RESEARCH ARTICLE

Clinico-Pathologic Subtypes of Breast Cancer Primary Tumors Are Related to Prognosis after Recurrence

Cesar Sánchez[1]*, Mauricio Camus[2], Lidia Medina[1], David Oddo[3], Rocío Artigas[1], Alejandra Pérez Sepúlveda[1], Francisco Domínguez[2], Dravna Razmilic[4], María Elena Navarro[4], Hector Galindo[1], Francisco Acevedo[1]

Abstract

Background: Pathological factors, based mainly on immunohistochemistry (IHC) and histological differentiation, are mostly used to differentiate breast cancer (BC) subtypes. Our present aim was to describe the characteristics and survival of a relapsing BC patient cohort based on clinico-pathologic subtypes determined for the primary tumors. **Methods:** We used a clinico- pathological definition of BC subtypes based on histological grade (HG), estrogen receptor (ER), progesterone receptor (PgR),and epidermal growth factor receptor type 2 (HER2) expression assessed by IHC. We determined variables associated with loco-regional recurrence (LRR), second primaries (SP), systemic recurrence (SR) and post-recurrence survival (PRS). **Results:** Out of 1,702 patients, 240 (14%) had an event defined as recurrence. Those with recurrent disease were significantly younger than those without,and were initially diagnosed at more advanced stages, with larger tumors, greater lymph nodal involvement and higher HG. With a median follow up of 61 months (1-250), 4.6% of patients without recurrence and 56.6% of patients with an event defined as recurrence had died. The median PRS for the LRR group was 77 months; 75 months for those who developed a SP and 22 months for patients with an SR ($p < 0.0001$). In SR cases, the median PRS was shorter for ER- tumors than for ER+ tumors (15 vs. 26 months, respectively; $p = 0.0019$, HR 0.44; CI: 0.25-0.44). **Conclusions**: Subtype, defined through classic histopathologic parameters determined for primary tumors, was found to eb related to type of recurrence and also to prognosis after relapse.

Keywords: Breast cancer- subtypes- prognostic- recurrence

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Introduction

Despite all efforts to control possible remaining deposit of disease, 20-30% of patients with an early BC, may suffer a relapse (Shim et al., 2014). Risk factors for recurrence have been extensively studied and are related to the stage at the initial presentation and the tumor biology (tumor size, lymph node involvement, tumor grade,presence of hormone receptors and growth factors receptors). Most of those factors are related to early relapse, but the identification of a subgroup of patients who continue to be at risk of recurrence long after completing the standard course of treatment, during the first and even the second decade after diagnosis, are still a developing clinical issue (Sestak et al., 2015).

In the last decade, improvements in available genetics tools have allowed the development of tumor genetic profiles especially for predicting late recurrences (Harris et al., 2016). Assays as PAM50 (Dowsett et al., 2013), OncotypeDx (Zelnak and O'Regan, 2013) and others (Harris et al., 2016) have been developed in order to analyze a group of genes related to recurrence, with remarkable results. However, high cost of these technologies has delayed the implementation of those tools in general population, mainly in developing countries. Further, an IHC-based score to determine which tumor marker is prognostically useful has been proposed (Dowsett et al., 2013). Classification of BC subtypes has been performed considering ER, PgR, HER2 and the expression of the cell cycle-regulated protein Ki67 (Goldhirsch et al., 2013). Even when the results obtained with this approach have given concordant results in comparison with the genomic profile of intrinsic subtypes determination, their correlation has been performed mainly in the prediction of therapeutic response and short term prognosis, specially for primary BC (Dowsett et al., 2013). Moreover, Ki67, which has been used usually to differentiate ER+ BC subtypes, is still not routinely used in many pathology labs due to a lack of standardization and pre- analytical and analytical issues (Jonat and Arnold, 2011). Instead, HG has been used as a surrogate marker for Ki67 widely (Petric et al., 2014), (Pathmanathan and Balleine, 2013). Taking into account all of these considerations, a better understanding of the correlation between primary tumor

*[1]Department of Hematology-Oncology, [2]Department of Surgery , [3]Department of Pathology, [4]Department of Radiology School of Medicine, Pontificia Universidad Catolica de Chile, Santiago, Chile. *For Correspondence: csanchez@med.puc.cl*

characteristics and patient outcomes after their treatment, in terms of specific type of recurrence, disease free interval (DFI), overall survival (OS) and survival after recurrence, would be useful as a clinical tool to define and control a high-risk population, especially in places where access to the latest technologies is still limited.

The aim of this study is to establish a correlation between the primary tumor subtype and prognosis after relapse. For this purpose, we have characterized a cohort of patients with different subtypes of recurrent BC in order to assess the impact of BC primary subtypes, determined though IHC in the primary tumor, on the specific type of recurrence, DFI, OS and post recurrence survival (PRS) in these patients, in comparison with a cohort of patients without recurrence.

Materials and Methods

This is a retrospective study performed at Cancer Center of the Pontificia Universidad Catolica de Chile, and approved by the Ethics Committee of this institution.

We analyzed all patients diagnosed with invasive BC in our institution from 1997 to 2014 included in our database. Epidemiological and clinical data were extracted from medical records. Vital status was obtained from the Civil Registry of Chile.

Pathological reports from the primary tumor were reviewed regarding histological type, tumor size, HG (according to Elston and Ellis (Elston and Ellis, 2002)), and nodal compromise. Status of ER, PgR and HER2 were determined through IHC. The cutoff value to determine if ER and PgR were positive was ≥1% of tumor cells with nuclear staining. Tumors with HER2 score of 3+ were considered as HER2 positive (Hammond et al., 2010), (Wolff et al., 2013). If the HER2 grading was reported as 2+, fluorescence in situ hybridization (FISH) study for HER2 was done. Since in our center the Ki67 study is not routinely indicated, we decided not to include it in our analysis.

The tumor stage at diagnosis was determined according to the American Joint Committee on Cancer 7th edition (Edge and Compton, 2010). Tumors were classified into 4 subtypes according to IHC markers and HG, as we have published before (Acevedo et al., 2015): Luminal A(LA) (ER positive and / or PgR positive, HG 1-2, HER2 negative), Luminal B (LB) (ER positive and/or PR positive, HG 3 and/or HER2 positive), triple negative (TN) (ER, PR and HER2 negative) and HER2-enriched (ER and PR negative, HER2 positive). We define LRR as a new tumor originatingon the same side of the previous BC; SP asa contra-lateral breast tumor and SR as tumor outside the loco-regional field, all identified>3 months from diagnosis, inorder to exclude synchronous tumors.

Statistical analysis and outcomes

DFI was measured from the date of first biopsy of the primary tumor to the date of first relapse (LRR, SP or SR). Overall survival was measured from the date of diagnosis of the primary tumor until death or the end of the study period. Post-relapse survival (PRS) was defined as the time from tumor relapse to death from any cause.

The patients'tumor and treatment characteristics were assessed for their influence on survival after a LRR, SP or SR using Kaplan-Meier estimates and the log-rank test for equality of survivor functions. To reveal differences in the characteristics between categorical variables, $\chi 2$ tests were used.Multivariate analysis was performed through a COX logistic regression. In case the hazards turned out to be non-proportional (crossed survival curves), Breslow test was used.

Significant difference was considered when p value <0.05. All data were analyzed using IBM® SPSS ® version 21 program.

Results

Association between primary BC characteristics and recurrence

We treated 2116 patients during this period, 339 patients were excluded from the study due to a loss of follow-up about recurrence status (16%) and 75 for being in stage IV at presentation. Of 1702 patients, we identified 240 (14%; 240/1702) relapsing during the follow-up interval. While we have ER information for all patients with recurrence, we only had enough information to use clinico-pathological subtype classification (LA, LB, TN or HER2) in 178 patients with recurrence.

Clinico-pathological characteristics of patients are described in Table 1.Patients who developed a recurrence were significantly younger at the moment of the primary diagnosis than those without recurrence (50 vs. 54 years, p<0.001), and were also initially diagnosed at more advanced stages (stage III: 46% vs. 14%,p <0.001), with larger tumors (2.5 vs. 1.8 cm, p<0.001), higher lymph nodal involvement (positive lymph nodes in 67% vs. 37%, in1672 patients analyzed, p<0.001) as well as higher HG (HG 3: 62% vs. 39% in 1478 patients analyzed, p<0.001). Primary tumors of patients with recurrence were less commonly ER positive (75% vs. 84% of 1647 patients, p<0.01), but no significant differences were observed in HER2 positivity (19% vs. 17% of 1416 patients, p=0.3).

Regarding the treatments indicated as part of the management of the primary tumor, total mastectomy (TM), chemotherapy (CT) and endocrine therapy (ET) were more frequentlyused in patients who developed recurrence than patients without relapse (47% vs. 26%, p< 0.001; 73% vs. 53%, p<0.001 and 73% vs. 53%, p < 0.003, respectively). After amedian follow up period

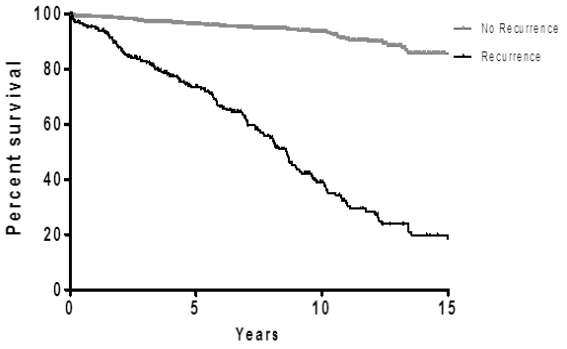

Figure 1. Overall Survival in 1,702 Patients with Breast Cancer, Based on Presence of Recurrence

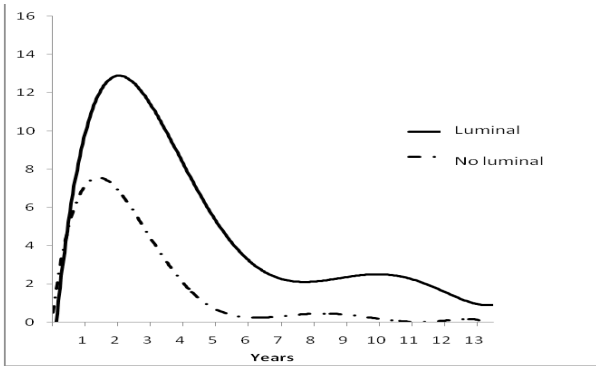

Figure 2. Time to Recurrence in 240 Breast Cancer Patients, Based on ER Expression on the Primary Tumor

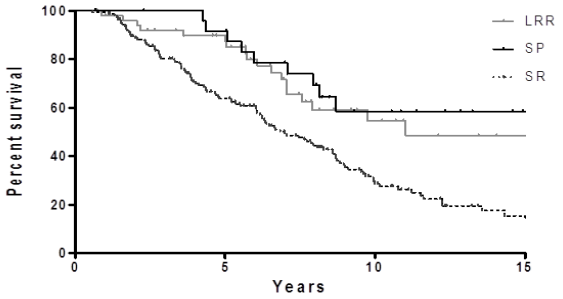

Figure 3. Overall Survival According to Type of Recurrence in 240 Patients with Breast Cancer

of 61 months, there was a significantly higher mortality in patients with recurrence in comparison with patients without recurrence, (54% vs. 4.6% in 1702 analyzed, p < 0.001). The median OS in patients without recurrence was not achieved, while in those who would develop recurrence the OS was 96.7 months (log rank test, p <0.001; HR: 0.027; 95% CI: 0019-0.04) (Figure 1).

Patients with aggressive tumors, classically defined as TN and HER2-enriched subtypes, showed a shorter DFI than less-aggressive subtypes, namely LA and LB subtypes (TN: median 22.5 months (4-148) and HER2-enriched: 23.5 months (5-129) vs. LA: median of 41 months (6-220) and LB: 34.5 months (4-162), p =0.003). While we found statistically significant differences for DFI between aggressive and not aggressive tumors similar as we described previously (Luminal vs. no luminal; figure 2), no differences were found between LA vs. LB or HER2-enriched vs. TN BC subtypes.

Association between primary BC characteristics and type of recurrence

In order to characterize the association between primary BC characteristics and a specific type of recurrence, clinic-pathological characteristics of primary tumors were compared between patients with LRR, SP tumor and SR (Table 2). In this regard, the first event determining the recurrence in our patients was more frequently the SR (67%), followed by LRR (22%) and SP (11%).

Patients who progressed with SR had primary tumor at more advanced stage (54% in stage III, 35% in stage II and 10% in stage I, considering a total of 162 patients who relapse in SR, p <0.001), while most of the patients who developed LRR or SP were diagnosed at earlier stage (LRR: 31% in stage III, 40% in stage II and 29% in stage I, considering a total of 52 patients who relapse in LRR, p = 0.5; SP: 27% in stage III, 31% in stage II and 42% in stage I, considering a total of 26 patients who relapse in SP, p=0.3). Further, patients with higher nodal involvement, larger tumor size and HER2 positivity at first diagnosis,developed more frequently a SR (Table 2). Moreover, patients who were first diagnosed with a HG 3 would represent around the half of all recurrence both

Table 1. Clinico-Pathological Characteristics of Patients with Recurrent Breast Cancer and a Cohort of Patients Free of Disease

	No recurrence (n=1,462)		Recurrence (n=240)	
Age, median (range)*	54 (19-93)		50 (24-91)	
Median time to recurrence in months (range)	-		35.5 (4-220)	
TNM stage*				
I	652/1462	45%	43/240	18%
II	596 /1462	41%	86/240	36%
III	214 /1462	14%	111/240	46%
Lymph node metastasis	533/911	37%	153/75	67%
Positive/negative*				
Tumor size (cm) median-range*	1.78 (0.02-14)		2.5(0.1-16.2)	
Histological grade				
1	256/1292	20%	15/186	8%
2	534 /1292	41%	54/186	29%
3	502/1292	39%	117/186	62%
ER+/ER-*	1194/223	84%	174/56	75%
HER2 positive/HER2 negative	202/1048	17%	32/134	19%
Alive/Death*	1392/68	95%	104/136	46%

*, P≤ 0.05

Table 2. Pathological and Clinical Characteristics of 240 Patients with BC Recurrence

	LRR		SP		SR	
Total events (276)	61	22%	38	14%	177	64%
First event (240)	52/240	22%	26/240	11%	162/240	67%
Age	49.0 (31-91)		53.0 (34-88)		50.0 (24-85)	
Time to first event(months)	33.5 (5-144)		46.5(7-153)		35.5 (4-220)	
Stage at diagnosis of primary tumor*						
I	15/52	29%	Nov-26	42%	17/162	10%
II	21/52	40%	Aug-26	31%	57/162	35%
III	16/52	31%	Jul-26	27%	88/162	54%
Lymphonodes positive/negative*	23/26	47%	09-Aug	53%	121/32	79%
Size (cm)*	2.5 (0.5-5.5)		1.7 (0.3-3.8)		3.0 (0.1-16.2)	
HG*						
1	Mar-40	7%	May-20	25%	7/126	5%
2	Oct-40	25%	Apr-20	20%	40/126	32%
3	27/40	67%	Nov-20	55%	79/126	63%
ER+/-	31/17	64%	22-Apr	85%	121/35	77%
HER2+/-*	Jul-30	19%	0/17	0%	25/66	27%
Alive/dead*	33/19	37%	Sep-17	35%	71/108	67%

LRR, Locoregional recurrence; SP, Second primary; SR, Systemic disease; &, number of patients with available information; *, P≤ 0.05

in SR, LRR and SP. This group of patients also showed a higher frequency of HER2 positivity (SR: 27%, LRR: 19%, SP: 0%; p=0.03). No significant differences in age at primary diagnosis, disease free interval (DFI), ER expression, type of surgery, or systemic therapy was found between these groups.

Regarding survival parameters, more than half of patients with SR have died during the follow up period (LLR: 37%; SP: 35% and SR: 67%, p<0.003) (Figure 3) and the median PRS in this group was significantly lower than in other types of recurrence evaluated (LRR: 77 months; SP: 75 months; SR: 22 months, p <0.001 (Figure 4). Moreover, in the subgroup of patients with SR, the median PRS was even shorter in those patients who had ER- tumors compared with those with ER+ tumors (26 vs. 15 months, respectively; p = 0.002, HR 0.44; CI: 0.25-0.44) (Figure 5).

Association between BC subtype and recurrence

We also have classified patients based on the IHC biomarker levels expressed in the primary tumor, in order to establish an association between this broad-used BC classification and specific type of recurrence. Most of the patients analyzed were classified as LB subtype, followed by LA, TN and HER2-enriched (LB: 54%, LA: 42.7%, TN: 16.3% and HER2-enriched 10.7% in a total of 178 patients analyzed). After a multivariate analysis performed through a COX logistic regression, considering node compromise, HER2 status, ER, BC subtype and HG, only lymph node compromise was predictive for SR (HR 3.5, IC: 2,2-5,3; p=0.01). The median PRS was higher in LA group, followed by LB, TN and HER2-enriched (Median survival: 75, 37, 34 and 19 months for LA, LB, TN and HER2-enriched respectively) but these differences did not reach statistical significance.

Discussion

The presence of recurrence after a diagnosis of BC defines a group at high risk of death from the disease. Several prognostic factors have been described for early recurrence especially for aggressive BC tumor (TN and HER2+) (Kennecke et al., 2010) and recently, risk factors for late recurrence, more common in ER+ tumors, have also been described (Sestak et al., 2015),(Harris et al., 2016).Tumor size, HG, and nodal status are currently used for risk assessment and decision making

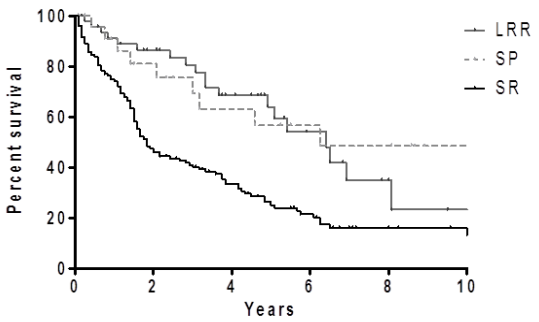

Figure 4.Post-Relapse Survival in 240 Patients with Breast Cancer Based on Type of Recurrence

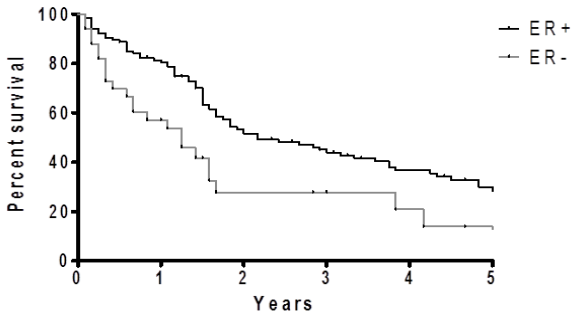

Figure 5. Post-Relapse Survival in 156 Breast Cancer Patients with Systemic Recurrence Based on ER Expression on the Primary Tumor

about whether adjuvant chemotherapy will be added to endocrine treatment or not,(Goldhirsch et al., 2013) and lately, multigene expression assays have been developed to achieve a more accurate assessment of prognosis and prediction of therapeutic benefit (Sparano et al., 2015), (Turner et al. , 2013). While these genetic profiles are able to determine prognosis of the disease and some of them even have some predictive value, they are still not widely used in clinical practice, given their associated cost and technical limitations. Therefore, it is imperative to develop new correlations between the widely-use IHC-tumor markers and patient outcomes, in order to make IHC a useful and effective tool,especially for those countries with limited resources. Our study was designed to apply a low cost IHC clinico-pathological subtype's classification in the primary tumor in order to determine outcome in recurrence.

Studies using IHC markers have succeeded to determine the prognosis and the risk of recurrence (Cuzick et al., 2011). Parameters such as age, tumor size, lymph node compromise, differentiation grade and receptor status may identify a higher risk group. However, most of these factors are associated with early recurrence risk. Molecular determinants of risk in patients with small tumors without nodal involvement have been developed recently, and are potentially the most important group to consider its use in order to avoid unnecessary therapies in low-risk population (Sparano et al., 2015).

The development of a score using four IHC factors (ER, PR, HER2 and Ki67) (Dowsett et al. , 2013) have been proposed to identify high risk patients. However, the lack of validation, particularly for Ki67 is a limitation in its use; particularly, in our laboratory, Ki67 measurement is not routinely performed (Jonat and Arnold, 2011).

While the receptor expression and differentiation may change over time in BC comparing primary tumor vs. relapse, most BC hold the initial expression pattern (Cardoso et al.,2014). In this regard, our results show that clinico-pathologic BC subtypes defined based on IHC and GH on the primary tumor, are related to type of relapse and prognosis after BC recurrence,clearly splitting patient with ER– from those with ER+ disease concerning time to relapse, overall survival and PRS. However, our paper has several limitations. It presented characteristic bias derived from its retrospective nature, we performed Ki67 study only in a minority of tumors, we did not analyze the IHC from recurrent tumor and we did not collect information about treatments. Also we reported OS, no disease specific survival rates.We must also consider the fact that we had to exclude 16% of our patients due to a lack of follow-up, which is very important for internal validity, as it is usual for patients who do not follow-up to have a different prognosis as those patients who do. However, it must also be noted that this loss of follow-up is solely on recurrence and not on overall survival. All patients within this 16% lost are currently alive, which might suggest a lack of recurrence. It must also be stated that there is no reason to believe that this subgroup, randomly created, behaves any differently from themain group of patients who did in fact remain throughout the whole study.

Besides that, our work reflects a real practice scenario, where subtype data are based on routine IHC information and mostly from the primary tumor.

We are working now prospectively to get enough information on recurrent BC patients studying BC subtypes on the primary tumor as in the samples obtained from the relapse.

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