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Original article

Survival and clinicopathological characteristics of breast cancer patient according to different tumour subtypes as determined by hormone receptor and Her2 immunohistochemistry. A single institution survey spanning 1998 to 2010

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ABSTRACT

As far as recent breast cancer molecular subtype classification is concerned, much work has dealt with clinical outcomes for triple negative and Her2 patients. Less is known about the course of patients in the remaining subtypes. Molecular classification based on immunohistochemistry is widely available and correlates well with genetic microarray assessment, but at a lower cost. The aim of our investigation was to correlate immunohistochemical subtypes of breast cancer with clinical characteristics and patient outcomes.

Since 1998, 1167 patients operated for 1191 invasive breast tumours were included in our database. Patients were regularly followed up until March 2010. Disease-free survival, overall mortality, and breast cancer-specific mortality at 5 years were calculated for the cohort.

72% of tumours were ER+PR±HER2− group, 13% triple negative (ER−PR−HER2−), 10% ER+PR±HER2+ group, and 5% Her2 (ER−PR−HER2+). Cancer-specific survival was 94.2% for the ER+PR+HER2− subtype, 84.8% for the Her2 subtype, 83.3% for the ER+PR−HER2− subtype, and 78.6% for triple negatives. Distant metastases prevalence ranged from 7% to 22% across subtypes, increasing stepwise from ER+PR+HER2−, ER+PR+HER2+, ER+PR−HER2−, ER+PR−HER2+, ER−PR−HER2+ through triple negative. Small, low-grade tumours with low axillary burden were more likely to belong to the ER+PR±HER2− group. Conversely, larger high-grade tumours with significant axillary burden were more likely to belong to Her2 or triple negative groups. ER+PR±HER2− group patients with negative PR receptors performed more like Her2 or triple negative than like the rest of ER+PR±HER2± groups patients.

Molecular classification of breast tumours based only on immunohistochemistry is quite useful on practical clinical grounds, as expected. ER+PR±HER2− group patients with negative PR receptors seem to be at high risk and deserve further consideration.

Introduction

Breast cancer is the most common cancer-related cause of death in women and the third most common tumour worldwide.[1] Breast cancer incidence and mortality may vary according to factors such as age, ethnicity, wealth and social status, as well as to tumour-related factors such as size, histological grade, and hormone receptor status.[2] Breast cancer is widely viewed as a multifactorial

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condition that consists of different and heterogeneous biological subtypes, each one of them associated with specific molecular and clinical characteristics that carry different prognostic and therapeutic implications.

The last few decades have witnessed major breakthroughs in the diagnosis and management of breast cancer patients. Population screening programs that allow early cancer detection on one hand and improvement in therapy on the other, have resulted in declining mortality rates and in better quality of life for those living with the disease.[3,4] On research grounds, however, much attention has been paid to newer molecular classifications of breast cancer,[5–14] which are based on genetic platforms or microarrays. Although very attractive for their prognostic power, these technologies are significantly limited from both availability and cost. On its own, immunohistochemical classification brings about important prognostic and therapeutic insights of breast cancer at a much lower cost. It is also widely available, and has been shown to correlate very well with intrinsic genetic expression microarray assessment as follows: ER±PR±HER2− and ER±PR± HER2+ as luminal, ER−PR−HER2+ as Her2, and ER−PR−HER2− as triple negative subtypes.[15]

The aim of our investigation was to correlate the different immunohistochemical subtypes of breast cancer, as well as the more classical prognostic factors, with patient disease-free survival, overall mortality, and breast cancer-specific mortality at 5 years.

Patients and method

Consecutive breast cancer patients referred to the Breast Unit of the University Hospital of Mútua Terrassa for surgical treatment of either primary or recurrent tumours were prospectively included in a database between January 1, 1998 and March 31, 2010. All patients had been referred either from the regional public health care system or from the Breast Cancer Screening Program of the Generalitat de Catalunya, West Valles Occidental section (Barcelona province). Patients with in situ carcinomas and those unfitted for surgery were excluded. The database of the Breast Unit included the following variables: age, tumour size, histologic type and grade (differentiation grade − G−, and histologic grade − HG), assessment of ER, PR, and Her2 status, as well as of nodal status, distant metastases occurrence, disease-free survival and mortality. This study was done in accordance with the Review Board and Ethics Committee of our centre. Written informed consent was always obtained before any invasive procedure as surgery.

All patients were treated according to the regularly updated protocol of the Breast Unit of the University Hospital of Mútua Terrassa, which follows both local and international guidelines. Chemotherapy regimes were based on anthracyclines and taxanes, and hormone therapy based on tamoxifen and aromatase inhibitors. From 2005 on, adjuvant trastuzumab was used for Her+ patients. Radiation therapy was performed at the nearby Hospital General de Catalunya using CT for bi-dimensional dosage planning until 2008. From then on, tri-dimensional planning was used, according to regularly updated protocols. In 2002, Sentinel Node (SN) biopsy was introduced for patients with tumours up to 3 cm in size and negative axilla, both clinically and sonographically.[16]

Hormone receptors were assessed by immunohistochemistry: DAKO Clone 1D5 was used for oestrogen receptors (ER), and DAKO Clone PgR 636 for progesterone receptors (PR). Assessment was based on the percentage of positive cell nuclei, independent of staining intensity. The positivity cut-off value was set at 5%. Her-2/neu protein over expression was determined by immunohistochemistry: DAKO HercepTest-TM,[15] and it was semi-quantitated based on staining of the cytoplasmic membrane rather than on cytoplasm itself. HercepTest was rated negative (0+ and 1+), indeterminate (2+) or positive (3+). In cases of 2+, FISH or CISH techniques were used to evaluate gene amplification.

For the purpose of the present study, breast cancer was classified into eight subtypes based on hormone receptor oestrogens and progesterone and Her2 values as follows: ER+PR+HER2−; ER+PR−HER2−; ER−PR+HER2−; ER+PR+HER2+; ER+PR−HER2+; ER−PR+HER2+; ER−PR−HER2+; ER−PR−HER2−.

Given the different views on ER negativity significance when PR are positive,[17,18] those cases with ER− and PR+ were added to the ER+PR+ groups (14 cases in ER±PR+HER2−; and 2 cases in the ER±PR+HER2+ group). Therefore, the analysis is now restricted to only six subtypes: ER+PR+HER2−, ER+PR−HER2−, ER+PR+HER2+, ER+PR−HER2+, ER−PR−HER2+ (Her2) and ER−PR−HER2− (triple negative).

We studied variations of patient prognosis between groups as defined by the modified classification of Sorlie and Perou,[5] including the most prevalent subtypes within the ER+PR±HER2± groups. Other variables considered were age, tumour size, histologic type and grade, disease-free survival, all distant metastases, and specific visceral metastases, including liver, lung or brain, as well as mortality.

Mortality was considered per se (overall), and also as specific mortality from breast cancer, once other causes of death unrelated to breast cancer had been excluded. Mortality figures were derived from the mortality register of our own centre as well as from the database from the Catalan Public Health Care System. Survival was determined as a function of the total number of cases over the natural year count from the surgery date.

The actual minimum follow-up period was 12 months. 82% of patients were followed for 24 months, 70% for 36 months, 60% for 48 months, 52% for 60 months, 42% for 72 months, 34% for 84 months, 25% for 96 months, 20% for 108 months, and 14% for ten or more years.

Statistics

Time intervals were defined as time elapsed from the diagnosis of cancer to the last uneventful control or to event occurrence: local or distant recurrence or death. Qualitative variables were expressed as “n” and percentage, whereas quantitative variables were expressed as their mean value and standard deviation (SD). For comparison of qualitative variables the Chi-square test was used, while for comparison between mean values, the ANOVA was used. Statistical significance was set at p value <0.05, with a two-tail approach. The Kaplan–Meier and log-rank tests were used to calculate and compare survival rates. A multivariate analysis was used based on the Cox proportional hazard method, including those variables that were significant, as well as those with potential clinical impact. Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Finally, 1167 patients suffering from 1191 invasive breast tumours that had been included in the breast cancer register of the University Hospital of Mútua Terrassa from January 1998 until March 2010 were entered into the study. Details of the exact number of analysed patients for each part of the study, as well as the exclusion causes are displayed in Table 1. Additionally, 188 patients that had been operated before HercepTest was available at our centre, but that indeed had hormone receptor assessment were also included. The mean patient age at diagnosis was 58 years. Most tumours were ductal carcinomas (91%), 50% were poorly differentiated, 58% were in the T1 size range, and 60% were node-negative. Baseline characteristics of study patients, including tumour subtypes are presented in Table 2. Out of the 1191 tumours, 72% were

Table 1
Summary of cases included in the analysis.

Summary of cases included in the analysis	*N*
Surgical breast cancer cases Jan 1, 1998–March 31, 2010	1382
Cases with at least one missing marker (ER or PR)	15
Excluded cases because DCIS	176
Cases before Her2 assessment availability	188
Immunohistochemical subtypes	1003
Cases included for survival analysis	1167
Synchronous bilateral breast cancer	24
Cases included for bivariate analysis	1191

ER+PR±HER2−, 13% triple negative, 10% ER+PR±HER2+, and 5% Her2. Within the ER+PR±HER2− group, the most prevalent subtype was ER+PR+HER2− (90%), followed by ER+PR−HER2− (10%). In the ER+PR±HER2+ group, the subtype with positive hormone receptors amounted 71%, followed by the PR− subtype (29%).

Local recurrence and distant metastases

Occurrence of both local recurrence and distant metastases, as well as per organ distribution of metastases is displayed in Table 3.

Table 2
Baseline characteristics of breast cancer patients ($N = 1191$).

Patient characteristic	Subjects *N* (%)
Age	58 ± 13 (25–103)
Differentiation grade	
Well differentiated (G1)	171 (14%)
Moderately differentiated (G2)	333 (28%)
Poorly differentiated (G3)	594 (50%)
Missing	93 (8%)
Histologic grade	
Grade 1/3 (GH1)	405 (34%)
Grade 2/3 (GH2)	524 (44%)
Grade 3/3 (GH3)	231 (19%)
Missing	31 (3%)
Tumour size (TNM)	
T1	688 (58%)
T2	394 (33%)
T3	61 (5%)
T4	48 (4%)
Histologic type	
Ductal carcinoma	1082 (91%)
Lobular carcinoma	94 (8%)
Mixed ductal-lobular carcinoma	15 (1%)
Lymph node status	
Positive	466 (39%)
Negative	712 (60%)
Not available	13 (1%)
Hormone receptors	
ER status positive vs negative	963 (81%) vs 228 (19%)
PR status positive vs negative	861 (72%) vs 330 (28%)
Her2 status positive vs negative	146 (15%) vs 857 (85%)
Breast cancer groups and subtypes	1003
ER±PR±HER2−	**724 (72%)**
ER+PR+HER2−	637 (89%)
ER+PR−HER2−	73 (10%)
ER−PR+HER2−	14 (1%)
ER±PR+HER2+	**97 (10%)**
ER+PR+HER2+	67 (69%)
ER+PR−HER2+	28 (29%)
ER−PR+HER2+	2 (2%)
Her2: ER−PR−HER2+	**49 (5%)**
Triple negative: ER−PR−HER2−	**133 (13%)**
Previous to Her2 availability	188 (16%)
ER±PR+	141 (12%)
ER+PR−	20 (2%)
ER−PR−	27 (3%)
Surgery	
Radical vs conservative surgery	741 (62%) vs 450 (38%)

The bold are the significance of Breast cancer groups.

Molecular subtype rates are shown in bold type, whereas the most conspicuous data across different groups and subtypes are displayed in shaded boxes.

Mortality and survival

The overall mortality rate in our study was 155 per thousand (181/1167), and the breast cancer-specific mortality was 108 per thousand (126/1167). At five years, overall mortality was 102 per thousand (119/1167), whereas the specific mortality for the same period of time was 72 per thousand (84/1167).

The accumulated survival, both overall and specific for all groups considered as well as for the six subtypes are displayed in Table 4 and in Fig. 1. In Fig. 2(a,b) overall and specific survival rates are displayed according to the four significant molecular groups. Detailed survival rates according to ER, PR, and Her2 status are displayed in Fig. 3(a, b, c). Also, in Tables 4 and 5, survival rates and hazard ratios are shown as adjusted for patient age, tumour size, histologic grade and nodal status. Multivariate analysis showed that triple negative tumours per se and all tumours with negative hormone receptors together were associated with worse prognosis. In Table 4, statistically significant survival differences are shown in dark grey shaded boxes, whereas those close to statistical significance ($p = 0.06$) are shown in light grey shaded boxes. At five years, patients in the ER+PR±HER2− group showed a significantly better specific survival than Her2 or triple negative patients, but not better than ER+PR±HER2+ patients. When tumour subtypes in ER+PR±HER2− group were considered, a significantly decreased survival was seen for the ER+PR±HER2− patients with negative PR, compared with positive PR patients (Fig. 4). Patients with negative ER had an accumulated 5-year specific survival significantly lower then patients with positive ER (Fig. 3a). The same applied for PR status (Fig. 3b). There were no statistically significant specific survival differences between Her2+ and Her2− patients at five years (Fig. 3c).

Clinicopathological analysis

Tumour characteristics for each one of the defined breast cancer subtypes were examined (Table 6). Significant differences are shown in grey shaded boxes. T1 tumours were significantly more common in the ER+PR±HER2± groups ($p < 0.001$), whereas T3 tumours were more often associated with Her2+ and triple negative ($p < 0.001$). The ER+PR−HER2− subtype had significantly less T1 tumours than the ER+PR+HER2− subtype ($p = 0.01$).

Patients under 50 had a significantly higher incidence of the ER+PR+HER2− subtype ($p = 0.007$) as well as of the triple negative subtype ($p = 0.04$). Between 50 and 69, patients had a significantly lower chance of belonging to the triple negative subtype ($p < 0.001$). Patients over 70 showed the least incidence of ER+PR±HER2+ group.

G3 and GH3 tumours were significantly more frequent among Her2+ and triple negative patients ($p < 0.001$). The ER+PR−HER2− subtype showed a significantly lower incidence of G1/GH1 than the ER+PR+HER2− subtype ($p = 0.04$). Incidence of ductal carcinomas was higher in the worse prognosis groups (Triple negative and HER2: $p = 0.02$ and $p = 0.05$, respectively), just as lobular carcinomas were higher in the most favourable prognosis group.

Only the ER+PR±HER2+ group had a significantly higher incidence of positive nodes as compared with the ER+PR±HER2− group ($p = 0.02$). However, if axillary tumour burden was considered (more than 3 positive nodes), then the triple negative subtype ranked the highest ($p = 0.03$).

ER+PR±HER2± tumours had a significantly lower chance of distant metastases than Her2 and triple negative tumours

Table 3
Metastases and local recurrence at 5 years follow-up according to breast cancer groups and subtype.

Subtype 1167	Organ distribution of metastases							
	Metastases overall 133 (11%)	Local recurrence 60 (5%)	Lung 32 (3%)	Liver 38 (3%)	Brain 25 (2%)	All visceral 69 (6%)	Lymph node 22 (2%)	Bone 30 (11%)
	N (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)
ER+PR±HER2− 706	**56 (8%)**	**24 (3%)**	**8 (1%)**	**15 (2)**	**7 (1%)**	**26 (4%)**	**7 (1%)**	**18 (3%)**
ER±PR+HER2− 635	46 (7%)	17 (3%)	7 (1%)	11 (2%)	6 (1%)	20 (3%)	6 (1%)	15 (2%)
ER+PR−HER2− 71	10 (14%)	7 (10%)	1 (1%)	4 (6%)	1 (1%)	6 (9%)	1 (1%)	3 (4%)
ER+PR±HER2+ 93	**9 (10)**	**2 (2%)**	**2 (2%)**	**2 (2%)**	**2 (2%)**	**4 (4%)**	**1 (1%)**	**2 (2%)**
ER±PR+HER2+ 66	5 (8%)	1 (2%)	1 (2%)	2 (3%)	2 (3%)	3 (5%)	1 (2%)	2 (3%)
ER+PR−HER2+ 27	4 (15%)	1 (4%)	1 (4%)	0	0	1 (4%)	0	0
Her2 49	**10 (20%)**	**3 (6%)**	**3 (6%)**	**2 (4%)**	**3 (6%)**	**6 (12%)**	**2 (4%)**	**0**
Triple negative 131	**29 (22%)**	**17 (13%)**	**12 (9%)**	**12 (9%)**	**8 (6%)**	**21 (16%)**	**8 (6%)**	**6 (5%)**
Previous to Her2 188	29 (15%)	14 (7%)	7 (4%)	7 (4%)	5 (3%)	12 (6%)	4 (2%)	8 (4%)
ER±PR+ 141	17 (12%)	8 (6%)	4 (3%)	5 (4%)	4 (3%)	7 (5%)	0	4 (3%)
ER+PR− 20	3 (15%)	2 (10%)	1 (5%)	2 (10%)	0	1 (5%)	0	3 (15%)
ER−PR− 27	9 (33%)	4 (15%)	2 (7%)		1 (4%)	4 (15%)	4 (15%)	1 (4%)

The bold are the significance of Breast cancer groups.

($p = 0.003$, and $p < 0.00001$, respectively). Within the ER+PR±HER2± group, the ER+PR−HER2− subtype was associated with a significantly increased rate of metastases, as compared with the ER+PR+HER2− subtype ($p = 0.003$), whereas no such differences were seen when compared with Her2 and triple negative tumours. Overall and organ-specific metastasis rates by groups and subtypes are displayed in Table 3. It can be seen that organ-specific metastasis distribution across tumour subtypes follows almost an identical pattern as that of the overall metastasis. Local recurrence is significantly more prevalent among triple negative and ER+PR±HER2± tumours with negative PR (Table 6).

Discussion

Knowledge of ER, PR and Her2 status allows convenient systemic therapy in breast cancer patients. Through the expression of ER and PR, endocrine sensitivity can be evaluated, a well-known factor predicting response to tamoxifen or ovarian suppression.[19–23] Also, assessment of Her2 over expression is useful in order to establish anti-Her2+ therapy.[17,22,24–26] It seems that tumour classification based on ER solely is less accurate for prognostic purposes than further subclassification based on ER, PR, and Her2. Some authors regard breast cancer as split into triple negative and the rest.[27] This is quite informative, albeit simplistic because grouping ER+PR+HER2−; ER+PR+HER2+; and ER−PR−HER2+ together might be misleading. Our own study shows some differences between subtypes within each group, as well as differences among Her2 patients.

Ever since 1998 the Allred score for hormone receptor status positivity has been used, even though not every clinical oncologist is willing to accept the bare 1% threshold value for a breast cancer patient to be entered in a tamoxifen-based therapeutic scheme. The large-scale California Parise study,[28] which included over 50,000 women sets-out a 5% threshold value. Recently, the American Society of Clinical Oncology (ASCO) and the American College of Pathologist have again recommended that ER status should be considered positive at 1%.[39]

Very few breast tumours with less than 10% ER positivity actually behave like ER+ from the molecular point of view. Rather, most behave like ER−. From a clinical perspective, it seems safer to use adjuvant endocrine therapy plus chemotherapy in this particular group of patients.[40]

The modified Perou molecular classification of breast tumours includes four groups according to hormone receptor and HER2-neu status values, without even accounting for any clinic-pathologic variable. Such classification is both simple and practical, and it brings about information that allows useful group characterization, especially as Her2 and triple negative are concerned. It does not however discriminate positive hormone receptor patients with or

Table 4
Specific and overall survival at 5 years follow-up according to tumour subtype, ER/PR and Her2 status.

Subtype ER/PR/HER2	*N*	Cancer-specific survival % (CI 95%)	Overall survival % (CI 95%)
- ER+PR±HER2−	**706 (61%)**	**93.1 (91.2–95.0)**	**89.7 (87.5–91.9)**
ER±PR+HER2−	635 (54%)	94.2 (92.4–96.0)	92.1 (90.0–94.2)
ER+PR−HER2−	71 (6%)	83.3 (74.6–92.0)	78.2 (68.6–87.8)
- ERPR±HER2+	**93 (8%)**	**91.1 (85.3–96.9)**	**88.7 (82.3–95.1)**
ER±PR+HER2+	66 (6%)	91.7 (85.0–98.4)	88.5 (80.8–96.2)
ER+PR−HER2+	27 (2%)	88.6 (75.3–100.0)	88.6 (75.3–100.0)
- Her2	**49 (4%)**	**84.8 (74.7–94.9)**	**81.5 (70.6–92.4)**
- Triple negative	**131 (11%)**	**78.6 (71.6–85.6)**	**73.8 (66.3–81.3)**

The bold are the significance of Breast cancer groups.

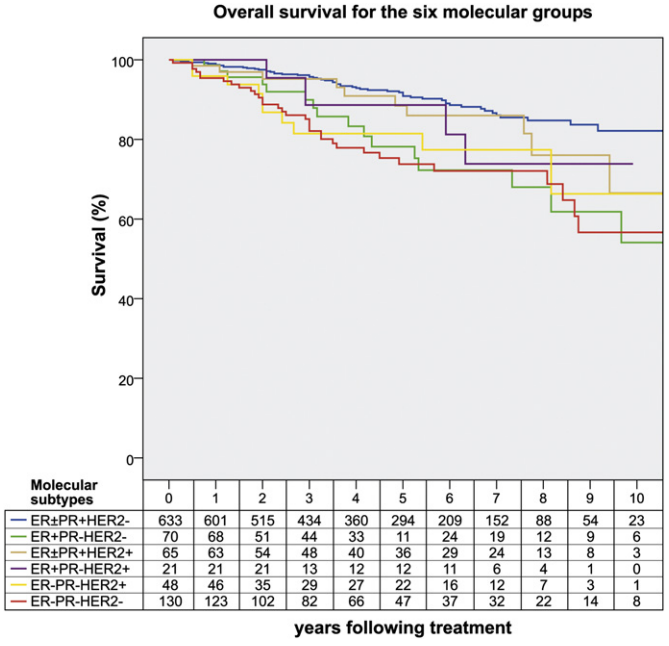

Fig. 1. Overall survival for the six molecular groups considered. Fractions of patients remaining in the study are displayed at annual intervals up to 10 years.

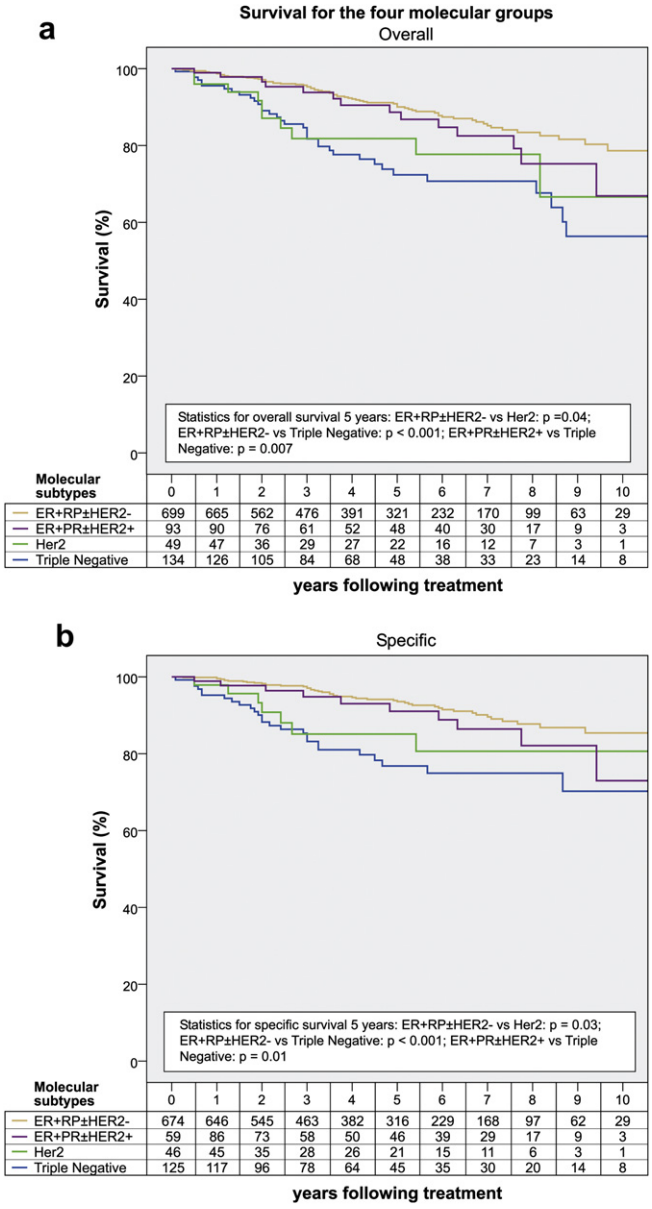

Fig. 2. Overall (a) and specific (b) survival for the four molecular groups considered. Fractions of patients remaining in the study are displayed at annual intervals up to 10 years.

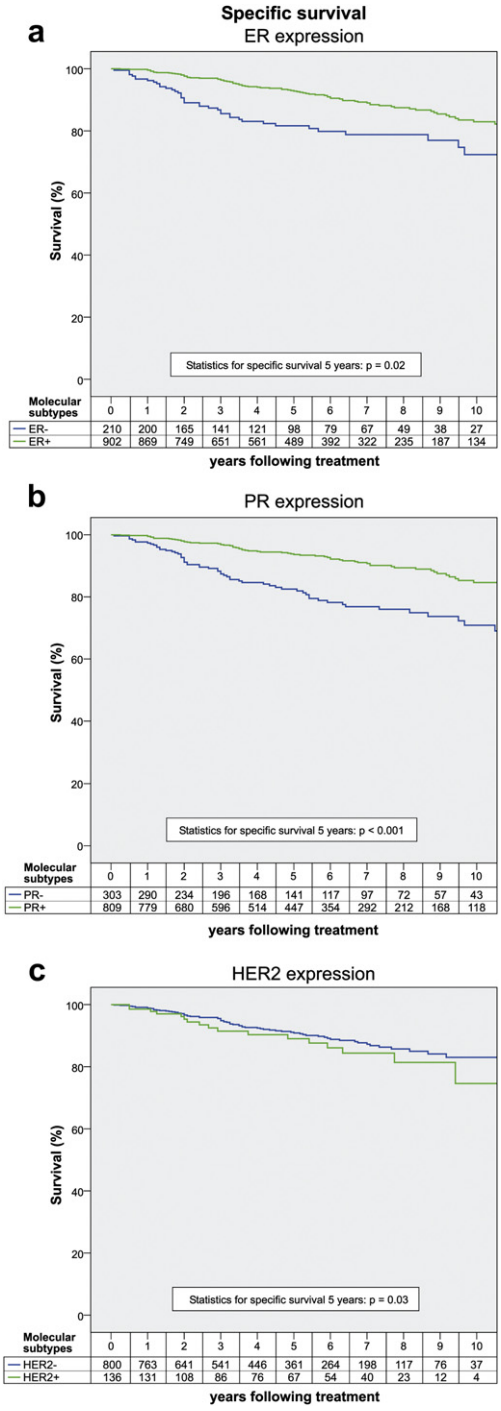

Fig. 3. Specific survival according to ER expression (a), PR expression (b) y HER2 expression (c). Fractions of patients remaining in the study are displayed at annual intervals up to 10 years.

without HER2+, due to the fact that such classification was actually built on the basis of only 42 patients.[5] Later, other authors modified the original classification by taking into consideration the proliferative factor, either grade 3 or the Ki 67 value,[41,42] in order to address the course of different breast tumours. However, profuse nomenclature changes make it difficult to compare the reported series.

Prevalence of both differentiation and histologic grade 3 is higher in triple negatives, Her2, and those ER+ subtypes with negative progesterone receptors. Probably because the aim of our study was not to establish an association between proliferation and hormone receptor or HER2 status, we could not compare our own results with those of Collins and Sotiriou[41,42]

Prevalence of Her2+ in younger women (under 50 in our study) was 6.6%, less than that in the studies by Parise,[28] Adedayo[26] and Collins,[41] whereas prevalence of triple negatives was similar to that of Collins,[41] Carvalho[43] or Eiermann.[44]

Our results are in line with those of previous studies[28,29] and confirm that breast cancer is indeed a multifaceted condition, consisting of different biologic subtypes each one with its own natural course, as well as with distinctive molecular, clinical, and pathologic features that obviously bear on therapy and prognosis. Taking the most prevalent group as the reference (ER+PR±HER2−) we were able to show important clinical, histopathological, and survival differences across groups and subtypes, both by bivariate and by multivariate analysis. As expected, the Her2 and triple negative groups were remarkable for their worse disease-free

Table 5
Multivariate analysis. Hazard ratios (95% CI) for overall and disease-free survival at 5 years, according to tumour subtype after adjusting for age, size (stage), histological grade, and lymph node status.

Subtype	Overall survival	Disease-free survival
ER+PR±HER2− ($n = 692$)	1.000	1.000
ER+PR±HER2+ ($n = 89$)	1.227 (0.638–2.557)	0.277 (0.037–2.071)
Her2 ($n = 49$)	1.227 (0.529–2.842)	1.472 (0.418–5.189)
Triple negative ($n = 123$)	1.889 (1.083–3.292)	3.357 (1.645–6.851)
ER/PR status		
ER/PR+ ($n = 895$)	1.000	1.000
ER/PR− ($n = 185$)	1.718 (1.117–2.641)	2.945 (1.622–5.380)
Her2 status		
Positive ($n = 132$)	1.000	1.000
Negative ($n = 776$)	0.887 (0.512–1.537)	0.417 (0.147–1.187)

survival, higher rate of distant metastases, and decreased overall survival at five years.[30,31] Some tumour characteristics deserve special consideration, more specifically with the PR negative subtypes. The potential predictive value of PR expression as a prognostic tool independent of ER expression is quite controversial. Nevertheless, the adjuvant therapy ATAC study (Arimidex and Tamoxifen alone or in combination) showed that ER+PR+ patients had lower recurrence rates than ER+PR− patients.[32]

Our by-group and subtype analysis is also in line with a previous large-scale report from a different geographical context,[29] showing that at five years ER+PR±HER2− and ER+PR±HER2+ groups of patients had better disease-free survival, less distant metastases, and greater overall survival, compared with the rest (Her2 and triple negative) with no significant differences between the two groups. However, these first two groups are obviously different as revealed by their immunohistochemistry divergence. Interestingly, the ER+PR−HER2− subtype had worse prognosis than the ER+PR+HER− and ER+PR+HER2+ subtypes. In fact, it was closer to Her2 and triple negative.

We did not find any significant difference in nodal status between the ER+PR±HER2− group and those groups with worse prognosis, namely Her2 and triple negative. However, we did notice significantly higher incidence of positive nodes in ER+PR±HER2+ patients compared with ER+PR±HER2− patients ($p = 0.02$).

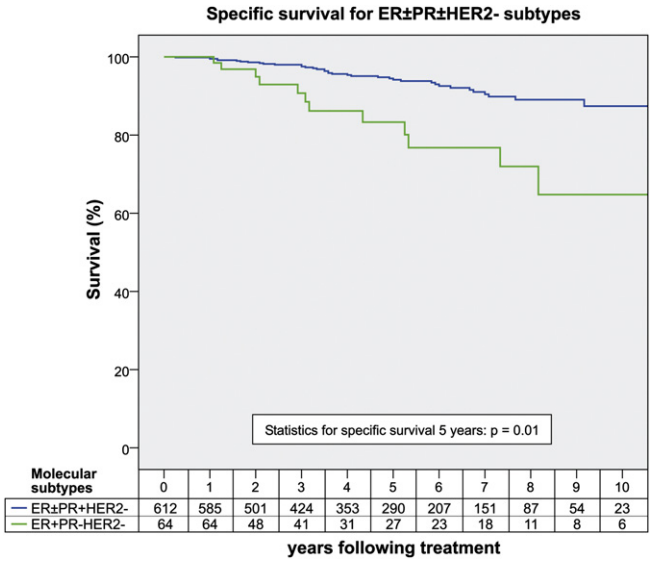

Fig. 4. Specific survival for both ER+PR±HER2− subtypes, differing in PR expression. Fractions of patients remaining in the study are displayed at annual intervals up to 10 years.

Noteworthy, we could not correlate lymph node status at surgery with distant metastases and overall survival. Subtypes with higher proliferation (TN, Her2, and ER+PR−HER2±) showed lower rates of nodal involvement, although they had the highest distant metastases rates and the lowest survival. Some authors[42,45] underline the importance of proliferation on relapse for ER+HER2− tumours, however we have only found such correlation for ER+PR− tumours.

As already reported by others,[33] our breast cancer patients with positive hormone receptor, both oestrogen and progesterone showed better survival. On the other hand, Her2+ and triple negative patients had higher rates of recurrence and breast cancer mortality.[34] In keeping with the work by Parise[28] and Adedayo,[26] our results show striking heterogeneity among Her2+ patients. Although both Her2+ subtypes are obviously different in genetic studies, they share similar clinicopathologic characteristics. However, compared with the Her2 group, the ER+PR±HER2+ group showed higher disease-free survival at five years as well as decreased rates of distant metastasis and mortality, both overall and specific. It seems that ER/PR, not Her2 status is the determinant factor governing patient survival in the short term,[35] although such a difference tends to peter out in the long run.

Just as with other studies,[29] our present work suggests that individual molecular markers are less important than their specific combinations, and that their individual effects on clinical outcomes seem to overlap in the long run. Additionally, our analysis confirms that there is a strong correlation between different molecular subtypes and the more conventional histopathologic variables. Superiority of sophisticated molecular technologies over routine immunohistochemistry is advocated on the basis of alleged improved quantitation and reproducibility. It seems clear, however, that a classification based only on the assessment of hormone receptors does not perform as well as that based on the combination of hormone receptors and Her2 expression, and that such classification provides also for improved therapeutic guidance. Immunohistochemical classification has evolved into an essential routine tool for breast cancer management. Immunohistochemical subtypes are a close reflection of the molecular breast cancer subtypes such as determined by microarray techniques,[6] but at a much lower cost and still holding great practical value for tailored treatment decision-making, notwithstanding the fact that perhaps a particular group of patients might further benefit from a more thorough knowledge of tumour genetic profile.

As for limitations in breast cancer classification systems, we know that the rate of false negative results for hormone receptor assays may be as high as 30%–60% overall, and 15%–20% for ER. Such a drawback may be multifactorial, including suboptimal fixation or procedural laboratory problems.[18,36,37] There are also some problems with Her2-neu assessment as shown by incongruous results between labs up to 80% of the time using immunohistochemistry, and up to 85% using fluorescence. Distribution of markers in our patients is however in keeping with other reported series[6,20,38]: 81% ER positive, 72% PR positive, 15% Her2+, as well as 72% ER+PR±HER2−, 13% triple negative, 9% ER+PR±HER2+, and 5% Her2.

To conclude, in our study immunohistochemical classification was able to differentiate three tumour types, namely HER2 and triple negatives as those with more somber prognosis and ER+PR+HER± that carry a better prognosis. Moreover, tumours with ER+PR−HER2± have a similar prognosis as HER2 and triple negatives, which brings the importance of PR status to the forefront.

When it comes to decide on systemic therapy for breast cancer patients, we have to take into account not only the immunohistochemical class, but also the proliferative grade.

Table 6
Baseline characteristics distribution according to tumour subtypes.

Groups	ER+PR±HER2− N = 724		ER+PR±HER2+ N = 97		Her2 N = 49	Triple negative N = 133
Subtypes	ER±PR+HER2− (651)	ER+PR−HER2− (73)	ER±PR+HER2+ (69)	ER+PR−HER2+ (28)	N (%)	N (%)
	N (%)		N (%)			
Age	651	73	67	28	49	133
<50 y	168 (26%)	9 (12%)	22 (33%)	6 (22%)	11 (23%)	44 (33%)
50–69 y	350 (54%)	45 (62%)	34 (51%)	18 (64%)	25 (51%)	49 (37%)
≥70 y	133 (20%)	19 (26%)	11 (16%)	4 (14%)	13 (26%)	40 (30%)
Differentiation grade	597	67	63	25	47	116
G1	129 (22%)	8 (12%)	7 (11%)	0 (0%)	0	8 (8%)
G2	199 (33%)	23 (34%)	23 (37%)	7 (28%)	10 (21%)	14 (12%)
G3	269 (45%)	36 (54%)	33 (52%)	18 (72%)	37 (79%)	94 (80%)
Histologic grade:	634	72	65	27	49	125
HG1	285 (45%)	25 (35%)	16 (25%)	3 (11%)	2 (4%)	13 (10%)
HG2	295 (47%)	35 (49%)	34 (52%)	18 (67%)	21 (43%)	45 (36%)
HG3	54 (8%)	12 (17%)	15 (23%)	6 (22%)	26 (53%)	67 (54%)
Size (T)	651	73	67	28	49	133
T1	422 (65%)	39 (53%)	34 (51%)	15 (53%)	19 (39%)	53 (40%)
T2	184 (28%)	20 (28%)	26 (39%)	12 (43%)	18 (37%)	62 (46%)
T3	23 (4%)	5 (7%)	6 (9%)	1 (4%)	7 (14%)	13 (10%)
T4	22 (3%)	9 (12%)	1 (1%)	0	5 (10%)	5 (4%)
Histologic type	651	73	67 calcular	28	49	133
Ductal carcinoma	579 (89%)	63 (86%)	66 (99%)	27 (96%)	48 (98%)	126 (95%)
Lobular carcinoma	60 (9%)	10 (14%)	1 (1%)	1 (4%)	1 (2%)	5 (4%)
Mixed ductal-lobular carcinoma	12 (2%)	0	0	0	0	0
Carcinosarcoma	0	0	0	0	0	2 (1%)
Lymph node status	643	73	67 calcular	28	49	133
negative	408 (63%)	41 (56%)	32 (48%)	16 (57%)	29 (59%)	81 (61%)
positive	235 (37%)	32 (44%)	35 (52%)	12 (43%)	20 (41%)	52 (39%)
1–3 nodes +	165 (70%)	20 (63%)	21 (60%)	8 (67%)	11 (55%)	28 (54%)
≥4 nodes +	70 (30%)	12 (37%)	14 (40%)	4 (33%)	9 (45%)	24 (46%)

Conflict of interest statement

None declared.

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