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RESEARCH

Female Breast Cancer Status According to ER, PR and HER2 Expression: A Population Based Analysis

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Abstract The aim of this study is to evaluate the prognostic values of some biological parameters in a population based series of female breast cancer patients. Through the Tuscan Cancer Registry all the invasive breast cancer cases diagnosed during the period 2004–2005 in the provinces of Florence and Prato, central Italy, were retrieved. Molecular subtypes were analyzed defined by immunohistochemical markers, by age, tumor size, lymph node status, histotype, grade of differentiation and proliferative marker. Out of 1487 patients 70.3% were luminal A subtype (ER/PR+HER2-), 15.6% luminal B (ER/PR+ HER2+), 8.1% triple negative (ER/PR-HER2-), 6.0% HER2+ (ER/PR-HER2+); the 3 year survival rates were 93.3%, 89.5%, 86.3%, 82.7% respectively ($p<0.001$). Analysis of survival by the Cox proportional hazards model showed an independent prognostic value of molecular classification. Our study revealed significant differences in clinicopathological characteristics among breast cancer molecular subtypes and confirmed their prognostic independent role.

Keywords Breast cancer subtypes · Immunohistochemical expression · Prognosis

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Introduction

Since 2000 characterization of breast cancer, apart from expression of biomarkers for estrogen receptors (ER) and progesterone receptors (PR), has included also the human epidermal growth factor 2 (HER2) [1].

In recent years, gene microarrays and immunohistochemical markers have been used to study molecular differences among different types of cancer. Among breast cancers patients, characteristic patterns of gene expression studies have demonstrated the heterogeneous character of breast cancer, using immunohistochemical selected stains [2–4].

Molecular characteristics are becoming more and more relevant not only in the diagnosis of groups of female breast cancer patients with different prognosis but also in their therapy. Hormonal positive receptors are predictive for response to endocrine therapy and positive HER2 are sensitive to target therapy with specific monoclonal antibody [5].

Several recent studies have identified different subtypes of morphologically similar breast cancer patients with different prognosis and different therapeutic response through the evaluation of the combination of ER, PR and HER2 status, currently reported in routine pathology reports of breast cancer [1, 5, 6].

The aim of this study is to evaluate in a population based series of female breast cancer patients, the frequency, the

Table 1 Breast cancer: clinical and pathological characteristics

	N cases	%
Age		
<50	340	22.8
≥50	1147	77.2
Subtypes		
Luminal A	1045	70.3
Luminal B	232	15.6
Triple negative	120	8.1
HER2+	90	6.0
Cell type		
Ductal	871	58.6
Lobular	207	13.9
Mixed	185	12.4
Other	224	15.1
Dimension		
<20	847	57.0
≥20	391	26.3
Unknown	249	16.7
pT		
pT1	885	59.5
pT2	365	24.5
pT3	53	3.6
pT4	23	1.6
Unknown	161	10.8
pN		
pN0	489	32.9
pN1	231	15.5
pN2	73	4.9
pN3	59	3.9
Unknown	635	42.8
Histology differentiation grade		
Well	157	10.5
Moderate	513	34.5
Poor	308	20.8
Unknown	509	34.2
Ki-67 expression		
<20%	974	65.5
≥20%	501	33.7
Unknown	12	0.8

characteristics and the prognostic values of these biological parameters through the evaluation of routinely reported data in pathology reports .

Materials and Methods

All cases of primary invasive breast cancer diagnosed among residents in the provinces of Firenze and Prato during the period 2004–2005 were retrieved from the Tuscan Cancer Registry (RTT). Cases diagnosed by death certificate only or for which histological report on primitive lesion was not available were excluded.

Data on age at diagnosis, stage, tumor size, lymph node status, histological grade of differentiation, histotype, hormonal status were already available in the RTT archive. Histological reports were re-examined for each case to collect information on the percentage of cells positive for immunohistochemical estrogen (ER) and progesterone (PR) receptors expression and on the score for human epidermal growth factor 2 (HER2).

Data on proliferation index, through the Ki-67 expression, and on fluorescence in situ hybridization (FISH) testing were also collected. Data on hormonal therapy and target therapy were not completely available, so they were not included in the analysis. Follow-up has been carried out up to the end of December 2008 or to patient's death whichever came first.

ER and PR were categorized as negative (if immunohistochemical staining of tumor cells was less than 5%) and positive (≥5%). Further more, also cut off at 10% and at 1% were analyzed. Tumors were considered positive for hormone receptors if found positive for at least one receptor.

HER2 expression was assessed through immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH). IHC was scored on a qualitative scale from 0 to 3+, based on interpretation of staining intensity, with 0 and 1+ categorized as negative, 2+ as borderline, and 3+ as positive [7]. FISH was scored on a quantitative scale. Cases with less than 2 copies of the HER2 gene were categorized as negative. Cases with IHC score 2+ and FISH unknown were considered positive [5].

In order to distinguish between high and low proliferating tumours the cut off for positive KI-67 was 20% of positive invasive breast cancer cells.

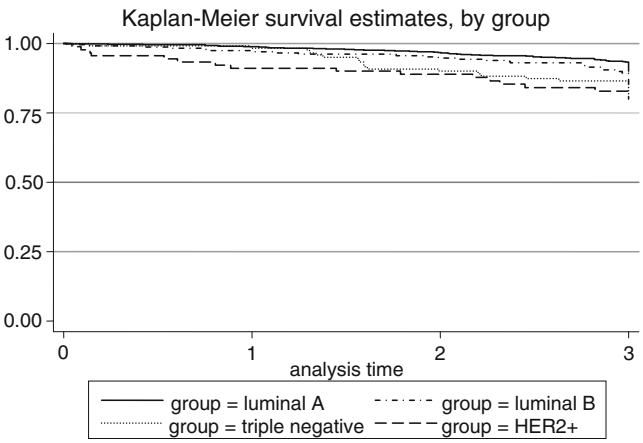

Fig. 1 Breast cancer: 3-year overall survival according to subtypes

Four subtypes were defined on the expression of ER or PR and HER2:

- luminal A if ER/PR+and HER2-,
- luminal B if ER/PR+and HER2+,
- triple negative if ER/PR-and HER2-,
- HER2 positive if ER/PR-and HER2+.

The subtypes were analyzed by age (<50, 50+), tumor size (<20, 20+ mm), lymph node status (positive/negative), histotype (ductal, lobular, ductal and lobular, others), histological grade of differentiation (poor, moderate, well differentiated) and proliferative marker (Ki-67 expression <20%, ≥20%).

The comparison of the distribution of categorical variables among cancer subtypes in the univariate analysis was based on Pearson chi test or Fischer's test. The survival rates were calculated using the Kaplan Meier method. Differences in survival among subtypes were tested using the log rank test. A multivariate Cox regression analysis was carried out to estimate hazard ratios, adjusting for main clinical and pathological variables.

Results

During the period 2004–2005 a total of 1984 invasive breast cancer cases were diagnosed in the RTT area. Data for ER were available for 1771 cases (89.2%) for PR for 1769 (89.1%) and for HER2 for 1497 (75.4%) cases, respectively. For 1487 cases (74.9%) all the three markers

Table 2 Breast cancer clinical and pathological characteristics: association with subtypes

	Luminal A n (%)	Luminal B n (%)	Triple negative n (%)	HER2+ n (%)	p
Age					0.3
<50	226 (21.6)	62 (26.7)	31 (25.8)	21 (23.3)	
≥50	819 (78.4)	170 (73.3)	89 (74.2)	69 (76.7)	
Cell type					<0.001
Ductal	538 (51.5)	167 (72)	93 (77.5)	73 (81.2)	
Lobular	183 (17.5)	19 (8.2)	3 (2.5)	2 (2.2)	
Mixed	161 (15.4)	19 (8.2)	3 (2.5)	2 (2.2)	
Other	163 (15.6)	27 (11.6)	21 (17.5)	13(14.4)	
Dimension					0.001
<20	620 (71.2)	130 (66.7)	53 (53.0)	44 (61.1)	
≥20	251 (28.8)	65 (33.3)	47 (47.0)	28 (38.9)	
T					0.05
pT1	645 (61.7)	132 (56.9)	60 (50.0)	48 (53.3)	
pT2	238 (22.8)	67 (28.4)	38(31.6)	23 (25.6)	
pT3	35 (3.4)	6 (2.6)	5 (4.2)	7 (7.8)	
pT4	14 (1.3)	2 (0.9)	5 (4.2)	2 (2.2)	
Unknown	113 (10.8)	26 (11.2)	12 (10.0)	10 (11.1)	
N					0.001
pN0	361 (34.5)	57 (24.6)	39 (32.5)	32 (35.6)	
pN1	165 (15.8)	39 (16.8)	18 (15.0)	9(10.0)	
pN2	44 (4.2)	14 (6.0)	13 (10.9)	2 (2.2)	
pN3	32 (3.1)	10 (4.3)	7 (5.8)	10(11.1)	
Unknown	433 (42.4)	112 (48.3)	43 (35.8)	37 (41.1)	
Histology differentiation grade					<0.001
Well	138(13.2)	13 (5.6)	4 (3.3)	2 (2.2)	
Moderate	413 (39.5)	78 (33.6)	13 (10.8)	9 (10.0)	
Poor	137 (13.1)	61 (26.3)	64 (53.3)	46 (51.1)	
Unknown	357 (34.2)	80 (34.5)	39 (32.6)	33 (36.7)	
Ki-67 expression					<0.001
<20%	803 (76.8)	109 (47.0)	36 (30.0)	26 (28.9)	
≥20%	236 (22.6)	119 (51.3)	84 (70.0)	62 (68.9)	
Unknown	6 (0.6)	4 (1.7)	0	2 (2.2)	

were available and only these cases were included in our study. The proportion of positive cells for immunohistochemical expression (level ≥5%) was 83.1% (1236 cases) for ER and 68.9% (1025 cases) for PR. Tumors scored by IHC most often stained as 0 (63.3%) or 1+ (13.5%), while 9.2% were scored as 2+.

The most prevalent (triple) subtype was ER/PR+/HER2- (luminal A, 70.3%), followed by ER/PR+/HER2+ (luminal B, 15.6%), triple negative tumors (8.1%) and ER-/PR-/HER2+ (HER2+group, 6.0%). The majority of patients were older than 50 years of age (77.2%) and ductal carcinoma was the most frequent histotype (58.6%), while lobular and mixed carcinoma represented 14% and 12% of cases, respectively. Tumors were poorly differentiated in 21% and showed high proliferation index in 34% of cases. Data on lymph node status and tumor dimension were available in 57.2% and 83.3% of cases respectively and showed that early stage was the most frequent (Table 1).

In Fig. 1 3-year survival rates are shown for different types of breast cancer. 3-year survival rates was 93.3% for the patients with luminal A, 89.5% for luminal B, 86.3% for triple negative and 82.7% for HER2+ group respectively ($p<0.001$) (Fig. 1).

Overall 3-year survival rates from our study (of all population of breast cancer patients) was similar to rates from Tuscan cancer registry: in fact, 3-year survival rates for women were 94.4% and 91.5%, respectively.

The median age at diagnosis was 62.7 years for luminal A, 60.8 for luminal B, 61.3 for triple negative tumors and 59.0 years for HER2+; the highest percentage of patients aged <50 years was detected in triple negative (25.8%) and luminal B (26.7%), without statistically significant difference. (Table 2)

Significant difference was found when subtypes were compared by tumor size at diagnosis. The highest percentage of patients with tumor size ≥20 mm was detected in triple negative (47.0%) and HER2+ (38.9%) groups (Table 2). The median size at diagnosis was 17 mm for luminal A, 19 for luminal B, 20 for HER2+ and 23 for triple negative tumors.

Axillary lymph node status at diagnosis was associated with subtypes. The highest percentage of negative lymph node cases was observed in luminal A and HER2 group and the differences by lymph node status were statistically significant ($p=0.001$). Compared with luminal A cases, triple negative and HER2+ tumors were more likely to be poorly differentiated at diagnosis ($p<0.001$). On the basis of histological examination ductal carcinoma cases were more frequent in HER2+ (81.1%) and triple negative (77.5%) groups (Table 2)

The expression of the Ki-67 marker differed significantly within the subtypes ($p<0.001$). Luminal A showed the highest percentage of cases with low expression of Ki-67 (76.8%), while the expression was higher than 20% in most triple negative (70.0%) and HER2+ (68.9%) cases (Table 2).

In Table 3 the prognostic effect of each variable is shown in comparison with the reference categories. The patients with HER2+ (HR=2.33), triple negative (HR=2.03) and luminal B (HR=1.68) tumors showed a significantly increased risk of dying in comparison with luminal A (reference). The multivariate Cox regression analysis (including age, pathological T and N status) revealed an important prognostic effect for immunohistochemical subtype. In fact triple negative and HER2+ tumor patients had an increased statistically significant risk of dying in comparison with luminal A (HR=1.68, CI 1.04–2.72 and

Table 3 Breast cancer subtypes

	HR	CI	HR[a]	CI
Age				
<50	1		1	
≥50	2.94	1.75–4.92	2.73	1.62–4.59
Histotype				
Ductal	1			
Lobular	0.69	0.42–1.12		
Mixed	0.39	0.20–0.75		
Other	0.87	0.56–1.34		
T				
pT1	1		1	
pT2	4.04	2.76–5.91	3.18	2.13–4.72
pT3	5.29	2.79–10.05	3.83	1.98–7.40
pT4	10.68	5.50–20.71	7.26	3.59–14.69
N				
pN0	1		1	
pN1	1.17	0.62–2.21	0.92	0.48–1.74
pN2	3.94	2.10–7.42	2.02	1.04–3.91
pN3	6.72	3.77–11.99	2.80	1.51–5.17
Histology differentiation grade				
Well	1			
Moderate	1.32	0 .70–2.47		
Poor	1.83	0.96–3.48		
Ki-67 expression				
<20%	1			
≥20%	2.04	1.49–2.78		
Subtype				
Luminal A	1		1	
Luminal B	1.68	1.14–2.50	1.65	1.11–2.46
Triple negative	2.03	1.26–3.26	1.68	1.04–2.72
HER2+	2.33	1.39–3.91	2.18	1.28–3.70

Univariate and multivariate analysis: hazard ratio of death (HR) and 95% confidence interval (CI)

[a] values were adjusted for T and N status in a Cox multivariate analysis

HR=2.18, CI 1.28–3.70, respectively), also when adjusted for main clinical and pathological variables; luminal B patients also showed higher hazard for death with respect to the same reference group (HR=1.65, CI 1.11–2.46) (Table 3).

Discussion

The status of biomarkers ER, PR and HER2 was analyzed in a population based series of invasive breast cancer. These markers have been considered predictive and prognostic and several studies have assessed risk factor profiles of tumor subtypes through their combined expression [1, 5, 8, 9]. Recent studies from population based analysis reported percentage of patients with IHC markers available ranging from 34% to 91% [9, 10]. In our registry we found all the three markers in routine histological reports for almost 75% of invasive breast cancer, confirming their availability in pathology reports and the possibility of a comprehensive retrieval of IHC markers in cancer registries [9].

In the present series the most frequent subtype is luminal A [11, 12], followed by luminal B, according to similar studies [9]. However, some authors reported that luminal B was less represented than other groups [11, 13], perhaps for reasons concerning selection of cases in trial setting.

According to recent studies [3, 9, 10] our analysis showed a significant association of immunohistochemical subtypes of breast cancer with histotype, histological grade of differentiation and stage at diagnosis. Moreover, a lower proliferation index in luminal A than in other subtypes was detected, according to the hypothesis that proliferation index could help in differentiation among subtypes, particularly between Luminal A and B, as recently suggested [6].

Triple negative and HER2+ patients tend to be younger than in luminal A and Luminal B cases [2, 3, 9]. In our study this association was not confirmed.

The four subtypes differed significantly in 3-year observed survival. HER2+ and triple negative subtype confirmed in our analysis a poorer prognosis then luminal A and luminal B, as recently reported [9, 11, 12]. Lately a poorer prognosis, among positive hormonal receptor tumors, for cases with high proliferation has been reported [14]. Consequently it has been suggested that Ki-67 is a candidate biomarker to define luminal B, although there is controversy on cut-off definition [6].

HER2+ showed the poorest prognosis among molecular subtypes, particularly among the first year of follow up as showed by Rakha et al. [14]. On the other hand other authors reported a poorer prognosis in triple negative [5, 9]. The prognosis of HER2 patients may be strongly influenced by the use of specific therapy but unfortunately we do not have any information on the matter.

Multivariate analysis on our data showed an increased risk of death for patients with triple negative and HER2+ immunohistochemical subtypes also adjusting for other variables (as stage and age), in agreement with previous reports [2, 9, 14]. The risk was also higher for luminal B in comparison with luminal A tumor patients .

A limitation that could affect the assessment of immunohistochemical markers, and consequently the distinction of prognostic and predictive subtypes of breast cancer, is the availability of clinically validated, reproducible and standardized cut off. We categorized as positive, according to recent studies [7, 14, 15], the cases with ≥5% of cells with ER/PR expression. However, the analysis with different cut off as 10%, commonly used, and 1%, as recently suggested [7, 16], showed that subtype categorization for some tumors and, therefore, the possible therapeutic options for these patients may change. With cut off at 10% for hormonal receptors, the number of patients in luminal A group became 1021 (instead of 1045 with cut off of 5%), while the number of triple negative patients who did not receive hormonal therapy became respectively 123 (instead of 120 with cut off of 5%) of the total. On the other hand, with a cut off of 1% the luminal A group would included 1031 and the triple negative group 113 patients, respectively. Furthermore, biomarkers were often analyzed by a variety of testing methods and sources without central review. Recently, a significant discordance of immunoistochemical data for both ER and HER2 determination was reported [17], with possible effect on therapeutic strategy.

Our data from a population based cancer registry study revealed significant differences in clinical and pathological characteristics among breast cancer molecular subtypes, as defined by the analysis of the expression of biological markers through immunohistochemical detection and confirmed their prognostic independent role. However, standardization of methods for immunohistochemical assessment to avoid limited reproducibility and adoption of common cut-off is still needed for molecular subtype definition in clinical practice.

Conflict of Interest We declare that we have no conflict of interest.

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