ORIGINAL ARTICLE

BREAST CANCER FROM THE PERSPECTIVE OF MOLECULAR SUBTYPES

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Abstract

Breast cancer is the most common malignancy in women and the second for both sexes, after pulmonary cancer. The definition of certain subtypes and the correlations with immunohistochemical markers provide new perspectives in terms of prognosis and may be useful in achieving individualized treatment for each patient. Our study aimed to determine, in a selected group of patients, which is the molecular profile of patients with breast cancer to validate or invalidate this distribution for Romanian patients. The research was retrospective, monocentric, and descriptive, and covered the period from January 1st, 2007 to December 31st, 2017. During this time, there were 2515 patients with breast cancer hospitalized in the Oncology Department of the University Emergency Hospital Bucharest, Romania, 438 of them meeting the inclusion criteria. Modern breast cancer management nowadays involves more than surgical and oncological chemotherapy and radiation therapy. It requires a deeper understanding of the mechanisms that lead to cancer initiation as well as of the targeted treatment methods for each cancer subtype.

Keywords*: breast cancer, molecular subtypes, hormonal receptors, HER2*

Introduction

Breast cancer is the most common malignancy in women and the second overall, after pulmonary cancer [1]. According to OMS in 2015 there were reported approximately 1.4 million of new breast cases worldwide and approximately 570.000 deaths (representing approximately 15% of total cancer related deaths in women) [2].

Classic breast cancer classification systems have been defined only using biological criteria such as the age of the Patient, the dimensions of the tumor, histological type, the status of the lymphatic ganglions or the presence of metastasis [3]. Only the histological aspect of the tumor seems not to be sufficient to establish he complex genetic modifications which underlying the bases of initiation and progression of cancer. Immunohistochemical examination has a very important role which offers the possibility of identification of molecular tumor characteristics which have the value of alternative markers, surrogates, which correspond with the genetic profile [4].

Modern histological exam criteria are represented by the immunohistochemical properties described by: estrogen receptors (ER), progesterone receptors (PR), human epidermal receptor 2 (HER2 or c-erbB2), KI 67 and E-cadherin [5-8].

The definition of certain subtypes and the correlations with immunohistochemical markers provide new perspectives in terms of prognosis and may be useful in achieving individualized treatment for each patient.

In 1970, Jensen is the first to describe the estrogen receptor and he proved that it isn't present in all types of breast cancer. Nearly a century before, in 1896, Beaston proved for the first time the regression of advanced breast cancer by removing the ovaries, in patients in premenopausal diagnose, while noting that it did not occur in all cases [9-13].

Perou and colleagues described four major molecular types. These are: estrogen receptor (ER), human epidermal growth factor receptor (HER2), basal-like and normal-like [3,14-18].

Our study aims to determine, in a selected group of patients, which is the molecular profile of patients with breast cancer to validate or refute this distribution for patients in Romania.

Materials and methods

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

The inclusion criteria in the study were:

- Patients with breast cancer (stage I, II and III)
- For whom treatment was with a curative visa
- And it consisted of local resection or mastectomy with or without axillary lymphadenectomy
- That have or have not undergone chimio-radiotherapy
- For which all the necessary data have been recorded in the observation charts

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

Results

The study group presented a distribution of the ages ranging from 23 to 90 years with an average of 58.97 years, a standard deviation of 12.49 years and a median of 59 years (Figure 1).

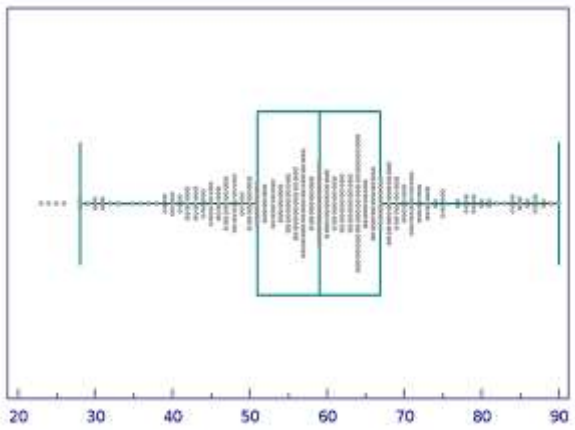

Figure 1 – Age distribution in the studied lot

Concerning the positivity of hormone receptors and of HER2, we notice 252 ER + cases, representing 57.35%, 216 PR + cases, representing 49.31%, respectively 110 cases of HER2 +, representing 25.11% of the total number of cases.

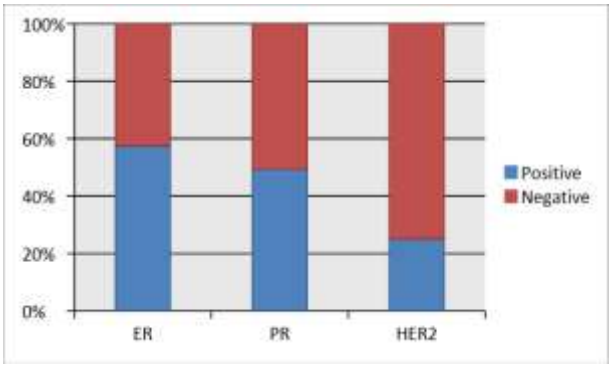

Figure 2 - Patient distribution according to receptor positivity

From the point of view of the molecular subtype, a number of 226 patients, representing 51.60%, were characterized as Luminal A subtype, 107 patients, representing 24.42%, were characterized as Luminal B subtype, 49 patients, representing 11.19% in the subtype of those with overexpression of the HER2 gene and 56 patients, representing 12.79%, were Triple Negatives (Figure 3).

A comparative analysis of the age statistics of patients enrolled in the study according to the molecular subtype revealed an average age with a minimum value of 50.64 years for triple negative patients. The other 3 groups (Luminal A, Luminal B and HER2)

showed an average age of approximately 60 years (Figure 4 and Table 1).

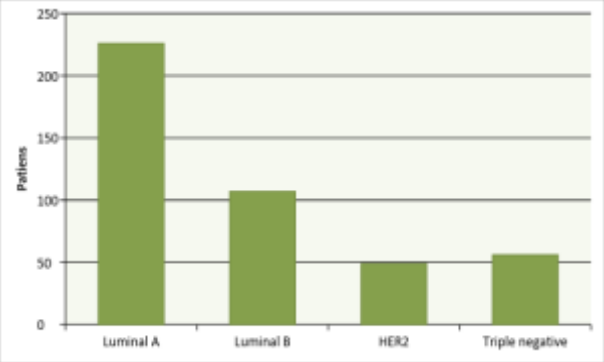

Figure 3 - Distribution of patients by molecular subtype

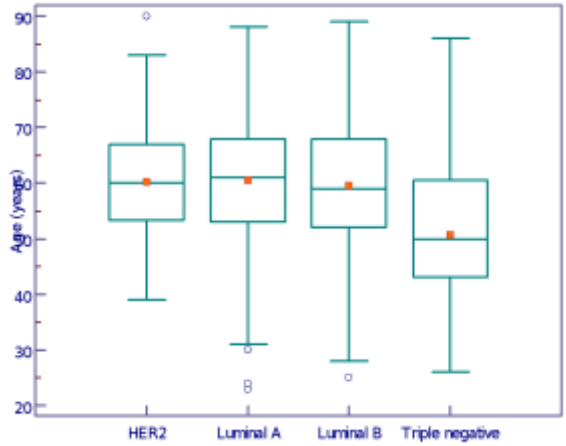

Figure 4 - Comparative statistical information by molecular subtype

	Age			
	HER2	Luminal A	Luminal B	Triple negative
N	49	226	107	56
Mean	60,16	60,50	59,53	50,64
SD	11,07	12,04	12,56	12,41
Median	60	61	59	50
Minimum	39	23	25	26
Maximum	90	88	89	86

Table 1 - Comparative statistical information by molecular subtype

Discussions

Estrogen receptors belong to the superfamily of steroid hormone receptors being a ligand-dependent transcription factor. They are composed of two receptors, alpha-estrogen (ER-α) and beta-estrogens (ER-β), both of which bind with high affinity to the estrogen ligand [19].

Estrogen receptors (ER) and/or progesterone (PR) are positive in about 75% of cases diagnosed with breast cancer. There are genes encoding cells of the luminal epithelium, being called luminal. These receptors comprise 2 main subtypes, luminal A and luminal B [20].

The luminal A subtype is seen in approximately 70% of cases. It includes tumors that typically have a low histological differentiation degree (G1-G2), being characterized by higher ER levels and lower levels of proliferation related genes. Luminal-A type is defined as ER-positive and / or PR-positive tumors with negative HER2 index and Ki67 <14% (proliferating nuclear antigen) by immunohistochemistry. Patients diagnosed with this subtype have a better prognosis, higher survival rate and a good response to hormone therapy; also, the risk of relapse is lower compared to the other subtypes [21].

Luminous subtype B - occurs in about 15-20% of mammary neoplasms, has a higher histological degree, increased aggression, increased number of proliferation genes; the most important genes involved in proliferation are: avian myeloblastosis viral oncogene homolog (V-MYB), gamma glutamyl hydrolase (GGH), transmembrane protein associated with 4-beta lysosome (LAPTMB4), nuclease-sensitive element binding protein 1(NSEP1) and cyclin E1 (CCNE1). This subtype is defined by ER positive, PR positive, HER positive / negative, ki67> 14%. It is correlated with a worse prognosis, a lower survival rate and a high relapse risk; there was also a low response to hormone therapy compared to luminal A subtype, but a better response to neoadjuvant therapy [21].

The HER-2, acronym for human epidermal growth factor receptor-2, is considered an important marker for the diagnosis and prognosis of breast cancer. It is expressed on the surface of normal cells. Its overexpression occurs in 20-25% of tumor cells of breast cancer because of alterations in ERBB2 amplification. Morphologically, these tumors are proliferative, have high histological and nuclear degrees, and more than 40% have mutations of the p53 gene [22-26]. Approximately 50% of HER2-positive mammary neoplasms are positive for ER, but generally express low ER levels. Tumors that

overexpress this gene are called HER-2 + and they represent the most aggressive type of breast cancer by aggressive metastasis (especially bone, cerebral and visceral) and increased resistance to therapy. This subtype is non-responsive to endocrine therapy but has a fairly high sensitivity to the administration of Trastuzumab (Herceptin) - monoclonal antibody or Doxorubicin; they significantly increase the survival rate; in the absence of treatment, the prognosis is poor [21].

Following in-depth studies, Staff et all subclasses HER2 + tumors according to prognosis in three distinct classes: one with poor prognosis and a survival rate of only 12% at 10 years compared to survival of 50-55% in the other two groups, using HER2 derived prognostic predictor (HDPP). HDPP is associated with genes related to the immune response to tumor invasion and metastasis [27-31].

Triple Negative - affects especially young women aged 45 and over, with a higher African American prevalence. This subtype represents 10-15% of all breast neoplasms characterized by a high histological grade, high mitotic and proliferative indexes and the presence of central areas of necrosis and fibrosis. Tumors have elevated levels of basal myoepithelial markers such as EGFR, CK5, CK14 and CK17 but do not express ER, PR or HER2, which is why they are termed triple negative [32].

The American Oncology Society defines triple negative tumors by lack of ER / PR expression (<1%) and HER2 expression (0 or 1+) and HER2 status confirmation by fluorescence in situ hybridization (FISH) if undetermined (2+) by IHC [33].

Macroscopically, these tumors are large, palpable, but with an increased risk of relapse and a reserved prognosis. Metastasis often occurs in the first 3 years from diagnosis and is localized especially at the visceral level [34].

In the treatment of these neoplasms, specific therapies are not momentarily available, so the standard is a combination of a taxane and an anthracycline. Although the prognosis is reserved, it seems that these tumors respond better to the treatment compared to the luminal subtype. This concept is termed the "triple negative paradox" [35].

Conclusions

Modern breast cancer management nowadays involves more than surgical and oncological chemotherapy and radiation therapy. It requires a deeper understanding of the mechanisms that lead to cancer initiation as well as of the targeted treatment methods for each cancer subtype.

References

[1]Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011;121:2750–2767.

[2]Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004;351:2817–2826.

[3]Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature. 2000;406(6797):747–752.

[4]Colditz GA, Rosner BA, Chen WY, Holmes MD, Hankinson SE. Risk factors for breast cancer according to estrogen and progesterone receptor status. J Natl Cancer Inst. 2004;96:218–228.

[5]Roessler J, Ammerpohl O, Gutwein J, Steinemann D, Schlegelberger B, Weyer V, Sariyar M, Geffers R, Arnold N, Schmutzler R, Bartram CR, Heinrich T, Abbas M, Antonopoulos W, Schipper E, Hasemeier B, Kreipe H, Lehmann U. The CpG island methylator phenotype in breast cancer is associated with the lobular subtype. Epigenomics. 2015;7:187–99.

[6]Millikan R, Newman B, Tse C, et al. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat. 2008;109:123–139.

[7]Iorio MV, Casalini P, Piovan C, Di Leva G, Merlo A, Triulzi T, Menard S, Croce CM, Tagliabue E. microRNA-205 regulates HER3 in human breast cancer. Cancer Res. 2009;69:2195–2200.

[8]Cronin KA, Harlan LC, Dodd KW, et al. Population-based estimate of the prevalence of HER-2 positive breast cancer tumors for early stage patients in the US. Cancer Invest. 2010;28(9):963–968.

[9]Haque R, Ahmed SA, Inzhakova G, et al. Impact of breast cancer subtypes and treatment on survival: an analysis spanning two decades. Cancer Epidemiol Biomarkers Prev. 2012;21(10):1848–1855.

[10]Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, Watson M, Davies S, Bernard PS, Parker JS, Perou CM, Ellis MJ, Nielsen TO. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009;101:736–50.

[11]Sotiriou C, Neo SY, McShane LM, Korn EL, Long PM, Jazaeri A, Martiat P, Fox SB, Harris AL, Liu ET.

Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci U S A. 2003;100:10393–10398.
[12]Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975–2010. Bethesda, MD: National Cancer Institute; 2013. http://seer.cancer.gov/csr/1975_2010/ Accessed June 17, 2013.
[13]Singh GK MB, Hankey BF, Edwards BK.Area Socioeconomic Variations in U.S. Cancer Incidence, Mortality, Stage, Treatment, and Survival, 1975–1999. NCI Cancer Surveillance Monograph Series, Number 4. NIH Publication No. 03-5417.Bethesda, MD: National Cancer Institute; 2003.
[14]SEER*Stat Software [computer program]. Version 7.0.4. Bethesda, MD: Surveillance Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute; 2010.
[15]Clarke CA, Keegan TH, Yang J, et al. Age-specific incidence of breast cancer subtypes: understanding the black-white crossover. J Natl Cancer Inst. 2012;104(14):1094–1101.
[16]Parise CA, Bauer KR, Brown MM, et al. Breast cancer subtypes as defined by the estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2) among women with invasive breast cancer in California, 1999–2004. Breast J. 2009;15(6):593–602.
[18]Porter PL, El-Bastawissi AY, Mandelson MT, et al. Breast tumor characteristics as predictors of mammographic detection: comparison of interval- and screen-detected cancers. J Natl Cancer Inst. 1999;91(23):2020–2028.
[19]Spitale A, Mazzola P, Soldini D, Mazzucchelli L, Bordoni A. Breast cancer classification according to immunohistochemical markers: clinicopathologic features and short-term survival analysis in a population-based study from the South of Switzerland. Ann Oncol. 2009;20:628–635.
[20]Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Arch Pathol Lab Med. 2010;134(7):e48–e72.
[21]Vallejos CS, Gomez HL, Cruz WR, Pinto JA, Dyer RR, Velarde R, Suazo JF, Neciosup SP, Leon M, de la Cruz MA, Vigil CE. Breast Cancer Classification According to Immunohistochemistry Markers: Subtypes and Association With Clinicopathologic Variables in a Peruvian Hospital Database. Clinical Breast Cancer. 2010;10:294–300.
[22]Pusztai L, Broglio K, Andre F, Symmans WF, Hess KR, Hortobagyi GN. Effect of molecular disease subsets on disease-free survival in randomized adjuvant chemotherapy trials for estrogen receptor-positive breast cancer. J. Clin. Oncol. 2008;26:4679–4683.
[23] Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature. 2000;406:747–752.
[24]Gatza ML, Lucas JE, Barry WT, Kim JW, Wang Q, Crawford MD, Datto MB, Kelley M, Mathey-Prevot B, Potti A, Nevins JR. A pathwaybased classification of human breast cancer. PNAS. 2010;107:6994–6999.
[25]Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61–70.
[26]Reis-Filho JS, Pusztai L. Gene expression profiling in breast cancer: classification, prognostication, and prediction. Lancet. 2011;378:1812–1823.
[27]Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin S, Dunning MJ, Barbosa-Morais NL, Teschendorff AE, Green AR, Ellis IO, Tavaré S, Caldas C, Miska EA. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. Genome Biol. 2007;8:R214
[28]Rakha EA, Putti TC, Abd El-Rehim DM, Paish C, Green AR, Powe DG, Lee AH, Robertson JF, Ellis IO. Morphological and immunophenotypic analysis of breast carcinomas with basal and myoepithelial differentiation. J Pathol. 2006;208:495–506
[29]Toussaint J, Sieuwerts AM, Haibe-Kains B, Desmedt C, Rouas G, Harris AL, Larsimont D, Piccart M, Foekens JA, Durbecq V, Sotiriou C. Improvement of the clinical applicability of the Genomic Grade Index through a qRT-PCR test performed on frozen and formalin-fixed paraffin-embedded tissues. BMC Genomics. 2009;10:424.
[30]Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, Cronin M, Baehner FL, Watson D, Bryant J, Costantino JP, Geyer CE Jr, Wickerham DL, Wolmark N. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J. Clin. Oncol. 2006;24:3726–3734
[31]Buyse M, Loi S, van't Veer L, Viale G, Delorenzi M, Glas AM, d'Assignies MS, Bergh J, Lidereau R, Ellis P, Harris A, Bogaerts J, Therasse P, Floore A, Amakrane M, Piette F, Rutgers E, Sotiriou C, Cardoso F, Piccart MJ, Consortium T. Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. J Natl Cancer Inst. 2006;98:1183–1192
[32] Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006;103:2257–2261.
[33]Van Roosbroeck K, Pollet J, Calin GA. miRNAs and long noncoding RNAs as biomarkers in human diseases. Expert Rev Mol Diagn. 2013;13:183–204.
[34]Carey L, Perou C, Livasy C, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. J Am Med Assoc. 2006;295:2492.
[35]Kurian A, Fish K, Shema S, et al. Lifetime risks of specific breast cancer subtypes among women in four racial/ethnic groups. Breast Cancer Res. 2010;12(6):R99.