17. Kurosumi M. Significance of histopathological evaluation in primary therapy for breast cancer–recent trends in primary modality with pathological complete response (pCR) as endpoint. Breast Cancer 2004; 11: 139–47.
18. Gounaris I, Provenzano E, Vallier AL et al.. Accuracy of unidimensional and volumetric ultrasound measurements in predicting good pathological response to neoadjuvant chemotherapy in breast cancer patients. Breast Cancer Res Treat 2011; 127: 459–69.
19. von Minckwitz G, Kümmel S, Vogel P et al.. Intensified neoadjuvant chemotherapy in early-responding breast cancer: phase III randomised GeparTrio study. J Natl Cancer Inst 2008; 100: 552–562.
20. Chagpar AB, Middleton LP, Sahin AA et al.. Accuracy of physical examination, mammography and ultrasonography in predicting residual pathologic tumour size in patients treated with neoadjuvant chemotherapy. Ann Surg 2006; 243: 257–264.
21. Yeh E, Slanetz P, Kopans DB et al.. Prospective comparison of mammography, sonography, and MRI in patients undergoing neoadjuvant chemotherapy for palpable breast cancer. AJR Am J Roentgenol 2005; 184: 868–877.
22. Singletary SE, McNeese MD, Hortobagyi GN. Feasibility of breast-conservation surgery after induction chemotherapy for locally advanced breast carcinoma. Cancer 1992; 69: 2849–2852.
23. Kim HJ, Im YH, Han BK et al.. Accuracy of MRI for estimating residual tumor size after neoadjuvant chemotherapy in locally advanced breast cancer: relation to response patterns on MRI. Acta Oncol 2007; 46: 996–1003.
24. Bear HD, Anderson S, Smith RE et al.. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 2006; 24: 2019–27.
25. Carey LA, Metzger R, Dees EC et al. American Joint Committee on Cancer tumor-node-metastasis stage after neoadjuvant chemotherapy and breast cancer outcome. J Natl Cancer Inst 2005; 97: 1137–42.
26. Wolmark N, Wang J, Mamounas E et al. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from the National Surgical Adjuvant Breast and Bowel Project B-18. J Natl Cancer Inst Monogr 2001; 30: 96–102.
27. Chollet P, Amat S, Cure H et al.. Prognostic significance of a complete pathological response after induction chemotherapy in operable breast cancer. Br J Cancer 2002; 86: 1041–1046.
28. Montagna E, Bagnardi V, Rotmensz N et al.. Pathological complete response after preoperative systemic therapy and outcome: relevance of clinical and biologic baseline features. Breast Cancer Res Treat 2010; 124: 689–99.

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Progesterone receptor loss identifies Luminal B breast cancer subgroups at higher risk of relapse

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Background: The immunohistochemical (IHC) evaluation of estrogen receptor (ER), progesterone receptor (PgR), Ki-67 and HER2 is considered a surrogate means for identifying the molecular subtypes of breast cancer with different prognosis.
Patients and methods: We explored patterns of recurrence in 4837 women with breast cancer defined as Luminal B (ER-positive and/or PgR-positive, HER2 positive and/or Ki-67≥14%) by IHC classification. We evaluated four subgroups within the Luminal B subtype according to HER2 expression and PgR status.
Results: Patients within the ER+/PgR+/HER2− subgroup presented a 5-year breast cancer-related survival (BCS) of 97% (95% confidence interval (CI), 96–97) and overall survival (OS) of 95% [95% CI, 95–96], the best survivals of the Luminal B subgroups. In the multivariate analysis, the ER+/PgR−/HER2− subgroup was associated with a reduced BCS (HR 1.71; 95%CI, 1.25–2.35) and OS (HR 1.47; 95%CI, 1.10–1.96) when compared with the ER+/PgR+/HER2− subgroup. Also patients within the ER+/PgR−/HER2+ subgroup had a reduced BCS (HR 1.93; 95%CI, 1.32–2.83) and OS (HR 1.62; 95%CI, 1.14–2.30) when compared with ER+/PgR+/HER2− subgroup. On the other hand, no statistically significant differences were found with regard to BCS and OS among patients with ER+/PgR+/HER2+ and patients with ER+/PgR+/HER2− disease.

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Conclusions: PgR loss identifies Luminal B breast cancer subgroups at higher risk of relapse and death, both with HER-2-positive and HER-2-negative disease.

Key words: breast cancer, HER2, immunohistochemistry, luminal, progesterone, prognosis

introduction

Breast cancer is a heterogeneous disease and gene expression studies have identified molecularly distinct subtypes with prognostic implications across multiple treatment settings [1–3].

These subtypes include estrogen receptor (ER)-positive—Luminal A (Luminal A), ER-positive—Luminal B (Luminal B), HER2-enriched (i.e. tumors that overexpress ERBB2-associated genes but do not express genes that define the luminal subtype), basal-like and normal breast-like. HER2-enriched and basal-like subtypes are hormone receptor negative and have poor prognosis [1, 4, 5].

The immunohistochemical (IHC) evaluation of ER, progesterone receptor (PgR), Ki-67 and HER2 may be considered a surrogate means for identifying the molecular subtypes of breast cancer [6].

Moreover, a recent head-to-head comparison of a four IHC biomarker panel of ER, PgR, HER2 and Ki-67 (IHC 4) has been shown to provide prognostic information, which could be considered at least equivalent to the genomic health recurrence score [7].

The IHC classification according to the four subtypes (Luminal A, Luminal B, HER2 and triple negative) appeared useful to define different prognostic subgroups in relationship with the different adjuvant treatments previously received [8].

Recommendations for selection of adjuvant systemic treatments in specific patient populations were recently proposed based on the recognition of intrinsic biological subtypes with different responses to systemic and local therapies [9].

Luminal B is characterized by higher grade as well as lower levels of ER-related genes and is characterized by having increased expression of HER2-associated genes (i.e. ERBB2 and GRB7) and a cell proliferation signature that includes the expression of MKI67, CCNB1 and MYBL2, which have been associated with tamoxifen resistance [2, 10].

Nevertheless, the Luminal B subtype still represents a heterogeneous group of breast cancers, if considered that only 30% of Luminal B tumors present an overexpression of HER2 including both the PgR-positive and PgR-negative disease.

PgR is a prognostic factor, although its prognostic value after long-term follow-up is considered weak and lose [11]. The absence of PgR may be a marker of aberrant growth factor signaling and, consequently, one mechanism for anti-estrogen resistance [12, 13]. ER+/PgR− tumors as defined by RNA profiling represent a distinct subset of breast cancer with aggressive features and poor outcome despite being clinically ER+ [14].

In order to get a deeper insight into the prognostic significance of subtypes of early breast cancer, we analyzed data according to PgR and HER2 status from a large series of well-characterized patients with breast cancer defined as Luminal B by IHC classification.

patients and methods

We collected information on all consecutive breast cancer patients who underwent breast surgery at the European Institute of Oncology between January 1997 and December 2005. Data on each patient's medical history, concurrent diseases, surgery, pathological evaluation and results of staging procedures (blood chemistry, hematological values, bone scan, chest film and upper abdominal ultrasound examination) were retrieved. The surgically removed breast lesions were thoroughly sampled for pathological examination.

Tissue sections from all previous needle biopsies (at least three sections/core, cut at 110–200 mm intervals) and from all surgical resections carried out elsewhere were reviewed. Tumors were classified histologically according to the World Health Organization Histological Classification of Breast Tumors, as modified by Rosen and Obermann [15]. Tumor grading was assessed according to Elston and Ellis [16]. We looked for peritumoral vascular invasion as recommended by Rosen and Obermann [17]. Microinvasive breast cancer was diagnosed according to the TNM classification and following the criteria of Rosen and Obermann [15]. ER and PgR status, Ki-67 labeling index determined with the MIB1 monoclonal antibody, and HER2/neu overexpression were evaluated immunohistochemically as previously reported [17]. In particular, HER2/neu overexpression was evaluated using a 1/800 dilution of a polyclonal antiserum (Dako, Glostrup, Denmark) and considering only complete and intense membrane staining of at least 10% of neoplastic cells as evidence of overexpression (3+). Tumors showing weak to moderate circumferential membrane immunoreactivity (2+) were further subjected to FISH assays for the assessment of gene amplification, as previously reported. For evaluation of ER, PgR status and Ki-67 labeling index, the percentage of cells exhibiting definite nuclear staining over 2000 neoplastic cells examined at ×400 magnification was recorded. The stained slides were evaluated independently by two of the authors. Only nuclear immunoreactivity was evaluated for ER, PgR and MIB1. The threshold for ER and PgR positivity was 1% [17]. Histological grade and biological features were evaluated on the invasive component of the tumor.

statistics

The Fisher's exact test and the Mantel–Haenszel chi-square test for trend were used to assess the association between categorical and ordinal variables, respectively. The primary end-points were the incidence of locoregional relapse (LRR), distant metastases (DM), breast cancer-related survival (BCS) and overall survival (OS).

LRR included ipsilateral breast cancer, breast cancer recurrence in the axilla, regional lymph nodes, chest wall and skin of ipsilateral breast. DM included all sites of recurrence except locoregional relapses and contralateral breast cancer as first of subsequent events. BCS included locoregional relapses, distant metastases, contralateral breast cancer or death from breast cancer. OS was determined as the time from surgery until the date of death (from any cause) or was censored at the date of last follow-up.

Cumulative incidence and survival plots were drawn using the Kaplan–Meier method.

We decided to focus our analysis on the Luminal B subtype defined as tumors with hormonal receptors positive (ER > 0 or PgR > 0) and [Ki-67 ≥ 14% or HER2 overexpressed/amplified (HER2+)].

We evaluated four subgroups within the Luminal B subtype according to HER2 expression and PgR status:

- ER and PgR positive and HER2 not overexpressed/amplified (ER+/PgR+/HER2−)
- ER and PgR positive and HER2 overexpressed/amplified (ER+/PgR+/HER2+) (*named also Triple Positive or TP*)
- ER positive, PgR negative and HER2 not overexpressed/amplified (ER+/PgR−/HER2−)
- ER positive, PgR negative and HER2 overexpressed/amplified (ER+/PgR−/HER2+)

results

A total of 4837 patients with early Luminal B subtype breast cancer who underwent breast surgery at the European Institute of Oncology between 1997 and 2005 were included in this analysis. Male patients and females with previous noninvasive breast cancers or bilateral tumors were excluded.

Patients' characteristics are shown in Table 1.

Triple positive tumors (ER+/PgR+/HER2+) were associated with very young age (<35 years), occurrence of peritumoral vascular invasion and poorer differentiation than other Luminal B subgroups. Finally, TP tumors were associated with more advanced tumor stage, namely with more extensive nodal involvement and larger tumor size than the other Luminal B subgroups

About 50% of patients in the Luminal B subtype received only endocrine therapy as adjuvant treatment and 2% of patients did not receive any adjuvant treatment. Patients with ER+/PgR+/HER2+ and ER+/PgR−/HER2+ tumors received more chemotherapy than the other two subgroups without overexpression of HER2. The ER+/PgR+/HER2− subgroup was treated with less chemotherapy and more endocrine therapy alone than the other subgroups (Table 2).

About 99% of patients with TP tumors did not receive trastuzumab as adjuvant treatment, while two patients received adjuvant trastuzumab in the subgroup ER+/PgR−/HER2+.

The ER+/PgR+/HER2− subgroup was the one with the lowest rate of LRR, DM and breast cancer-related events as well as with the best BCS and OS when compared with the other Luminal B subgroups (Figures 1 and 2).

On the contrary, the ER+/PgR−/HER2+ subgroup was the one that showed the worst BCS and OS when compared with the other Luminal B subgroups. At the same time, the TP subgroup showed a better BCS and OS than the ER+/PgR−/HER2+ but also ER+/PgR−/HER2− subgroup, as shown in Figure 2.

At 5 and 10 years, patients with TP tumors had a 95.8% (94.1–97.4) and 85.4% (80.5–90.4) of BCS and an OS of 94.8% (92.9–96.6) and 83.5% (78.5–88.6) at 5 and 10 years, respectively.

The ER+/PgR−/HER2+ subgroup had HR of 2.39 (95% CI, 1.65–3.47) and 1.94 (95% CI, 1.37–2.75) for BCS and OS, respectively, while the TP one had HR of 1.43 (95% CI, 1.06–1.95) and 1.22 (95% CI, 0.92–1.61) at the univariate analysis. The ER+/PgR−/HER2− subgroup showed an intermediate increased risk when compared with the ER+/PgR+/HER2− subgroup at the univariate analysis, with HR of 1.96 (1.44–2.68, 95% CI) and 1.65 (95% CI, 1.24–2.19) for BCS and OS, respectively (Figure 2).

At the multivariate analysis, the TP subgroup had an increased risk of locoregional relapses in comparison with the ER+/PgR+/HER2− subgroup but no statistical significant differences could be found for DM, contralateral breast cancer, BCS and OS. On the other hand, the ER+/PgR−/HER2− subgroup was associated with an increased risk with respect to all outcomes considered, including BCS and OS, when compared with the ER+/PgR+/HER2− subgroup. Finally, the analysis showed that the ER+/PgR−/HER2+ subgroup had an increased risk of DM, BCS and OS compared with the ER+/PgR+/HER2− subgroup with HR of 1.93 (1.32–2.83; 95% CI)) and 1.62 (1.14–2.30; 95% CI) for BCS and OS, respectively (Table 3).

The multivariate analysis carried out in 1715 women with Luminal B breast cancer treated after 2003, to exclude the potential effect of trastuzumab therapy, confirmed the results obtained in the whole population (Table 4).

discussion

Subtypes with different epidemiological risk factors, different natural histories and different responses to systemic and local therapies have been identified. Clinicians managing breast cancer should consider cases within the various distinct subtypes in order to properly assess the relevant evidence and reach an appropriate therapeutic choice. Endocrine therapy is part of the treatment of the 'Luminal B' subtype. Chemotherapy is also considered indicated for most patients with 'Luminal B' disease with the addition of trastuzumab in 'HER2-positive' disease [9].

However, the tumor subtypes identified in these analyses include heterogeneous groups of tumors, and the identification of further tumor subtypes amenable to targeted treatments represents a research priority. Specifically, there is lack of consensus on the threshold indication for inclusion of chemotherapy for patients with 'Luminal B (HER2-negative)' disease.

The results of the present study indicate that PgR is an important prognostic factor in order to properly define subgroups with different prognosis within the Luminal B subtype, irrespective of HER2 overexpression or amplification.

Other studies reported and analyzed the prognostic and predictive role of progesterone receptor in breast cancer, especially of the subgroup of tumors ER+/PgR−.

The prognostic and predictive value of PgR has been for a long time ascribed to the dependence of PgR expression on ER activity, with the absence of the PgR reflecting a nonfunctional ER and resistance to hormonal therapy, However, alternative molecular mechanisms potentially explaining the different outcome and selective estrogen receptor modulator resistance in ER-positive/PgR-negative tumors have been suggested by experimental indications that growth factors may reduce PgR levels. Thus, the absence of PgR may reflect hyperactive cross talk between ER and growth factor signaling pathways that reduce PgR even as they activate other ER functions.

Previous experimental studies have shown that ER+PgR− tumors have high growth factor signaling [12, 18, 19]. In

Table 1. Characteristics of 4837 women with Luminal B breast cancer according to PgR and HER2 status

Characteristics	Total	Histological subtype				*P* value
		ER+PgR−HER2+	ER+PgR+HER2+	ER+PgR−HER2−	ER+PgR+HER2−	
	N	*N* (%)	*N* (%)	*N* (%)	*N* (%)	
All patients	4837	236 (4.9)	591 (12.2)	435 (9.0)	3575 (73.9)	
Age group (years)						
<35	222	12 (5.4)	42 (18.9)	15 (6.8)	153 (68.9)	<0.0001
35–49	1953	66 (3.4)	311 (15.9)	100 (5.1)	1476 (75.6)	
50–69	2183	143 (6.6)	213 (9.8)	261 (12.0)	1566 (71.7)	
70+	479	15 (3.1)	25 (5.2)	59 (12.3)	380 (79.3)	
Tumor size (cm)						
≤1	853	49 (5.7)	93 (10.9)	82 (9.6)	629 (73.7)	<0.0001
1–2	2170	83 (3.8)	224 (10.3)	188 (8.7)	1675 (77.2)	
2–5	1607	85 (5.3)	239 (14.9)	146 (9.1)	1137 (70.8)	
>5	180	16 (8.9)	32 (17.8)	17 (9.4)	115 (63.9)	
Unknown	27	3 (11.1)	3 (11.1)	2 (7.4)	19 (70.4)	
pT						
pT1	3026	133 (4.4)	319 (10.5)	271 (9.0)	2303 (76.1)	<0.0001
pT2	1606	85 (5.3)	235 (14.6)	147 (9.2)	1139 (70.9)	
pT3/4	196	17 (8.7)	36 (18.4)	17 (8.7)	126 (64.3)	
Unknown	9	1 (11.1)	1 (11.1)	0 (0.0)	7 (77.8)	
Number of positive nodes						
None	2401	134 (5.6)	270 (11.2)	235 (9.8)	1762 (73.4)	0.03
1–3	1555	59 (3.8)	195 (12.5)	126 (8.1)	1175 (75.6)	
4–9	467	26 (5.6)	71 (15.2)	34 (7.3)	336 (71.9)	
10 or more	324	11 (3.4)	47 (14.5)	33 (10.2)	233 (71.9)	
PNx	90	6 (6.7)	8 (8.9)	7 (7.8)	69 (76.7)	
Tumor Grade						
G1	360	8 (2.2)	21 (5.8)	21 (5.8)	310 (86.1)	<0.0001
G2	2723	98 (3.6)	217 (8.0)	220 (8.1)	2188 (80.4)	
G3	1630	125 (7.7)	341 (20.9)	179 (11.0)	985 (60.4)	
Unknown	124	5 (4.0)	12 (9.7)	15 (12.1)	92 (74.2)	
Histology						
Ductal	4004	219 (5.5)	536 (13.4)	354 (8.8)	2895 (72.3)	<0.0001
Lobular	417	6 (1.4)	20 (4.8)	42 (10.1)	349 (83.7)	
Ductal + lobular	212	3 (1.4)	17 (8.0)	22 (10.4)	170 (80.2)	
Cribriform	59	0 (0.0)	6 (10.2)	1 (1.7)	52 (88.1)	
Mucinous	70	3 (4.3)	4 (5.7)	7 (10.0)	56 (80.0)	
Tubular	2	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	
Papillary	24	1 (4.2)	5 (20.8)	2 (8.3)	16 (66.7)	
Tubulo-lobular	8	0 (0.0)	0 (0.0)	0 (0.0)	8 (100)	
Apocrine	16	2 (12.5)	2 (12.5)	3 (18.8)	9 (56.3)	
Micropapillary	17	2 (11.8)	0 (0.0)	2 (11.8)	13 (76.5)	
Metaplastic	3	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)	
Medullary	2	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	
Adenoido-cistic	1	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	
Other	2	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	
PVI						
Absent	3260	159 (4.9)	353 (10.8)	313 (9.6)	2435 (74.7)	0.0001
Present	1577	77 (4.9)	238 (15.1)	122 (7.7)	1140 (72.3)	
Proliferative fraction (Ki-67)						
<14%	71	22 (31.0)	49 (69.0)	0 (0.0)	0 (0.0)	<0.0001
14–30%	3370	106 (3.1)	234 (6.9)	301 (8.9)	2729 (81.0)	
≥30%	1396	108 (7.7)	308 (22.1)	134 (9.6)	846 (60.6)	
Type of surgery						
Conservative	3823	160 (4.2)	421 (11.0)	348 (9.1)	2894 (75.7)	<0.0001
Mastectomy	1014	76 (7.5)	170 (16.8)	87 (8.6)	681 (67.2)	
Radiotherapy						
No	799	55 (6.9)	116 (14.5)	74 (9.3)	554 (69.3)	0.002
Yes	4038	181 (4.5)	475 (11.8)	361 (8.9)	3021 (74.8)	

PVI, peritumoral vascular invasion.

Table 2. Medical adjuvant treatment of Luminal B breast cancer subgroups

	No treatment (%)	Endocrine therapy alone (%)	Non anthracycline-containing chemotherapy (%)	Anthracycline-containing chemotherapy (%)	*P* value
Overall	103 (2.1)	2643 (54.6)	542 (11.2)	1549 (32.0)	<0.0001
ER+PgR−HER2+	10 (4.2)	67 (28.4)	45 (19.1)	114 (48.3)	
ER+PgR+HER2+	8 (1.4)	173 (29.3)	109 (18.4)	301 (50.9)	
ER+PgR−HER2−	11 (2.5)	212 (48.7)	90 (20.7)	122 (28.1)	
ER+PgR+HER2−	74 (2.1)	2191 (61.3)	298 (8.3)	1012 (28.3)	

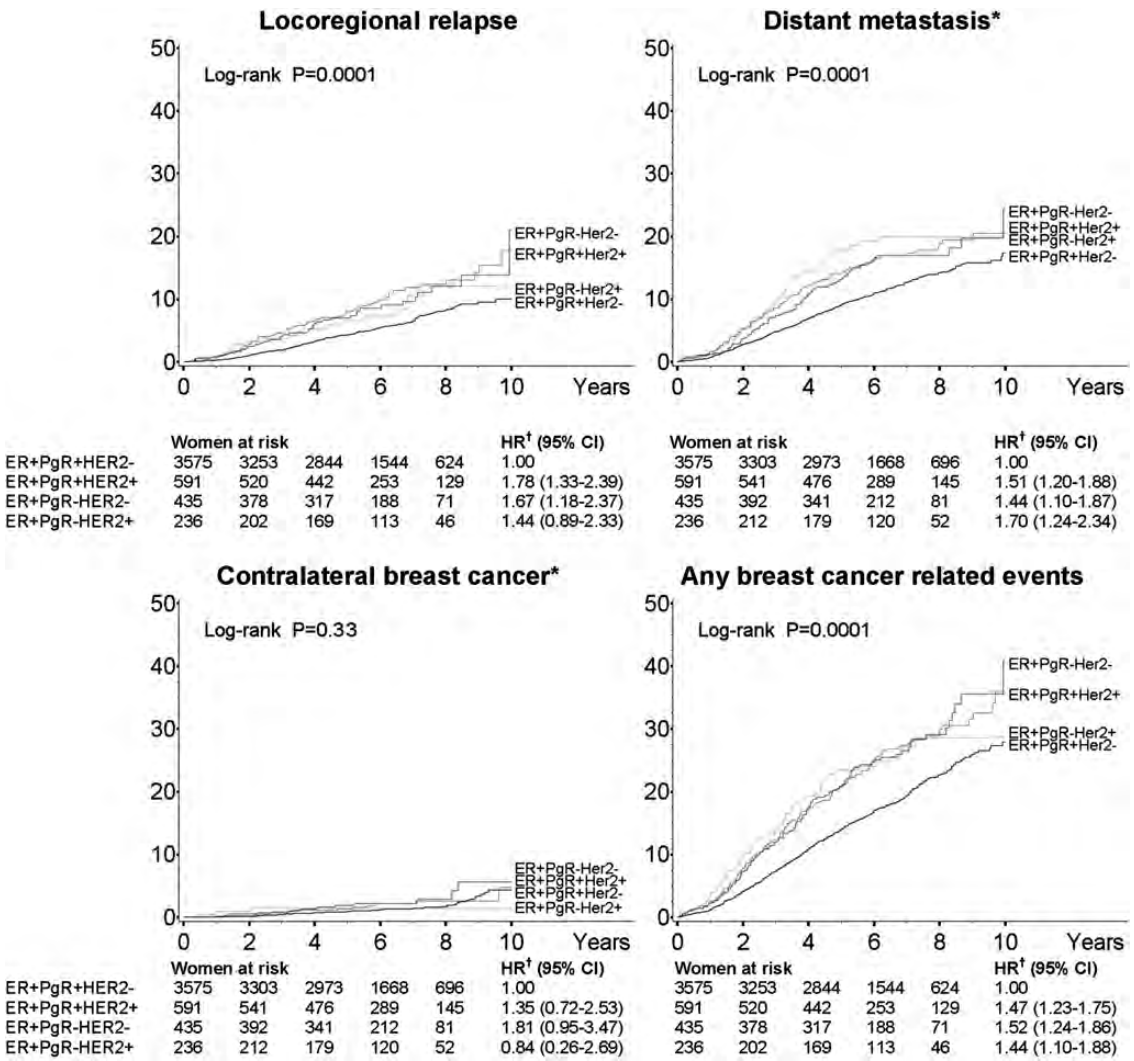

Figure 1. Clinical outcomes of 4837 women with Luminal B breast cancer according to PgR and HER2 status. *As first or second event; †Hazards ratios (HR) and 95% confidence intervals (CI) obtained from a univariate Cox proportional Hazards regression model.

particular ER+/PgR− tumors have higher levels of epidermal growth factor receptor (EGFR) and HER2 than ER+PgR+ tumors [19–21]. In the large series of Arpino et al, HER2 overexpression was associated with a significantly shorter disease-free survival (DFS) interval in patients with ER+PgR− tumors, whereas in ER+PgR+ disease, HER2 overexpression was not associated with DFS, among tamoxifen-treated women [19].

Two large randomized trials evaluated the efficacy of aromatase inhibitors, letrozole (BIG 1–98 trial) and anastrazole (ATAC trial), compared with tamoxifen as adjuvant treatment of patients with early breast cancer. In BIG 1–98, patients treated with letrozole had a better outcome than those treated with tamoxifen regardless of their PgR status [22]. Subsequent analyses of the ATAC trial have shown that quantitative expression of ER and PgR and HER-2 status did not identify

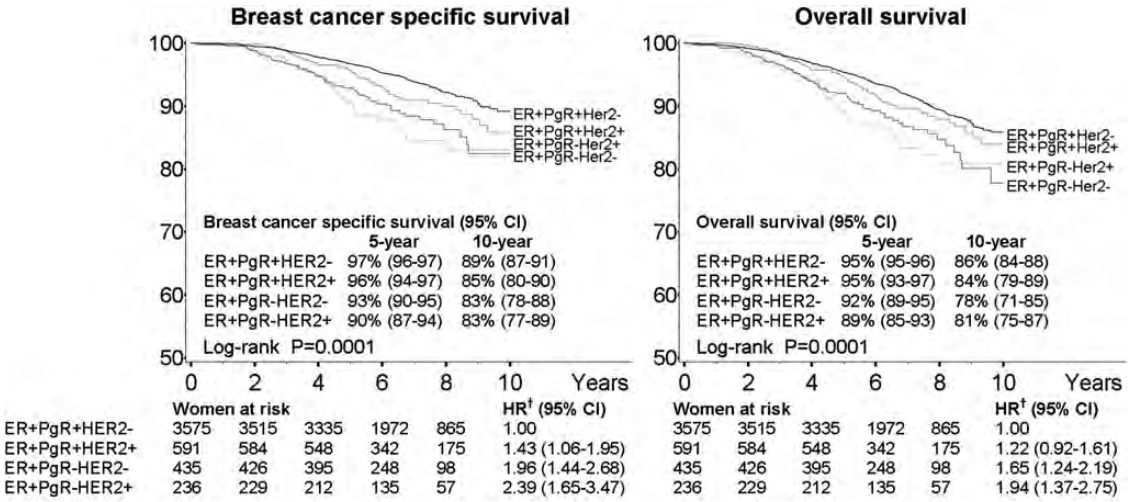

Figure 2. Survival of 4837 women with luminal B breast cancer according to PgR and HER2 status. †Hazards ratios (HR) and 95% confidence intervals (CI) obtained from a univariate Cox proportional Hazards regression model.

Table 3. Multivariate analysis in 4837 women with Luminal B breast cancer

Number of events/deaths	Locoregional relapse 308	Distant metastasis 597	Contralateral breast cancer 79	Breast cancer-related event (BCS) 939	Breast cancer-specific survival 338	Overall survival (OS)
	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]
IHC subgroup						
ER+PgR+HER2−	1.00	1.00	1.00	1.00	1.00	1.00
ER+PgR+HER2+	1.41 (1.04–1.92)	1.11 (0.88–1.40)	1.39 (0.73–2.65)	1.18 (0.98–1.41)	1.02 (0.74–1.39)	0.98 (0.74–1.31)
ER+PgR–HER2–	1.68 (1.18–2.39)	1.34 (1.03–1.74)	1.94 (1.00–3.77)	1.41 (1.15–1.73)	1.71 (1.25–2.35)	1.47 (1.10–1.96)
ER+PgR–HER2+	1.32 (0.81–2.16)	1.58 (1.14–2.18)	1.02 (0.31–3.33)	1.32 (1.00–1.74)	1.93 (1.32–2.83)	1.62 (1.14–2.30)
Age group (years)						
<35	1.93 (1.25–2.99)	1.42 (1.04–1.92)	2.53 (1.15–5.57)	1.35 (1.04–1.76)	1.10 (0.71–1.69)	0.91 (0.60–1.39)
35–49	1.24 (0.97–1.60)	0.85 (0.71–1.02)	0.92 (0.56–1.51)	0.91 (0.79–1.05)	0.67 (0.53–0.87)	0.61 (0.49–0.77)
50–69	1.00	1.00	1.00	1.00	1.00	1.00
70+	1.05 (0.67–1.67)	1.32 (0.99–1.77)	1.19 (0.50–2.84)	1.61 (1.30–2.00)	1.73 (1.22–2.44)	2.32 (1.77–3.04)
Tumor size (cm)						
≤2	1.00	1.00	1.00	1.00	1.00	1.00
>2	1.67 (1.31–2.13)	2.23 (1.86–2.66)	1.27 (0.77–2.09)	1.99 (1.73–2.29)	2.39 (1.88–3.05)	2.15 (1.74–2.65)
Nodal status						
pN0	1.00	1.00	1.00	1.00	1.00	1.00
pN+	0.94 (0.73–1.22)	2.06 (1.68–2.52)	0.93 (0.56–1.55)	1.46 (1.25–1.70)	1.91 (1.46–2.49)	1.89 (1.50–2.39)
pNx	4.51 (2.37–8.59)	2.63 (1.39–4.98)	–	2.62 (1.72–4.00)	3.01 (1.50–6.04)	3.95 (2.46–6.35)
Histology						
Ductal	1.00	1.00	1.00	1.00	1.00	1.00
Lobular	1.22 (0.80–1.86)	1.69 (1.28–2.34)	0.83 (0.32–2.13)	1.39 (1.11–1.75)	1.74 (1.21–2.49)	1.56 (1.14–2.13)
Ductal + lobular	1.15 (0.67–1.98)	1.25 (0.86–1.82)	2.00 (0.85–4.69)	1.31 (0.97–1.75)	1.35 (0.83–2.19)	1.27 (0.82–1.96)
Other	0.51 (0.22–1.17)	0.55 (0.27–1.12)	1.04 (0.35–3.05)	0.74 (0.47–1.16)	0.41 (0.13–1.31)	0.68 (0.32–1.45)
Tumor Grade						
G1	1.00	1.00	1.00	1.00	1.00	1.00
G2	1.04 (0.59–1.83)	1.23 (0.71–2.14)	0.71 (0.32–1.59)	1.06 (0.75–1.51)	1.50 (0.65–3.46)	1.24 (0.70–2.21)
G3	1.24 (0.68–2.27)	1.95 (1.11–3.43)	0.44 (0.17–1.13)	1.53 (1.062.22)	3.30 (1.41–7.72)	2.54 (1.40–4.61)
Unknown	1.45 (0.58–3.64)	1.50 (0.65–3.43)	1.37 (0.38–4.97)	1.44 (0.83–2.49)	1.03 (0.25–4.24)	1.02 (0.37–2.79)
PVI						
Absent	1.00	1.00	1.00	1.00	1.00	1.00
Present	1.38 (1.06–1.79)	1.39 (1.16–1.66)	1.47 (0.87–2.48)	1.30 (1.12–1.51)	1.44 (1.13–1.82)	1.30 (1.05–1.60)
Proliferative fraction (Ki-67)						
<20%	1.00	1.00	1.00	1.00	1.00	1.00
≥20%	1.64 (1.21–2.22)	1.77 (1.39–2.24)	1.33 (0.79–2.24)	1.79 (0.57–5.64)	1.47 (1.08–2.02)	1.25 (0.96–1.61)

IHC, immunohistochemical; PVI, peritumoral vascular invasion.

[a]Hazards ratio (HR) and 95% confidence intervals (CI) obtained from a multivariable COX proportional hazards regression model.

Table 4. Multivariate analysis in 1715 women with Luminal B breast cancer treated after 2003 (introduction of Trastuzumab)

	Locoregional relapse	Distant metastasis	Contralateral breast cancer	Breast cancer-related event (BCS)	Breast cancer-specific survival	Overall survival (OS)
Number ofevents/deaths	97	171	12	266	73	93
	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]
IHC subgroup						
ER+PgR+HER2−	1.00	1.00	1.00	1.00	1.00	1.00
ER+PgR+HER2+	2.49 (1.49–4.14)	1.28 (0.82–1.99)	2.65 (0.51–13.7)	1.47 (1.03–2.09)	0.81 (0.36–1.82)	0.74 (0.35–1.57)
ER+PgR−HER2−	1.27 (0.57–2.82)	2.19 (1.35–3.53)	1.34 (0.16–11.2)	1.75 (1.17–2.61)	2.03 (1.02–4.03)	1.44 (0.75–2.77)
ER+PgR−HER2+	2.02 (0.90–4.53)	2.29 (1.32–3.99)	1.95 (0.23–16.7)	2.08 (1.30–3.31)	2.42 (1.15–5.10)	2.89 (1.14–4.60)

IHC, immunohistochemical.

[a]Hazards ratio (HR) and 95% confidence intervals (CI) obtained from a multivariable COX proportional hazards regression model adjusted for age, tumor size, nodal status, histology, tumor grade, PVI and Ki-67.

patients with differential relative benefit from anastrozole over tamoxifen [23].

In the present study, we observed that TP breast cancer patients had a better survival than those in the ER+/PgR−/HER2+ but also in the ER+/PgR−/HER2− subgroup.

The ER+/PgR−/HER2− subgroup received less chemotherapy than TP, so the different survival outcome among these two subgroups could be related to the positive impact of chemotherapy on the risk of breast cancer-related events and deaths in the TP subgroup. However, these data confirm the importance of PgR status, prompting us to consider more chemotherapy in the ER+/PgR−/HER2− subgroup.

About 30% of patients with TP and ER+/PgR−/HER2+ tumors received endocrine therapy alone and ∼65–70% received chemotherapy plus endocrine therapy as adjuvant treatments. Moreover, the same percentage of patients (about 1%) in both the TP and ER+/PgR−/HER2+ subgroups received trastuzumab as adjuvant therapy.

The difference in the outcomes of these two subgroups appeared, therefore, related more probably to the PgR status. In 2003 and during the subsequent years, the use of aromatase inhibitors progressively increased and some patients received trastuzumab as experimental adjuvant treatment. However, multivariate analysis carried out in only the 1715 women with Luminal B breast cancer treated after 2003 confirmed the results obtained in the whole population.

Exploratory analysis of the magnitude of trastuzumab effects within the patient subgroups in the HERA trial showed that adjuvant trastuzumab therapy reduces the risk of relapse similarly across subgroups defined by nodal status and steroid hormone receptor status, even those at relatively low risk for relapse [24].

Interestingly, in this exploratory analysis of the HERA trial, patients with TP tumors had an important benefit from trastuzumab with 3-year DFS of 85% compared with 77% of patients in the observational arm, and reduction of recurrence risk of 37%. Apparently, the better survival outcome for patients in our analysis compared with the TP patients in the HERA trial can mostly likely be related to the different nodal involvement. About 32% of all patients in the HERA trial did not have nodes involvement; moreover, in the same trial, only the 12%–13% of patients had hormone receptor-positive tumors without nodes involvement. In the present analysis, the number of patients without nodal involvement ranged between 46% with TP tumors and 57% with ER+/PgR−/HER2+ tumors.

Subgroup and retrospective analyses must be interpreted with caution due to the increased likelihood of false-positive and false-negative results arising from the play of chance. However, our data together with the HERA subgroup analysis make us believe not to consider mandatory a triple combination of treatments (endocrine + chemotherapy + anti-HER2 therapy) in all patients with TP tumors, differently from the subgroup of patients with ER+/PgR−/HER2+ tumors.

In conclusion, after dividing the Luminal B subtype group into four subgroups according to PgR and HER2 status, we provided evidence of a relatively good prognosis of the TP subgroup, and highlighted and confirmed the significant impact of progesterone receptor status on the outcome of patients with early breast cancer.

disclosure

The authors have declared no conflicts of interest.

references

1. Sorlie T, Tibshirani R, Parker J et al.. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA 2003; 100: 8418–8423.
2. Oh DS, Troester MA, Usary J et al.. Estrogen-regulated genes predict survival in hormone receptor-positive breast cancers. J Clin Oncol. 2006; 24: 1656–1664.
3. Rouzier R, Perou CM, Symmans WF et al.. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 2005; 11: 5678–5685.
4. Perou CM, Sorlie T, Eisen MB et al.. Molecular portraits of human breast tumors. Nature 2000; 406: 747–752.
5. Sorlie T, Perou CM, Tibshirani R et al.. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 2001; 98: 10869–10874.
6. Cheang MC, Chia SK, Voduc D et al.. Ki67 Index, HER2 Status, and prognosis ofz patients with luminal B breast Cancer. J Natl Cancer Inst 2009; 101: 736–750.
7. Cuzick J, Dowsett M, Pineda S et al.. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the genomic

health recurrence score in early breast cancer. J Clin Oncol. 2011; 29: 4273–4278.
8. Hugh J, Hanson J, Cheang MC et al.. Breast cancer subtypes and response to docetaxel in node-positive breast cancer: use of an Immunohistochemical definition in the BCIRG 001 Trial. J Clin Oncol 2009; 27: 1168–1176.
9. Goldhirsch A, Wood WC, Coates AS et al.. Strategies for subtypes: dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the primary therapy of early breast cancer 2011. Ann Oncol. 2011; 22: 1736–1747.
10. Marcom PK, Isaacs C, Harris L et al.. The combination of letrozole and trastuzumab as first or second-line biological therapy produces durable responses in a subset of HER2 positive and ER positive advanced breast cancers. Breast Cancer Res Treat. 2007; 102: 43–49.
11. Bardou VJ, Arpino G, Elledge RM et al.. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. J Clin Oncol 2003; 21: 1973–1979.
12. Cui X, Schiff R, Arpino G et al.. Biology of progesterone receptor loss in breast cancer and its implications for endocrine therapy. J Clin Oncol 2005; 23: 7721–7735.
13. Rakha EA, El-Sayed ME, Green AR et al.. Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. J Clin Oncol 2007; 25: 4772–4778.
14. Creighton CJ, Kent Osborne C, van de Vijver MJ et al.. Molecular profiles of progesterone receptor loss in human breast tumors. Breast Cancer Res Treat 2009; 114: 287–299.
15. Rosen PP, Oberman H. Tumors of the Mammary Gland. Washington, DC: Armed Forces Institute of Pathology 1993.
16. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991; 19: 403–410.
17. Viale G, Regan MM, Mastropasqua MG et al.. Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. J Natl Cancer Inst 2008; 100: 207–212.
18. Osborne CK, Shou J, Massarweh S et al.. Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. Clin Cancer Res 2005; 11: 865s–870s.
19. Arpino G, Weiss H, Lee AV et al.. Estrogen receptor-positive, progesterone receptor-negative breast cancer: Association with growth factor receptor expression and tamoxifen resistance. J Natl Cancer Inst 2005; 97: 1254–1261.
20. Taucher S, Rudas M, Mader RM et al.. Do we need HER-2/neu testing for all patients with primary breast carcinoma? Cancer 2003; 98: 2547–2553.
21. Huang HJ, Neven P, Drijkoningen M et al.. Association between tumor characteristics and HER-2/neu by immunohistochemistry in 1362 women with primary operable breast cancer. J Clin Pathol 2005; 58: 611–616.
22. Viale G, Regan MM, Maiorano E et al.. Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1-98. J Clin Oncol 2007; 25: 3846–3852.
23. Dowsett M, Allred C, Knox J et al.. Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial. J Clin Oncol. 2008; 26: 1059–1065.
24. Untch M, Gelber RD, Jackisch C et al.. HERA study team. Estimating the magnitude of trastuzumab effects within patient subgroups in the HERA trial. Ann Oncol 2008; 19: 1090–1096.

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The role of radioactive iodine-125 seed localization in breast-conserving therapy following neoadjuvant chemotherapy

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Background: Neoadjuvant chemotherapy (NAC) is increasingly used in the framework of breast-conserving therapy (BCT). Localization of the initial tumor is essential to guide surgical resection after NAC. This study describes the results obtained with I-125 seed localization in BCT including NAC.

Patients and methods: Between January 2009 and December 2010, 85 patients treated with NAC and BCT after I-125 seed localization were included. Radiological and pathological response and resection margins were retrospectively evaluated.

Results: BCT was carried out in 85 patients without secondary local excisions. Nineteen patients with unifocal tumors and seven patients with multifocal tumors showed a complete pathological response ($P = 0.18$). Tumor-free resection

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