancer.

The mean age of our study population was independent of HER2 status, although young women overexpressed HER2. Similar results were observed by several authors [11–14].

The expression of estrogen receptors (RE) and progesterone (RP) is currently determined by IHC. It is currently recommended to specify the percentage of positive cells, which reflects the sensitivity of the cells to hormone therapy. In some centers, the results are given in the form of a score taking into account not only the percentage of positive cells, but also intensity of the labeling. However, this last parameter is highly dependent on the technical conditions (fixation and antigenic restoration). During the analysis, invasive carcinoma must be distinguished from carcinoma in situ. The reliability of a negative result implies a positive internal control (marked normal ductal epithelium)

The Ki-67 cut-off value for young breast cancer patients was identified as 36%. Tumors with a Ki-67> 14% are considered proliferative tumors and a correlation was observed between young age, high tumor grade, ER negativity, and HER-2 positivity (Figure 1).

"Triple negative" breast cancers represents about a quarter of breast cancers and is characterized by the absence of hormone receptors for estrogen and progesterone and the lack of HER-2 growth factor overexpression in immunohistochemistry. This breast cancer subtype is associated with a clinical profile unfavorable with a high risk of early metastatic relapse due to the aggressive nature of these tumors, of their part response High Ki-67 levels are often observed in triple-negative subtype cases. Were studied 496 triple-negative subtype womans with lymph node-negative and a mean age of 52 years and a median Ki-67 value of 48%. The study showed that the proliferation index increased with decreasing age and histologic grade [15].

The overexpression of HER2, the high tumor grade, and the young age of our study population are unfavorable clinicopathologic factors supported by data from the literature [16-22].

4. Conclusions

Although the incidence of breast cancer young patient continues to increase in many countries, the mortality linked to this pathology has significantly decreased.

This favorable development is certainly the consequence of the earlier diagnosis by mammographic screening, but also of the progress made in adjuvant treatments. Breast cancer is a heterogeneous disease. The risk of relapse varies enormously depending on the age of the patient, the stage of tumor extension at diagnosis, and the biology of tumor cells which is increasingly seen as a deciding factor. The Ki-67 cut-off value for young breast cancer patients was identified as 36%. Tumors with a Ki-67> 14% are considered proliferative tumors and a correlation was observed between young age, high tumor grade, ER negativity, and HER-2 positivit. Better discrimination between patients who would

benefit from adjuvant therapy and those who could be spared is essential for optimizing the benefit / risk and benefit / cost ratios. The molecular approach, by analyzing the expression of a large number of genes, could soon lead to better efficiency, but still requires further clinical validation.

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