ARE TUMORAL MARKERS PREDICTIVE FACTORS FOR MOLECULAR SUBTYPES IN BREAST CANCER?

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Abstract

Breast cancer kills more women annually than any other type of neoplasm. Even though he has shown a particular interest in the medical body since ancient times, the very heterogeneous nature of the tumors and their versatility have made it difficult to find a diagnostic and therapeutic consensus that fully characterizes this pathology and describes its evolution. Immunohistochemical methods manage to outline a gene-molecular profile depending on the biomarkers expressed by each tumor. Correlations between clinical aspects and molecular characteristics of tumors are of particular importance in the management of such a case. The study examined the impact of breast cancer molecular subtypes on therapeutic management for a group of 438 patients treated on the Oncology Department of the Bucharest University Emergency Hospital during 2007-2017.

Keywords: breast cancer, molecular subtypes, luminal A, luminal B, Her2

Introduction

Breast cancer is the most common female neoplasia, accounting for more than 25% of all diagnosed malignancies. Breast cancer ranks first among the causes of cancer deaths in women, accounting for about 32% of all female cancers and 18% of cancer deaths, with over 1,000,000 new cases/year worldwide, and about 373,000 deaths/year [1].

The natural history of breast cancer is characterized by long duration and marked heterogeneity among patients. Breast cancer is one of the slowest tumors and, as a consequence, both the preclinical period - before diagnosis, and the clinical phase is measured in years and decades. There are also sick people with very aggressive forms. Some have so slowly evolving forms that it is hard to

demonstrate that therapy has any effect on survival. During the clinical phase, there is the possibility of clonal mutations that determine the progression of the disease, with almost all patients having multiple clones, each with its growth, metastasis, sensitivity to drug therapy.

The first step in the individualized diagnostic approach is the accuracy and correctness of the immunohistochemical examination, which complements the clinical-morphological diagnosis. It uses four markers - the "surrogate" method for the gene-molecular profile (ER, PR, HER2, Ki67) [2]. Improving mammalian malignant lesions taxonomy as well as a superior therapeutic response is a consequence of reporting intra- and intergenerational heterogeneity.

It is preferable to have a correct and complete diagnosis, including molecular

compliance, although this is deficient in Romania. What led to the start of this Ph.D. study was the absence of clinical trials on extended batches of patients at both European and Romanian level, studies that highlighted correlations between pathological and clinical aspects and other biomarkers, as well as an assessment of heterogeneity of malignant lesions breast.

The aim of the paper is to analyze the impact of breast cancer's molecular subtypes on therapeutic management.

Materials and Method

The research was retrospective, unicentric and descriptive and covered the period from January 1st, 2007 to December 31st, 2017. During this period, 2515 patients with breast cancer were identified in the Department of Oncology of the University Emergency Hospital Bucharest, Romania.

The inclusion criteria in the study were:

- Patients with a unilaterally expressed or clinically unilateral mammary neoplasm (Stage I, II and IIIA),
- For whom the treatment was with a curative visa,
- And it consisted of local resection or mastectomy,
- With or without axillary lymphadenectomy,
- Who have benefited or not from chemotherapy/radiotherapy,
- For which all the necessary data have been identified in the observation sheets

Exclusion criteria from the study were:

- Stage T0, Tx, Tis or T4
- The presence of metastases (M1)
- Other history of neoplasia

Applying the inclusion criteria to the total number of patients, we obtained the study group of 438 patients.

Results

In the studied group of 438 patients, a number of 226 (representing 51.59%) were classified as Luminal A molecular type and 107 patients (representing 24.42%) were classified

as Luminal type B. 56 patients (representing 12.81%) were triple negative. Fewer patients were classified as HER2, i.e. 49 patients (representing 11.18%).

The age of the patients included in the study ranged from 23 to 90 years with an average of 58.97 and a standard deviation of 12.49 years.

The alpha-fetoprotein was a tumor marker measured together with the carcinoembryonic antigen (ACE) and CA 15-3. Its values ranged between 12.2-273.6 ng/ml with an average of 129.87 ng/ml (Table 1).

Sample size	438
Lowest value	<u>12,20</u>
Highest value	<u>273,60</u>
Arithmetic mean	129,8703
95% CI for the mean	124,687 to 135,053
Median	128,8000
95% CI for the median	122,400 to 135,599
Variance	3046,420
Standard deviation	55,194

Table 1 – Statistical data regarding AFP values

The distribution of the cases studied in relation to AFP is approximately uniform, with a maximum number of cases in the range of 120-140 ng/ml - 63 patients, representing 14.38% followed by 100-120 ng / ml - 52 of cases, representing 11.87% and 140-160 ng / ml - 50 cases representing 11.42% (Figure 1).

Figure 1 – Patient's distribution in relation to AFP value

Table no. 2 and Figure no. 2 show approximate values of the statistical parameters (minimum, maximum, mean, median) relative

to the molecular subtype, and there is no statistically significant predisposition. This empirical observation is confirmed by Table no.

2 where one can observe the lack of statistical correlation.

Molecular subtype	Luminal A	Luminal B	Triplu negativ	HER2
N	226	107	56	49
Mean	130,837	130,776	130,164	123,100
95% CI	123,86 - 137,81	119,41 - 142,13	115,04 - 145,28	107,39 - 138,81
SD	53,2238	59,2780	56,4547	54,6959
Median	129,900	135,200	126,950	122,400
95% CI	118,83 - 138,94	119,35 - 147,31	110,84 - 146,28	105,01 - 138,43
Minimum	23,000	12,200	20,900	26,800
Maximum	273,60	252,60	257,40	270,80

Table 2 – Statistical data regarding the AFP value in relation with the molecular subtype

Figure 2 – Statistical data regarding the AFP value in relation with the molecular subtype

The evaluation of carcinoembryonic antigenic values in our research revealed a minimum value of 0.8 ng/ml as well as a maximum of 10.9 ng/ml with an average of 3 ng / ml (Table no. 3, Figure 3).

Sample size	438
Lowest value	<u>0,8000</u>
Highest value	<u>10,9000</u>
Arithmetic mean	3,0023
95% CI for the mean	2,7719 to 3,2326
Median	1,9500
95% CI for the median	1,8000 to 2,1000
Variance	6,0157
Standard deviation	2,4527

Table 3 – Statistical data regarding the ACE value

The distribution of patients based on the ACE value showed a maximum number of cases in the range of 1-2 ng/ml, 72 patients, representing 40.41%, followed by the range of 2-3 ng/ml with 88 cases representing 20.09 % (Figure 3).

Figure 3 – The distribution of patients based on the ACE value

Analysis of the batch studied in terms of ACE value relative to the four molecular subtypes highlights in the Luminal A and Luminal B subtypes a series of cases outside the normal distribution range, with values much higher than average (2.53, respectively 2.52). In the case of triple negative and HER 2 subtypes, a higher mean value is observed compared to the first two categories (4.75 and 4.21 respectively) (Table 4 and Figure 4).

Molecular subtype	Luminal A	Luminal B	Triplu negativ	HER2
N	226	107	56	49
Mean	2,534	2,522	4,752	4,212
95% CI	2,277 - 2,790	2,093 - 2,952	3,885 - 5,619	3,446 - 4,978
SD	1,9563	2,2410	3,2380	2,6666
Median	1,900	1,600	3,950	3,600
95% CI	1,700 - 2,022	1,500 - 1,800	2,400 - 5,762	2,600 - 4,583
Minimum	0,800	0,800	0,800	1,000
Maximum	10,900	10,500	10,700	10,

Table 4 – Statistical data regarding the ACE value in relation to the molecular subtype

Figure 4 – Statistical data regarding the ACE value in relation to the molecular subtype

The evaluation of CA15-3 showed values ranging from 4.3 - 92.5 ng/ml, with an average of 43.57 ng/dl and a median of 43.25 ng/ml (Table 5 and Figure 5).

Sample size	438
Lowest value	<u>4,3000</u>
Highest value	<u>92,5000</u>
Arithmetic mean	43,5788
95% CI for the mean	41,5371 to 45,6205
Median	43,2500
95% CI for the median	39,9002 to 47,2998
Variance	472,6681

Table 5 – Statistical data regarding the CA 15-3 value

The graph in Figure no. 5 shows the distribution of the patients included in the study relative to the value of CA 15-3 tumor marker. Thus, an equal number of cases was observed in the 20-30, 30-40 and 40-50 ng/ml ranges (60 patients, representing 13.7%) and a higher

number of cases in the 60-70 ng/ml range (66 patients, representing 15.07%).

Regarding the value of this tumor marker relative to the molecular subtype, an average value of this higher parameter is observed for the negative triple and Her2 subtypes (18.2 and 15.2 ng/ml as compared to 38.83 and 41, 96 ng/ml in the Luminal A and Luminal B lots). (Figures 6 and Table 6).

Figure 5 – The distribution of patients relative to the CA 15-3 value

Figure 6 – Statistical data about the CA 15-3 value in relation to the molecular subtype

Molecular subtype	Luminal A	Luminal B	Triplu negativ	HER2
N	226	107	56	49
Mean	38,834	41,969	54,366	56,649
95% CI	36,309 - 41,359	37,630 - 46,308	49,388 - 59,344	49,529 - 63,769
SD	19,2618	22,6387	18,5887	24,7882
Median	37,700	43,500	56,250	60,400
95% CI	35,378 - 41,422	36,559 - 49,837	48,037 - 61,927	47,217 - 70,065
Minimum	4,300	6,000	18,200	15,200
Maximum	72,800	81,900	87,300	92,500

Table 6 – Statistical data about the CA 15-3 value in relation to the molecular subtype

	AUC	SE ^a	95% CI ^b
AFP	0,508	0,0278	0,460 to 0,556
ACE	0,576	0,0277	0,528 to 0,623
CA_15__3	0,624	0,0268	0,576 to 0,669

Figure 7 – Tumor markers' correlation with Luminal A subtype

	AUC	SE ^a	95% CI ^b
AFP	0,500	0,0423	0,452 to 0,548
ACE	0,688	0,0428	0,642 to 0,731
CA_15__3	0,659	0,0347	0,612 to 0,703

Figure 9 – Tumor markers' correlation with the Triple negative subtype

	AUC	SE ^a	95% CI ^b
AFP	0,513	0,0339	0,465 to 0,561
ACE	0,614	0,0318	0,567 to 0,660
CA_15__3	0,522	0,0337	0,474 to 0,569

Figure 8 – Tumor markers' correlation with Luminal B subtype

	AUC	SE ^a	95% CI ^b
AFP	0,555	0,0321	0,507 to 0,603
ACE	0,591	0,0312	0,544 to 0,638
CA_15__3	0,587	0,0336	0,539 to 0,634

Figure 10 – Tumor markers' correlation with the HER2 subtype

Discussions

Benign or malignant breast tumors are diagnosed by classical staining - hematoxylin-eosin. Additional immunohistochemical tests are required for cases where diagnostic problems occur - both epithelial and mesenchymal proliferation - to accurately determine histogenesis, tumor subtype, and other histological features. As mentioned above, the current 4 biomarkers are used: Ki67, ER, PR, and Her-2. Additionally, other biomarkers may be useful in highlighting the histopathological subtype - E-cadherin or to highlight molecular features with important implication for prognosis and therapy [3].

International therapeutic guidelines recommend adjuvant endocrine therapy or Herceptin for patients with mammary carcinoma in which the tumor expresses estrogen (ER) and/or progesterone (PR) receptors as well as HER2 [4]. An increase in the Ki67 proliferative index, with a high histopathological grade (G2-G3), and the ER / PR negativity are factors indicating the use of chemotherapy[5]. The current diagnostic guidelines recommend reporting the size, type, and histological degree as well as immunohistochemical characters only in the case of the index tumor.

Conclusions

In our study no correlation of tumor markers with the molecular subtype could be demonstrated. Advances in the understanding of carcinogenesis and custom management, both at patient and tumor specificity level, have led to decreasing mortality and achieving oncological desideratum in an increasing number of patients. We consider mandatory to further investigate the implications of molecular characteristics of the tumor in the management of breast cancer.

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