

ORIGINAL PAPER

Clinicopathologic features and five years survival analysis in molecular subtypes of breast cancer

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Abstract

Purpose. In the last years, the incidence of breast cancer has been increasing; characteristic patterns of gene expression have emerged, reflecting molecular differences between previously known as well as newly defined subtypes of breast cancer. This study aimed to classify the molecular subtypes of breast cancers based on the expression profile of immunohistochemical markers and to evaluate their association with clinicopathological features. **Material and Methods.** A total of 173 cases of breast carcinoma were examined retrospectively using immunostains for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2). Because the triple-negative phenotype, when defined by IHC using only these three markers, is not the optimal method for defining basal-like breast cancer, we need to use an additional marker – CK 5/6. **Results.** The luminal type was the most common subtype in breast cancer (71.6%), which was followed by the basal subtype (21.9%). HER2 subtype were 2.8% from the total of cases, being associated with the highest rate of high-graded cases. Basal type is presented largely in premenopausal women and displayed aggressive features, such as large tumor size and poorly differentiated cancers. Luminal A included the highest percentage of patients older than 60 years, the highest proportion of stage I–II tumors and well/moderately differentiated lesions. HER2-type was more frequent in premenopausal women and showed a high percentage of positive lymph nodes. **Conclusions.** These molecular differences have been shown to correlate very well with clinical features and survival, or even better than traditional histopathological parameters. The discovery of certain molecular characteristics of breast cancers has helped us to understand better the pathophysiology of disease and to develop more direct therapeutic strategies.

Keywords: breast cancer, molecular classification, clinicopathologic features.

Introduction

Invasive breast cancer is the most common carcinoma in women; it accounts for 22% of all female cancers. The areas of high-risk are the populations of North America, Europe and Australia; the risk is low in the less developed regions of sub-Saharan Africa and Southern and Eastern Asia, including Japan. Breast cancer is a heterogeneous disease from a clinical, pathological, and therapeutic point of view; classical histological classifications do not fully capture the various clinical courses of this disease. Breast cancer incidence, as with most epithelial tumors, increases rapidly with age. Histological type, grade, tumor size, lymph node involvement, and estrogen receptor (ER), progesterone receptor (PR) and HER2-receptor status, all influence prognosis and the probability of response to systemic therapies.

Based on the recent DNA-microarray studies on breast cancer cases, distinct molecular subtypes of breast carcinoma were identified with different clinical outcomes [1]. Reverse transcription polymerase chain reaction and DNA-microarrays are increasingly used in the clinic and in clinical research as prognostic or predictive tests. Results from these tests led to novel risk stratification methods and to new molecular

classification of breast cancer. However, large-scale subtyping using gene expression profiling from formalin-fixed, paraffin-embedded samples is not currently feasible. Therefore, immunohistochemical (IHC) markers have been used as surrogates for DNA-microarray in subtyping breast cancer. ER, PR, HER2 are determined in most pathology labs, which allows for framing the breast cancer cases in the known molecular classes. The HER2/neu-gene is a member of a gene family encoding transmembrane receptors for growth factors, including EGFR, HER2, HER3 and HER4. Approximately 25 to 30% of invasive female breast cancers over-express HER2 [2].

By using a panel of four antibodies including estrogen receptor (ER), HER1, HER2 and cytokeratin 5/6 (CK5/6), Nielsen *et al.* characterized three IHC-defined subtypes: luminal (ER+, HER2-), HER2 (HER2+) and basal-like (ER-, HER2-, CK5/6+ or HER1+). Based on more recent gene expression studies, Carey *et al.* [3] updated IHC subtype definition as luminal A (ER+ and/or progesterone receptor PR+, HER2-), luminal B (ER+ and/or PR+, HER2+), HER2+/ER- (ER-, PR-, HER2+), basal-like (ER-, PR-, HER2-, CK5/6+) and unclassified (negative for all five markers). These molecular differences have been shown to correlate with clinical features, such as survival,

prognosis and treatment sensitivity. In addition to these subtypes, the prognosis of breast cancer may be affected by expressions of other additional molecular markers. One from those is the epidermal growth factor receptor (EGFR) which was reported to be overexpressed in aggressive breast cancer [4, 5] and is currently being evaluated in clinical trials as a potential therapeutic target [6, 7]. This paper reports our attempt to subclassify breast carcinomas based on the immunoprofile and to evaluate the association of the subtypes with histological type and grade, lymph node status, clinical stage and survival.

Material and Methods

Slides and paraffin-embedded tissue blocks from 173 patients with breast cancer were retrieved from the surgical pathology archives in the Departments of Pathology of The Municipal Clinical Hospital from Timisoara, between September 2000 and November 2005. The histologic diagnosis was completed with clinical and therapeutic data from medical records from the Oncological Surgery, Radiotherapy and Chemotherapy Departments, and other information obtained through a questionnaire sent by mail. Data include: grade according to the Scarff–Bloom–Richardson scale, lymph node involvement; estrogen receptor (ER), progesterone receptor (PR) status, HER2; CK5/6 status was determined immunohistochemically.

Based on these, breast tumors were categorized in the five known molecular classes: basal-like, luminal A, B, HER2, and unclassified. Hundred and seventy-three cases were selected which can be completely assessed morphologically in the classic manner. The histological classification was based on the criteria set by the *World Health Organization*. Survival time was calculated from the date of surgery until time of death or confirmation of survival, and survival rate was represented by the percentage of survivals at the end of the observed interval (a mean of 68 months). Tissue sections (4 µm) from each case were prepared for immunostaining. After incubation in a 60°C oven overnight and deparaffinization, the tissue sections were treated with 3%

hydrogen peroxide in methanol for five minutes. Following a brief pretreatment with 0.02% protease XXIV for two minutes, the slides were incubated with one of the following antibodies: anti-human ER, PR, HER2 and CK5/6. The color was visualized by incubation with DAB chromogen for 16 minutes. The slides were then counterstained with Mayer's Hematoxylin. The staining patterns and intensities for each of the markers were interpreted by two pathologists. For the evaluation of ER, PR, the cut-off positivity was 10% tumor cells. A positive HER2-stain was determined by membranous staining of tumor cells greater than 2+. An estimation of more than 10% of tumor cells with membranous or cytoplasmic staining was required for a positive interpretation of CK5/6. The IHC-based definition of breast cancer subtypes used in this study was as follows: luminal A (ER+ and/or PR+, HER2-), luminal B (ER+ and/or PR+, HER2+), HER2+/ER- (ER-, PR-, HER2+), and basal-like (ER-, PR-, HER2-, CK5/6+) [4].

Results

The most common findings (97%) in these patients are breast lumps, which may or may not be associated with pain. Nipple abnormalities (discharge, retraction, distortions or eczema) are present and other forms of presentation are absent. Only a few cases (5) were positive for regional lymph node metastasis.

Based on immunophenotyping, the most common subtype in our group (which included only women), was luminal A subtype (53%), followed by basal subtype – 21% (Figure 1). The clinicopathological features of breast carcinoma from the 173 cases are summarized in Table 1.

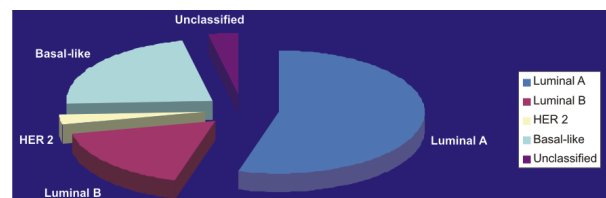


Figure 1 – Distribution of cases.

Table 1 – The clinicopathological features of breast carcinoma

Characteristics	Luminal A	Luminal B	HER2	Basal-like	Unclassified
Number of cases (173)	92	32	5	38	6
Age of patients	31–85	36–83	37–68	34–78	41–84
Mean age	58	54	52	53	59
Menopausal status					
Premenopausal	21	13	4	26	2
Postmenopausal	71	19	1	12	4
Clinical stage at diagnosis					
Stage I	6	1	0	1	0
Stage IIa	41	8	0	13	2
Stage IIb	22	8	2	8	2
Stage IIIa	13	6	3	6	2
Stage IIIb	8	7	0	10	0
Stage IV	2	2	0		0
Tumoral size [cm]					
Mean	2.5	3.1	4.4	4.5	3.2
Under 2 cm	48	8	0	3	3

Characteristics	Luminal A	Luminal B	HER2	Basal-like	Unclassified
<i>Between 2 and 5 cm</i>	36	13	1	21	2
<i>More than 5 cm</i>	8	11	4	14	1
Grading					
<i>Low</i>	9	2	0	1	0
<i>Medium</i>	81	26	2	16	4
<i>High</i>	2	4	3	21	2
Lymph node involvement					
<i>Absent</i>	38	11	2	10	2
<i>Present</i>	54	21	3	28	4
Histology					
<i>Ductal</i>	58	19	5	24	4
<i>Lobular</i>	7	1	0	0	0
<i>Mix (ductal and lobular)</i>	18	8	0	4	1
<i>All other</i>	9	4	0	10	1
<i>Number of death at the end of 5 year follow-up</i>	12	8	2	15	2
<i>Five-year relative survival rates (RSR) [%]</i>	86	75	60	60	66

Luminal-like cancers represent the highest proportion (71.6%); they tend to have the most favorable long-term survival, the overall five-year relative survival being between 86–75%. The luminal subtypes are low/medium grade (97% for luminal A and 86% for luminal B). Luminal tumors, both A and B, express hormone receptors, but these two luminal subtypes present distinguishing characteristics. Luminal A cancers have a high expression of ER and PR, HER2-negative; including the highest percentage (53%), the highest proportion of stage I–II (75%) and well/moderately-differentiated lesions (97%). Ages of the patients ranged from 31 to 85-year-old with a mean age of 58 years. Luminal B cancers (18%) have a lower expression of ER and PR with HER2-positive. Patients with luminal B subtype tumor were between the same age limits, but younger than those with luminal A subtype, mean age being 54 years. Luminal B cases have shown poorly differentiated cancers, in a larger percentage than luminal A cases, this probably being one of the explanations for the difference between the two kinds of survival rates (Figure 2).

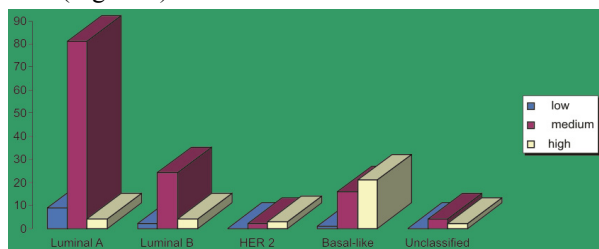


Figure 2 – Distribution of cases according to tumor differentiation.

Most of the cases are postmenopausal: 77% in the case of luminal A type, 59% in the cases of luminal B. Patients with luminal B subtype carcinomas had an increased tendency to involve lymph nodes (65%) compared with patients with luminal A subtype tumors (58%). The histological versions of luminal A and luminal B were as follows: 63% and 59%, respectively, invasive ductal carcinomas; the luminal B subtype presented only one lobular carcinoma option, but eight cases were mixed (lobular and ductal). In up to 45 luminal cases, foci of associated ductal carcinoma in

situ (DCIS) were present and foci of elastosis, in a periductal or perivenous distribution. In a minority of cases, a distinct lymphoplasmacytoid infiltrate can be identified. Most of mucinous carcinoma cases were recorded in the luminal subtype (six out of seven).

A relatively high percentage (21.9%) represents the basal-like cases. Most women with BRCA1-mutations generally develop basal-like breast cancer. In this study, BRCA mutations could not be determined, but 36% cases had first-degree relatives with history of breast cancer. Patients' age was between 34 and 78, with an average of 53 years. This type is presented largely in premenopausal women (68%) and displays aggressive features, such as large size, poorly differentiated cancers (Figure 2). Most cases were invasive ductal carcinomas and 73% presented nodal metastasis at the time of diagnosis. Nine cases were of medullar type: poorly differentiated cells arranged in large sheets, with no glandular structures, scant stroma and a prominent lymphoplasmocytic infiltrate. Recent data show the fact that the lymph node status is not a major prognostic factor in the case of basal-like tumors. Multiple data sets have revealed that the basal-like subtype has a poor prognosis; more commonly it develops soft tissue and visceral relapses including CNS-metastases. More than half of them metastasized first in the brain. As expected, survival was poor; the average survival rate in 5 years was 60%.

ErbB2 (HER2) cancers have high levels of expression of HER2, with minimal expression of ER and PR. The HER2-array subtype (2.8%) is more likely to be high-graded and poorly differentiated, and more likely to involve axillary lymph nodes (67%). Age of patients ranged from 37 to 68 years with a mean age of 52 years, and 80% were premenopausal. All five tumors of HER2 subtype were histologically invasive ductal carcinomas and had the poorest prognosis, the overall 5-year relative survival being 60%.

Unclassified cancers refer to negative triple tumors where the negative reaction for CK5 is added; the prognostic of these tumors is slightly better than the basal subtypes. In our study, only six cases were framed in this type, moderately/poorly differentiated and postmenopausal. All cases are of ductal invasive type.

While breast cancer survival rates are generally better than those for many other types of cancer, they vary with each patient and with tumor characteristics. Subtype comparison revealed significant differences in outcomes (Figure 3).

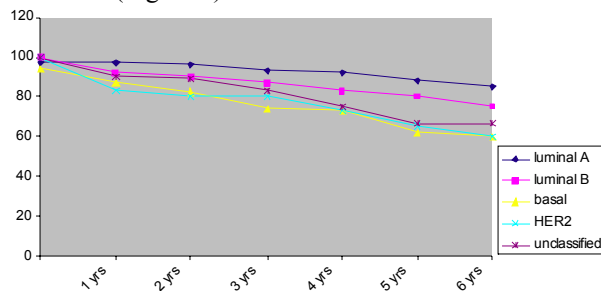


Figure 3 – Survival rates according to the molecular subtype.

The five-year relative survival rate for luminal A was the highest (86%) and the lowest survival rate (60%) in patients with basal-like subtype. Similar survival rates can be noticed at HER2 and basal-like subtypes, but the number of the HER2 cases is small. Lowest survival rates are associated with large tumor size, younger age, grading 3 characteristics, HER2 and basal-like subtypes.

Discussion

Although the molecular subtypes of breast cancer were initially identified by gene expression analysis using DNA-microarrays, IHC markers were currently used as surrogates for DNA-microarray in subtyping breast cancer. Based on recent updated IHC-subtype definitions [3], we identified five molecular subtypes of breast carcinoma: in our group as luminal A (53%), luminal B (18%), HER2, basal-like and unclassified subtypes. In the current study, we detected only five out of 173 (5%) breast cancers with HER2-gene amplification determined using IHC (3+). Overexpression of HER2 is a well-known prognostic factor associated with poor survival in women with breast carcinoma [1, 2].

Age is one of the most important risk factors for breast cancer. The entire studied group has in common the mean age of 55 years, smaller than literature data, which according to the maximum disease incidence, is around 75–79 years according to some authors, or above 60 years, according to others. This shows that all patients were significantly younger (Figure 4).

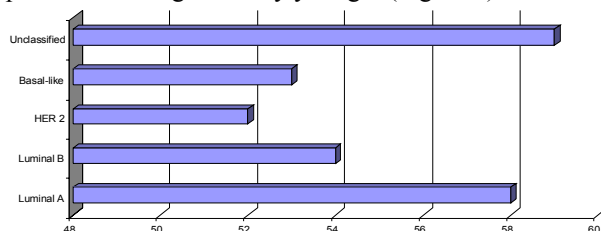


Figure 4 – Mean age of patients [years].

A possible explanation of this fact could be the younger age for menopause onset, or hormonal replacement therapy. Although the average age difference between the five subtypes is relatively small, there are differences concerning survival. The registered

deaths are situated at the extremes of the age intervals.

Tumoral size played an important prognostic role; this is directly related to an increasing probability of regional metastasis, an increasing average number of involved axillary lymph nodes and an increasing probability of recurrence and death. The influence of primary tumor size on prognosis can be appreciated in both node-negative and node-positive cases. The relationship probably reflects increasing vascular and lymphatic dissemination with progressive tumor growth. Patients with small tumors and either regional nodal involvement or direct extension of the tumor proved a survival rate equal or better than those with large tumors confined to the breast. We should note the different between molecular classes and the correlation with five-year rates: good survival rates for luminal cases were more likely to be diagnosed with smaller tumors than basal-like and HER2 cases (Figure 5).

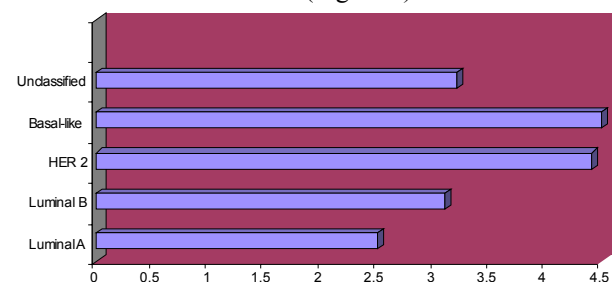


Figure 5 – Average tumor size [cm].

Five-year relative survival rates were high for patients with small tumors and negative lymph nodes; they were lower for women with large tumors and positive nodes. Among node negative patients, the prognostic influences of nuclear and histological grades are clearly obvious. Unfortunately, a high percentage (63%) shows positive lymph nodes at the moment of diagnosis (Figure 6).

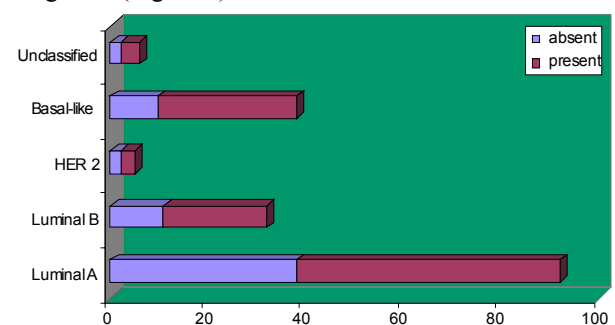


Figure 6 – Lymph node involvement.

The differences between molecular groups about the implication of the axillary lymph nodes are relatively reduced, but they prevail in the case of basal subtypes, correlating with the reduced survival rates. On the other hand, the luminal cases with a high number of positive lymph nodes have shown good survival rates. This demonstrates that the recorded lymphonodal status does not represent a major prognostic factor. Further, there is a survival relationship between tumor size, extension of tumor, and lymph node involvement. Also, these findings suggest that carcinoma with HER2 overexpression and basal-like subtype may be associated with unfavorable prognostic factors. The five-year

survival rates are decreasing with the anatomic stage, as known from the specialty literature. If in stage I, the five-year average survival rate was 88%, in the IVth stage it was only 25%, noted in only four cases.

It is equally important if the same subtypes of breast carcinoma have similar responses to therapy. It should be noted that basal and ERBB2-subgroups have been found to respond better to 5-fluorouracil, doxorubicin and cyclophosphamide chemotherapy, compared with other breast cancer subgroups [9]. To answer these questions, further investigation by comparison of prognosis and therapy responses with matched subtypes of breast carcinoma is warranted. Following our study, we also found worth noting that that women develop breast cancer at a younger age.

In summary, the current study attempts to characterize subtypes of breast cancer by using IHC markers. It provides new information for future study on prognosis and clinical management of breast cancer. However, our data needs to be interpreted with caution, for several reasons. First, the study was designed based on published data from breast cancer studies that allowed sub-classification of these tumors according to their characteristic features in DNA-microarray and expression profiles [1, 8]. Secondly, the current study was unable to provide very good correlative data between the immunosubtypes of breast cancer and their clinical behavior and survival information due to a relatively short period of follow-up (five years follow-up). It is certainly critical in future studies to identify the subtypes of breast carcinoma and their association with survival as well as the candidate markers with potential prognostic and predictive values. Finally, the small number of cases collected in this group may affect validation of its conclusion and the statistical power of the observations. Additional studies with larger numbers of patients are needed to achieve sufficient statistical power.

✉ Conclusions

Our study demonstrates that luminal subtypes are the major subtype of breast carcinoma, these being associated to better survival rates. Unfortunately, in our study a relatively high percentage is represented by the basal-like subtype associated to a reduced survival rate. However, there are several potential limitations to the study design that could affect interpretation of the results. Finally, we do not know to what extent these results can be generalized for the general population; it should be further evaluated in larger and more representative community samples. Better understanding of the molecular classes of breast cancer, independent of their prognostic and predictive values, may also

lead to new biological insights and eventually to better therapies that are directed toward particular molecular subsets. Also, not only can a target be identified, but one can also develop simultaneously a method for defining the patient population for therapy.

References

- [1] SØRLIE T, PEROU CM, TIBSHIRANI R, AAS T, GEISLER S, JOHNSEN H, HASTIE T, EISEN MB, VAN DE RIJN M, JEFFREY SS, THORSEN T, QUIST H, MATESE JC, BROWN PO, BOTSTEIN D, EYSTEIN LØNNING P, BØRRESEN-DALE AL, *Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications*, Proc Natl Acad Sci U S A, 2001, 98(19):10869–10874.
- [2] 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.
- [3] CAREY LA, PEROU CM, LIVASY CA, DRESSLER LG, COWAN D, CONWAY K, KARACA G, TROESTER MA, TSE CK, EDMISTON S, DEMING SL, GERADTS J, CHEANG MC, NIELSEN TO, MOORMAN PG, EARP HS, MILLIKAN RC, *Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study*, JAMA, 2006, 295(21):2492–2502.
- [4] IWASE H, ITOH Y, KUZUSHIMA T, YAMASHITA H, IWATA H, TOYAMA T, HARA Y, KOBAYASHI S, *Simultaneous quantitative analyses of c-erbB-2 protein, epidermal growth factor receptor, cathepsin D, and hormone receptors in breast cancer*, Cancer Detect Prev, 1997, 21(1):29–35.
- [5] TOYAMA T, YAMASHITA H, KONDO N, OKUDA K, TAKAHASHI S, SASAKI H, SUGIURA H, IWASE H, FUJII Y, *Frequently increased epidermal growth factor receptor (EGFR) copy numbers and decreased BRCA1 mRNA expression in Japanese triple-negative breast cancers*, BMC Cancer, 2008, 8:309.
- [6] GEE JM, HARPER ME, HUTCHESON IR, MADDEN TA, BARROW D, KNOWLDEN JM, MCCLELLAND RA, JORDAN N, WAKELING AE, NICHOLSON RI, *The antiepidermal growth factor receptor agent gefitinib (ZD1839/Iressa) improves antihormone response and prevents development of resistance in breast cancer in vitro*, Endocrinology, 2003, 144(11):5105–5117.
- [7] CAMERON D, CASEY M, PRESS M, LINDQUIST D, PIENKOWSKI T, ROMIEU CG, CHAN S, JAGIELLO-GRUSZFIELD A, KAUFMAN B, CROWN J, CHAN A, CAMPONE M, VIENS P, DAVIDSON N, GORBOUNOVA V, RAATS JI, SKARLOS D, NEWSTAT B, ROYCHOWDHURY D, PAOLETTI P, OLIVA C, RUBIN S, STEIN S, GEYER CE, *A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses*, Breast Cancer Res Treat, 2008, 112(3):533–543.
- [8] 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(18):10393–10398.
- [9] ROUZIER R, PEROU CM, SYMMANS WF, IBRAHIM N, CRISTOFANILLI M, ANDERSON K, HESS KR, STEC J, AYERS M, WAGNER P, MORANDI P, FAN C, RABIUL I, ROSS JS, HORTOBAGYI GN, PUSZTAI L, *Breast cancer molecular subtypes respond differently to preoperative chemotherapy*, Clin Cancer Res, 2005, 11(16):5678–5685.

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Received: September 20th, 2009

Accepted: February 18th, 2010