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**Prognosis and adjuvant treatment effects in
very young women (below 35 years) with
operable breast cancer**

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List of Publications

Giuseppe Canello, Patrick Maisonneuve, Nicole Rotmensz, Giuseppe Viale, Paolo Veronesi, Rosalba Torrisi, Emilia Montagna, Anna Cardillo, Alberto Luini, Mattia Intra, Oreste Gentilini , Raffaella Ghisini, Aron Goldhirsch and Marco Colleoni. **Prognosis and adjuvant treatment effects in selected breast cancer subtypes of very young women (below 35 years) with operable breast cancer.** *Submitted*

Abstract

Background: There is limited knowledge about prognosis of selected breast cancer subtypes among very young women

Patients and Methods: We explored patterns of recurrence by age according to four immunohistochemically-defined tumor subtypes: triple negative, HER2 positive (and) endocrine receptor absent, Luminal A and Luminal B (ER-positive and/or PR-positive and either HER2-positive and/or high Ki67) in 2970 premenopausal patients with pT1-3, pN0-3 and M0 breast cancer.

Results: Patients below 35 years of age (315, 11%) presented a significantly increased risk of recurrence and death (HR=1.65, 95%CI 1.30-2.10 and HR=1.78, 95%CI 1.12-2.85, respectively) when compared with older patients (2655, 89%) with similar characteristics of disease. This was true considering patients with luminal B (HR 1.62, 95%CI, 1.21-2.18, for DFS; HR 2.09, 95%CI, 0.96-4.53, for OS) and with triple-negative (HR 2.04, 95%CI, 1.11-3.72, for DFS; HR 2.20, 95%CI, 1.10-4.41, for OS) breast cancer, observing the highest risk of recurrence in the younger patients with HER2 positive breast cancer (HR 2.37, 95% CI, 1.12-5.02), when compared with older patients.

Conclusions: Very young patients with triple negative, luminal B, or HER2 positive breast cancer have a worse prognosis when compared with older patients with similar characteristics of disease.

1. Introduction

Breast cancer at a young age has been reported to have a more aggressive biological behaviour compared with the disease in older patients (1-6).

Walker evaluated pathological features, oestrogen and progesterone receptor status, proliferation as determined by Ki-67 labelling and the presence of c-erbB-2 and p53 protein of one hundred and sixty-three breast carcinomas occurring in women aged between 26 and 44 years comparing with a control group of carcinomas from women in the 50-67 years age group. In this analysis carcinomas occurring in women aged under 35 years had a significantly high incidence of being poorly differentiated and of having high proliferation rates. This group also had a significantly high incidence of p53 protein staining. Carcinomas in the under 30 years age group had a lower incidence of oestrogen and progesterone receptor positivity. No differences were found in c-erbB-2-positive staining between the groups (1)

Kroman investigated whether young age at diagnosis is a negative prognostic factor in primary breast cancer and how stage of disease at diagnosis and treatment influences such an association with a retrospective cohort study based on a population based database. Subjects were 10356 women with primary breast cancer who were less than 50 years old at diagnosis. As result, overall, young women with low risk disease who did not receive adjuvant treatment had a significantly increased risk of dying; risk increased with decreasing age at diagnosis (adjusted relative risk: 45-49 years (reference): 1; 40-44 years: 1.12 (95% confidence interval 0.89 to 1.40); 35-39 years: 1.40 (1.10 to 1.78); <35 years: 2.18 (1.64 to 2.89). However, no similar trend was seen in patients who received adjuvant cytotoxic treatment. The increased risk in younger women who did not receive adjuvant treatment compared with those who did remained

when women were grouped according to presence of node negative disease and by tumour size (4).

Colleoni evaluated biological features and stage at presentation for 1427 consecutive premenopausal patients aged ≤ 50 years with first diagnosis of invasive breast cancer referred to surgery at the European Institute of Oncology from April 1997 to August 2000. A total of 185 patients (13%) were aged < 35 years ('very young') and 1242 (87%) were aged 35–50 years ('less young'). In this analysis, compared with less young patients, the very young patient group had a higher percentage of tumours classified as ER negative ($P < 0.001$), PgR negative ($P = 0.001$), higher expression of Ki-67 $\geq 20\%$ of cells stained; 62.2% versus 53%, ($P < 0.001$), vascular or lymphatic invasion (48.6% versus 37.3%, $P = 0.006$), and pathological grade 3 ($P < 0.0001$). There was no difference between the two groups for pT, pathological tumor size (pN) and number of positive lymph nodes.

Authors concluded that compared with less young premenopausal patients, very young women have a greater chance of having an endocrine-unresponsive tumour, and are more likely to present with a higher grade, more extensively proliferating and vessel invading disease. Pathological tumour size, nodal status and number of positive axillary lymph-nodes have a similar distribution among the younger and the older cohorts, thus not supporting previous data indicating more advanced disease in younger patients at diagnosis of operable disease (6).

Although controversy exists about the definition of "very young age" or "very young patients".and different cut off have been proposed it has been showed that younger age is associated with a more unfavourable prognosis and that the relationship between recurrence hazard and age was continuous with a 4% decrease in recurrence and a 2% decrease in cancer-specific death for every year of increase in age (7). In a recent publication of Han W. for patients aged < 35 years, the risk of death rose by 5% for every 1-year reduction in age, whereas there was no significant change in death risk with age in patients aged 35–50 years. (8).

Chemotherapy was commonly offered to the younger patients due to the fact that adjuvant therapies were prescribed in the past according to risk factors: the higher the risk the more intensive the treatment.

However, endocrine therapies appear to be an essential component of an effective adjuvant therapy program and retrospective analyses suggest that the endocrine effects of chemotherapy alone are insufficient for the younger patients with endocrine-responsive breast cancer (9). Whether use of complete endocrine therapy (e.g. ovarian function suppression plus tamoxifen) may be sufficient for these patients is a hypothesis that has not been tested adequately.

Recently, the Early Breast Cancer Overview group reported a meta-analysis of individual patient data on the use of LHRH agonists. In patients 40 years old or younger, the addition of an LHRH agonist to chemotherapy significantly reduced the risk of recurrence and death (HR 0.74; $p = 0.01$) versus chemotherapy alone. This effect was greatest in the group 35 years old or younger, whereas in the group older than 40 years, the addition of an LHRH agonist did not improve outcome. When chemotherapy alone was compared with LHRH agonist with or without tamoxifen in younger premenopausal patients with hormone receptor-positive tumors, the endocrine therapy improved outcome (mortality HR 0.82; $P = 0.15$) (10).

1.1 Molecular classification of Breast Cancer

Breast cancer is a molecularly heterogeneous disease. Evidence from gene expression microarrays suggests the presence of multiple molecular subtypes of breast cancer. Gene expression studies have identified five molecularly distinct subtypes of breast cancer that have prognostic value across multiple treatment settings (11-13). Using complementary DNA (cDNA) microarrays representing 8,102 human genes to characterize gene expression patterns in a set of 65 surgical specimens of human breast tumours from 42 different individuals, Perou et al. demonstrated that the phenotypic diversity of breast tumours was associated with corresponding gene expression diversity. From

the genes in the 65 tissues samples, the investigators selected a subset of 456 genes, which were termed the “intrinsic” gene subset, and consisted of genes with significantly greater expression variation between different tumours than between paired samples from the same tumour. Using this subset, the authors were then able to identify 4 different molecular subtypes of breast cancer: estrogen receptor (ER)-positive/luminal-like, basal-like, Erb-B2-positive (ie, tumours that overexpress ERBB2-associated genes but do not express genes that define the luminal subtype), and normal breast. Subsequent data expanded the classification to distinguish between luminal A and luminal B. Sorlie et al. examined a subset of 49 patients with locally advanced breast cancer who were treated with doxorubicin and had a median follow-up of 66 months and found that the recurrence-free survival and overall survival differed significantly among the breast cancer subtypes, with the luminal A tumours having the longest survival times, the basal-like and HER2-positive subtypes having the shortest survival times, and the luminal B tumors having an intermediate survival time (11,14,15). The expression of ER-associated genes characterizes the luminal breast cancers, with luminal B tumours having poorer outcomes than luminal A tumours. Recent study defined the best Ki67 index cut point to distinguish luminal B from luminal A tumours (16). In this study, authors developed a clinically practical immunohistochemistry assay to distinguish luminal B from luminal A tumors and investigated its ability to separate tumors according to breast cancer recurrence-free and disease-specific survival. Tumors from a cohort of 357 patients with invasive breast carcinomas were subtyped by gene expression profile. The best Ki67 index cut point to distinguish luminal B from luminal A tumors was 13.25%. In an independent cohort of 4046 patients with breast cancer, 2847 had hormone receptor – positive tumors, then HER2 immunohistochemistry and the Ki67 index were used to subtype these 2847 tumors; Luminal B and luminal – HER2-positive breast cancers were statistically significantly associated with

poor breast cancer recurrence-free and disease-specific survival in all adjuvant systemic treatment categories (16).

Moreover, data from the study BCIRG 001 were analyzed dividing tumours in four subtypes according to immunohistochemical evaluation of ER, PgR, Ki-67 and HER2. The four subtypes were Luminal A, Luminal B, HER2 and triple negative; this classification appeared useful to define different prognostic subtypes with different relationship with adjuvant treatment received (17).

Although this "new" classification has limitations, it could be useful in the clinical practice, allowing not only a more accurate prognosis in breast cancer patients but also a selective treatment for each predefined subtype.

We therefore investigated the most recently available details of biological characteristics and prognosis of very young patients (<35 years of age) with operable breast cancer and the effects of adjuvant treatment programs according to immunohistochemically (IHC) defined subsets.

2 Patients and methods

We prospectively collected information on all consecutive premenopausal breast cancer patients operated at the European Institute of Oncology between April 1997 and December 2004.

Data on the 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 required. Pathological assessment included evaluation of the primary tumour size, histological type and of lymph nodes status including a sentinel node biopsy (18), when applicable. Tumour grade was evaluated according to Elston and Ellis (19) and peritumoral vascular invasion (PVI) was assessed according to Rosen (20) Estrogen (ER) and progesterone receptor (PgR) status, Ki-67 labeling index (assessed with the MIB 1 monoclonal antibody), and HER2/ neu over-expression (routinely performed since 1999) were evaluated immunohistochemically as previously reported (21). The threshold for ER and PgR positivity was 1% and for Mib1 positivity 20%, as previously published (21). The threshold for ER and PgR was based on published data indicating a different pattern of outcome according to the degree of potential endocrine responsiveness (22, 23).

2.1 Statistics

The Fisher exact test and the Mantel-Haenszel Chi-Square test for trend were used to assess the association between respectively, categorical and ordinal variables. The primary endpoints were the incidence of locoregional relapse (LRR), distant metastasis (DM), breast cancer related event (BCE), disease-free survival (DFS) and overall survival (OS). DFS was defined as the length of time from the date of surgery to any relapse (including ipsilateral

breast recurrence), the appearance of a second primary cancer (including contralateral breast cancer), or death, whichever occurred first. OS was determined as the time from surgery until the date of death (from any cause) or the date of last follow-up. Cumulative incidence and survival plots according to age were drawn using the Kaplan-Meier method. The log-rank test was used to assess the survival difference between strata. Multivariate Cox proportional hazard regression analysis was used to assess the independent prognostic significance of various clinical and histopathological characteristics of the tumor on event free or overall survival. Factors included in multiple regression analyses included tumor diameter, lymphnodal involvement, ER and PgR expression, Ki-67 expression, Her2/neu overexpression, vascular invasion, grade, histotype and immunohistochemical classification:

- Luminal A (ER>0 or PgR>0) and (Ki67<14%) and (Her2Neu 0/+/+)
- Luminal B (ER>0 or PgR>0) and ((Ki67≥14%) or (Her2Neu +++))
- HER2 (ER=0 and PgR=0) and (Her2Neu +++)
- Triple Negative (ER=0 and PgR=0) and (Her2Neu 0/+/+)

In patients with Luminal B tumors and with Triple Negative tumors, we assessed the effect of adjuvant therapy (respectively hormonal and chemotherapy) on outcome. All analyses were performed with the SAS software, version 8.2 (Cary, NC).

2.2 Treatment received

All patients received adequate local treatment (breast conserving surgery or total mastectomy) plus axillary sentinel lymph node biopsy (SLNB) or complete axillary dissection. SLNB was followed by axillary dissection only if the sentinel node contained metastasis or minimal node involvement. The SLN was identified and isolated using a gamma probe as a guide as previously

published (24). Postoperative breast irradiation (RT) was proposed to all the patients that received breast-conserving surgery (25). Systemic adjuvant therapy was recommended according to St. Gallen's treatment guidelines (25-27). For patients with endocrine responsive disease, adjuvant endocrine therapy alone was indicated (the combination of tamoxifen for 5 years plus LH-RH analogue for a minimum of 2 years) (21). In patients at higher risk (i.e. occurrence of peritumoral vascular invasion, younger age, large tumors) and/or with features of uncertain endocrine responsiveness [(e.g. low levels of ER positivity, lack of PgR expression, overexpression of HER2/neu, and increased proliferation markers, (28)], chemotherapy was added. Anthracycline containing chemotherapy was considered as the first option in patients with higher risk (i.e. AC, adriamycin and cyclophosphamide, for four courses (29); in case of comorbidities or patients preferences classical CMF (oral cyclophosphamide, methotrexate and fluorouracil) for a duration of three to six courses was considered (30). In case of endocrine non-responsive disease 6 months of chemotherapy was commonly indicated [classical CMF for six courses or AC for four courses followed by classical CMF for three courses (23) according to the degree of the patient risk]

Results

A total of 12,281 patients with invasive breast cancer were referred to the interdisciplinary evaluation and their data were included in the institutional database between 1997 and 2004.

We selected 4,524 consecutive premenopausal patients, of age 50 or less.

We subsequently excluded 1,213 patients, 473 that presented with neoadjuvant therapy, 52 had a previous other primary, 136 bilateral tumours and 552 operated with recurrent or metastatic tumours and 341 for lack of information on endocrine receptor status, ki76 or Her2Neu which did not allow to perform the biological classification of the tumors.

The final analysis is based on data from 2970 patients

The number and characteristics of evaluable patients are given in **Table 1**

Table 1: Characteristics of breast cancer patients according to age at diagnosis

	All Patients	Age at diagnosis		p- value
		<35	≥35	
ALL	2970	315 (100.0)	2655 (100.0)	
Histology				
Ductal	2398	283 (89.8)	2115 (79.7)	<.0001
Lobular	253	7 (2.2)	246 (9.3)	
Ductal+lobular	120	4 (1.3)	116 (4.4)	
Other	199	21 (6.7)	178 (6.7)	
Tumour size				
≤1cm	515	33 (10.5)	482 (18.2)	

	1-2cm	1286	129 (41.0)	1157 (43.6)	
	2-4cm	906	115 (36.5)	791 (29.8)	
	>4cm	227	34 (10.8)	193 (7.3)	<.0001
	Unknown	36	4 (1.3)	32 (1.2)	
Tumour grade					
	G1	432	21 (6.7)	411 (15.5)	
	G2	1348	99 (31.4)	1249 (47.0)	
	G3	1083	180 (57.1)	903 (34.0)	<.0001
	Unknown	107	15 (4.8)	92 (3.5)	
Number of positive nodes					
	None	1454	146 (46.3)	1308 (49.3)	
	1-3	996	107 (34.0)	889 (33.5)	
	4-9	324	33 (10.5)	291 (11.0)	
	10 or more	186	29 (9.2)	157 (5.9)	0.08
	pNx	10	-	10 (0.4)	
PVI					
	Absent	1951	189 (60.0)	1762 (66.4)	
	Present	1015	126 (40.0)	889 (33.5)	0.02
	Unknown	4	0 (0.0)	4(0.2)	
ER					
	Absent	413	72 (22.9)	341 (12.8)	
	Present	2557	243 (77.1)	2314 (87.2)	<.0001
PgR					
	Absent	613	101 (32.1)	512 (19.3)	
	Present	2357	214 (67.9)	2143 (80.7)	<.0001
Ki67					
	<20%	1173	71 (22.5)	1102 (41.5)	
	≥20%	1786	239 (75.9)	1547 (58.3)	<.0001
	Unknown	11	5 (1.6)	6 (0.2)	
Her2/Neu					
	0/+/>++	2144	208 (66.0)	1936 (72.9)	
	+++	451	66 (21.0)	385 (14.5)	0.003
	Unknown	375	41 (13.0)	334 (12.6)	
Molecular classification					
	Luminal A	592	29 (9.2)	563 (21.2)	
	Luminal B	1986	217 (68.9)	1769 (66.6)	

	HER2	141	18 (5.7)	123 (4.6)	
	Triple Negative	251	51 (16.2)	200 (7.5)	<.0001
Surgery					
	Quadrantectomy	2194	227 (72.1)	1967 (74.1)	
	Mastectomy	776	88 (27.9)	688 (25.9)	0.44
Sentinel node biopsy					
	No	1123	157 (49.8)	966 (36.4)	
	Yes	1847	158 (50.2)	1689 (63.6)	<.0001

Table 1 (cont')

In the 'very young' group, when compared with the 'less young' patients, there were higher percentages of tumours of high grade (57.1% versus 34.0%; $P < 0.0001$), and tumours classified ER (22.9% versus 12.8%; $P < 0.0001$) and PgR (32.1% versus 19.3%; $P < 0.001$) absent,

Moreover in younger patients (aged <35 years) there were higher percentage of tumors with perivascular invasion (40.0% vs 33.5% $P= 0.02$), and HER2-overexpression (21% versus 14.5% $P=0.003$) than patients with age 35-50 years.

According to the immunohistochemical classification, in the group of patients aged < 35 years there were less tumors defined as Luminal A (9.2% versus 21.2%) and more triple negative tumors (16.2% versus 7.5%, $P < 0.0001$) than older patients .

3.1 Adjuvant treatment

As shown in Table 2, very young patients in the Luminal A subtype received more LHRH agonist alone but less Tamoxifen alone ($p=0.036$) than older patients; no difference was showed about the chemotherapy; in the Luminal B subtypes there were more patients who received LHRH alone and the combination of Tamoxifen and LHRH agonist than patients 35-50 years old ($p < .0001$); while , in the same subtype, younger patients received more chemotherapy than older patients , above all anthracyclines-based regimen (

p<0.0001). No significant difference was showed about treatments of the patients in the HER2 and Triple-negative subtypes.

Table 2: Adjuvant treatment modalities in breast cancer patients according to age at diagnosis and molecular classification

	Luminal A			Luminal B		
	<35	35-50	<i>P</i> *	<35	35-50	<i>P</i> *
ALL	29	563		217	1769	
Hormonotherapy						
None	2	12		16	80	
TAM alone	1	84		8	428	
LHRH alone	3	18		29	48	
TAM+LHRH	22	431		162	1117	
OTHER/NOS	1	18	0.036	2	96	<.0001
Chemiotherapy						
None	22	430		45	702	
Antracycline	7	102		132	776	
CMF	0	17		21	197	
Other/NOS	0	14	0.76	19	94	<.0001
		HER2		Triple Negative		
	<35	35-50	<i>P</i>*	<35	35-50	<i>P</i>*
ALL	18	123		51	200	
Hormonotherapy						
None	18	114		45	193	
TAM alone	0	2		1	1	
LHRH alone	0	3		5	2	
TAM+LHRH	0	3		0	4	
OTHER/NOS	0	1	1.00	0	0	0.006
Chemiotherapy						
None	1	11		3	13	
Antracycline	14	81		21	87	
CMF	1	8		22	88	
Other/NOS	2	23	0.90	5	12	0.79

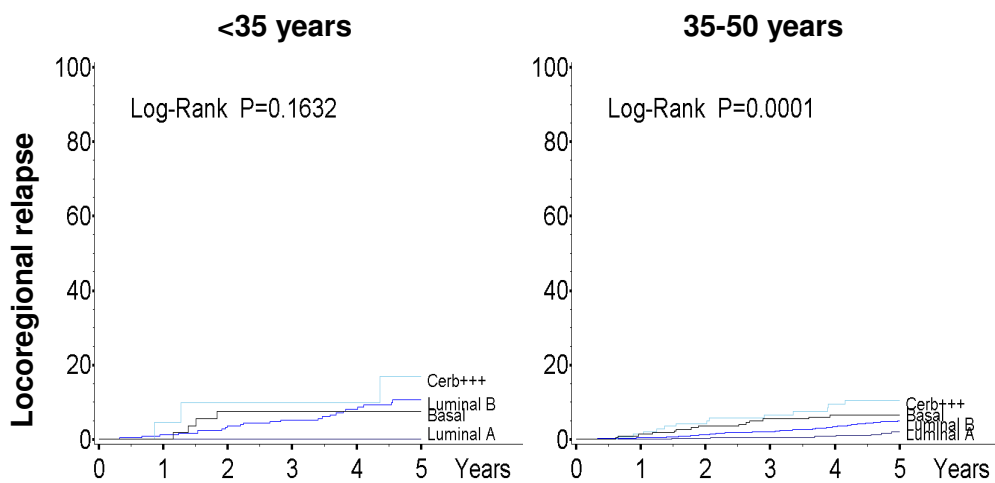
*Fisher's exact test

3.2 Clinical Outcomes according to age and immunohistochemical (IHC) classification

3.2.1. Locoregional Relapse (LRR), Distant Metastases (DM), Breast Cancer related Events(BCE)

As shown in the Figure 1, incidence of LRR , DM and BCE were different between the four IHC subtypes both in very young and 35-50 years old group; HER2 appeared the subtype with the highest incidence of events.

Figure 1: Outcome of breast cancer patients according to age at diagnosis and molecular classification.



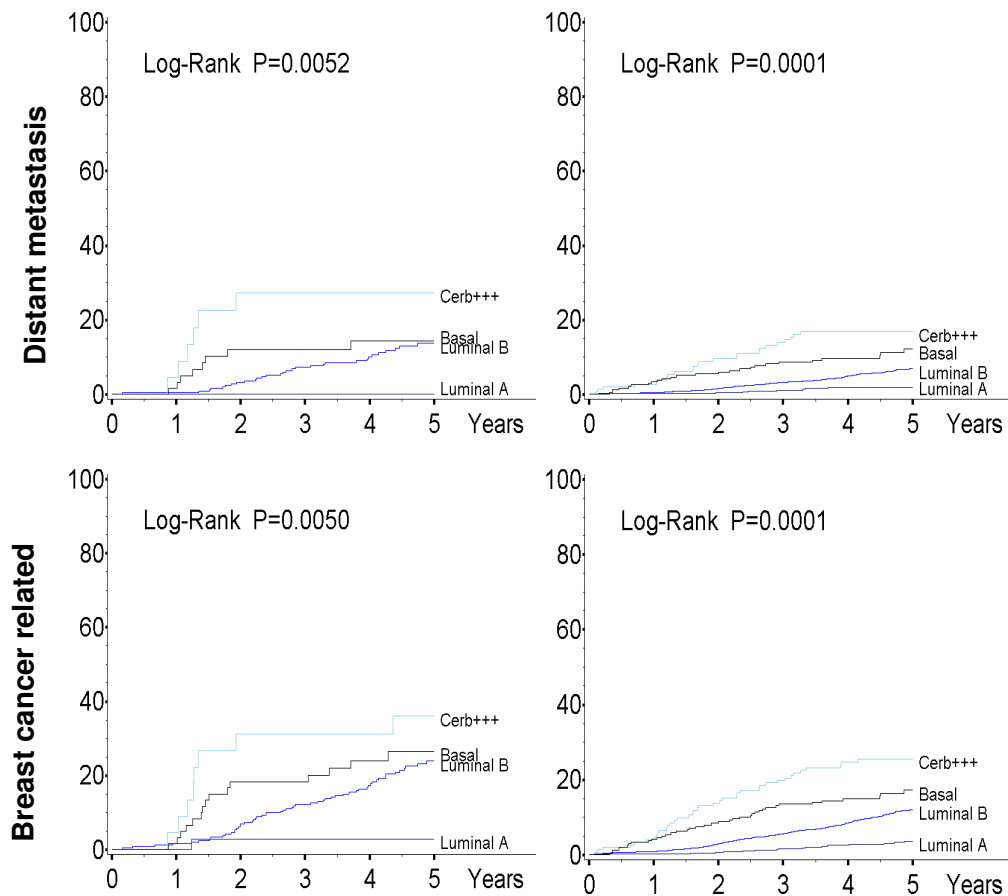


Figure 1(cont')

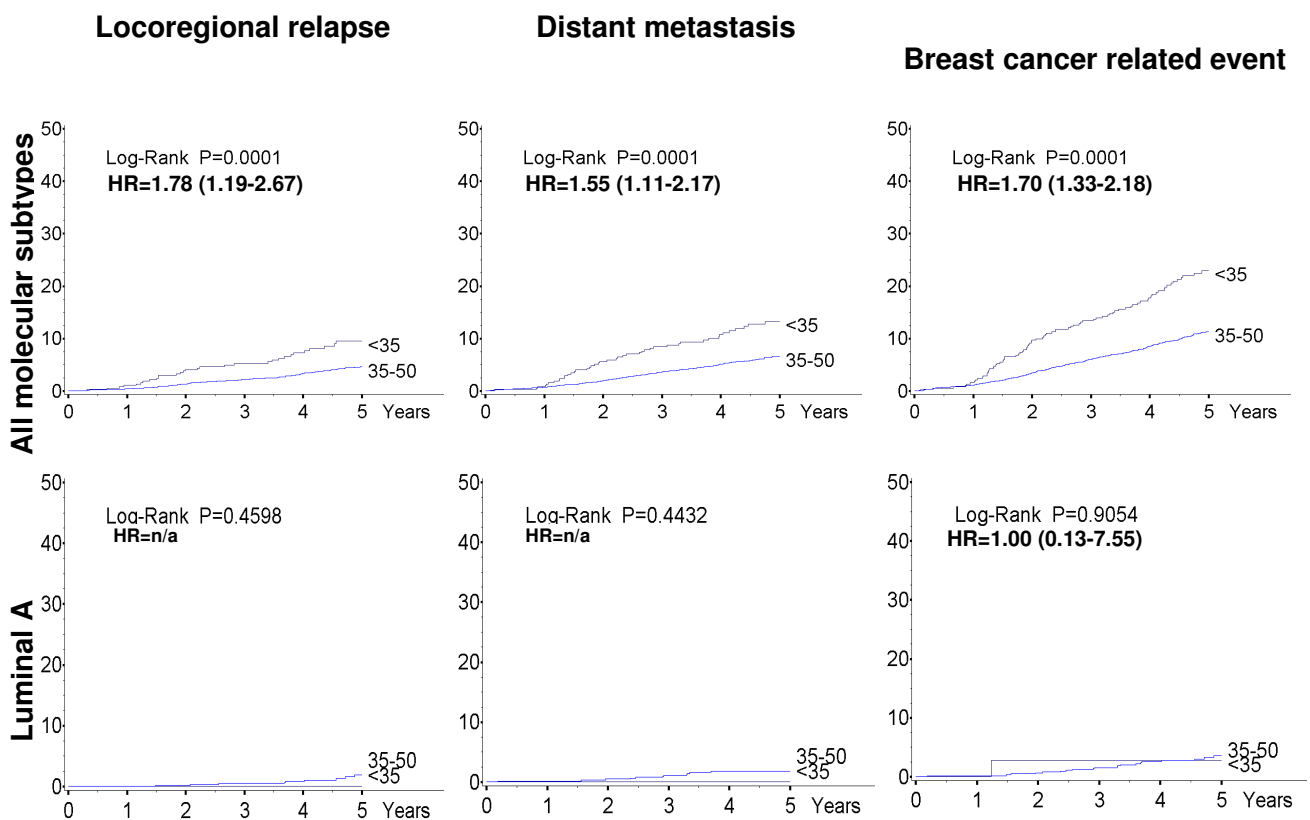
When we analyzed data to compare very young with older patients, we showed, for all biological subtypes, a statistically significant difference of incidence of LRR, DM and BCE ($p=0.0001$) between patients below 35 years and 35-50 years old patients. At multivariate analysis, age < 35 years was a risk factor for an increased incidence of locoregional relapse (HR=1.78, 1.19-2.67), distant metastasis (HR=1.55, 1.11-2.17) and breast cancer related events (HR=1.70, 1.33-2.18) (Figure 2).

Analyzing each subtype, our data did not show a difference of LRR, DM and BCE in the Luminal A subtype. The difference between very young

patients and older patients appeared evident in the Luminal B subtype with an increased risk of LRR (HR=1.82 ,1.13-2.94), DM (HR=1.57, 1.04-2.37) and BCE (HR=1.71, 1.26-2.32) (Figure 2).

In the HER2 and Triple Negative subtype, very young patients were at increased risk of LRR, DM and BCE with a statistically border-line significance. (Figure 2).

Figure 2: Cumulative incidence (%) of locoregional relapse, distant metastases and breast cancer related events in breast cancer patients according to age at diagnosis and molecular classification.



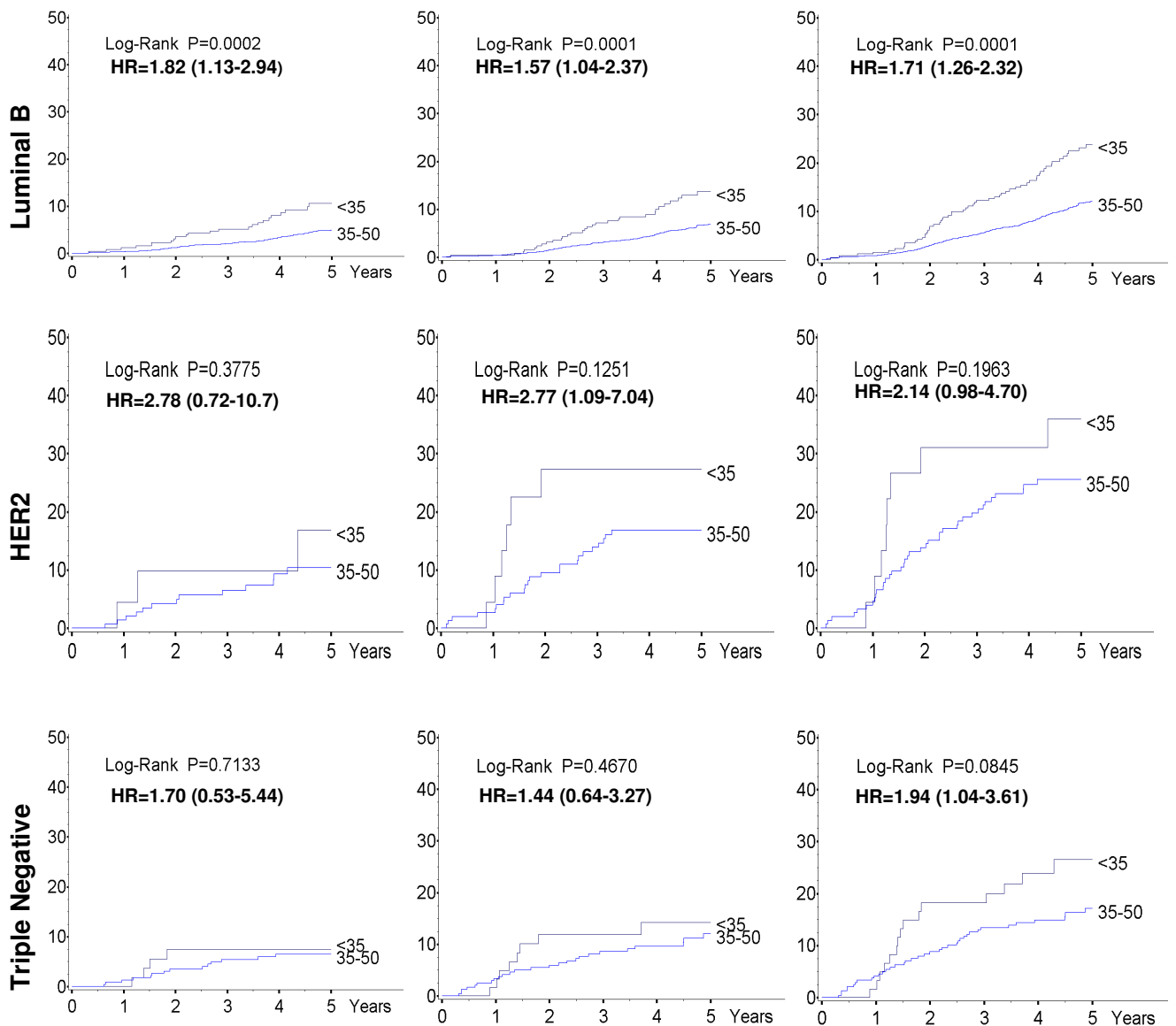


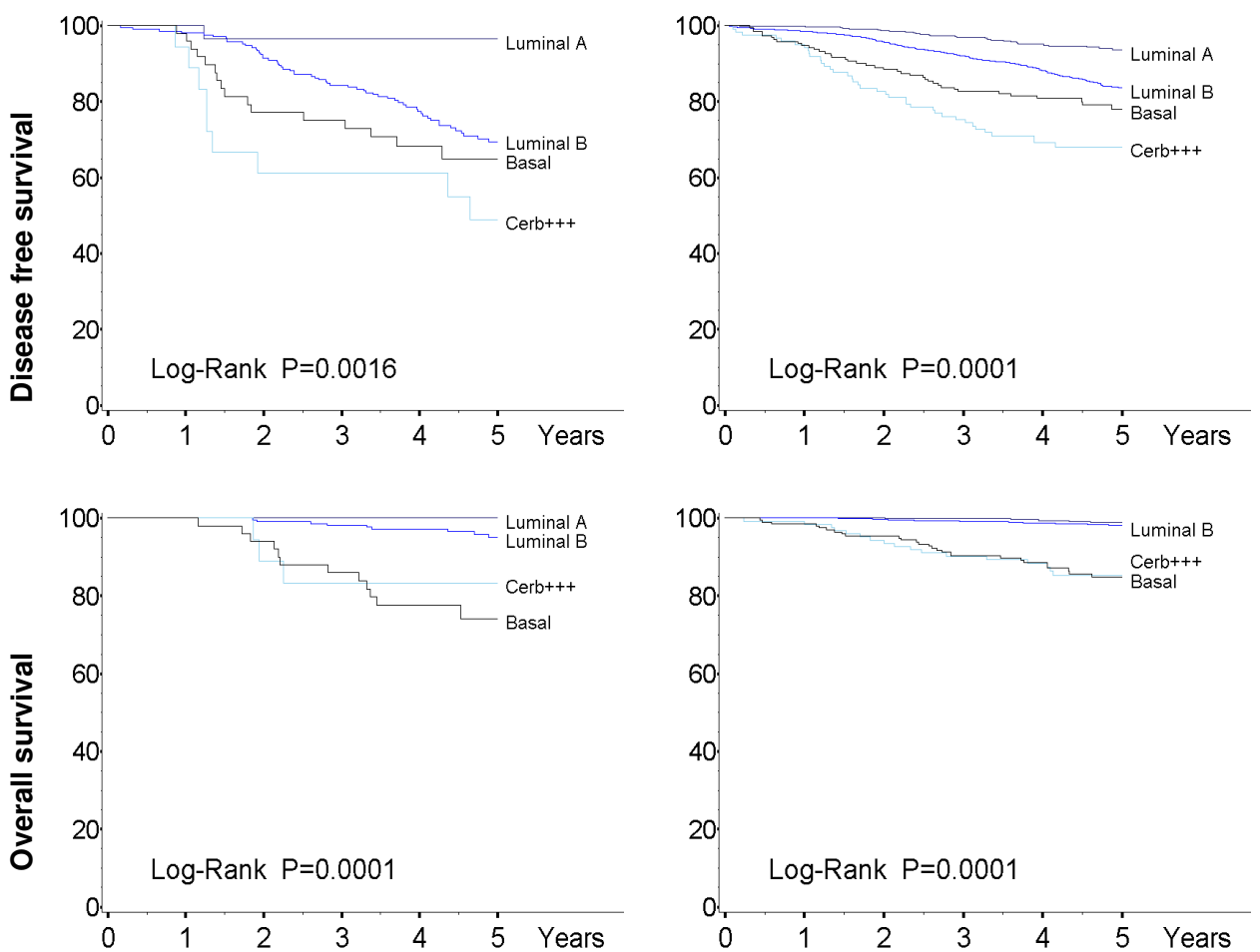
Figure 2 (cont')

Legend: Hazards Ratio (HR) and 95% confidence intervals obtained from multivariable Cox proportional hazards regression model adjusted for hormonal receptor status, proliferative index (ki-67), peritumoural vascular invasion, tumour size, nodal status and Her2Neu overexpression, chemotherapy (none/ CMF/Anthracycline containing therapy, other regimen) and hormonotherapy (none, LHRH or Tamoxifen alone, LHRH+Tamoxifen, other regimen).

3.2.2 Survival Outcomes: Disease-Free Survival (DFS), Overall Survival (OS)

DFS and OS were statistically different between the four IHC defined subtypes both in the group of very young and in the group of older patients; HER2 and Triple negative were the subtypes with the lowest survival in both age groups (Figure 3).

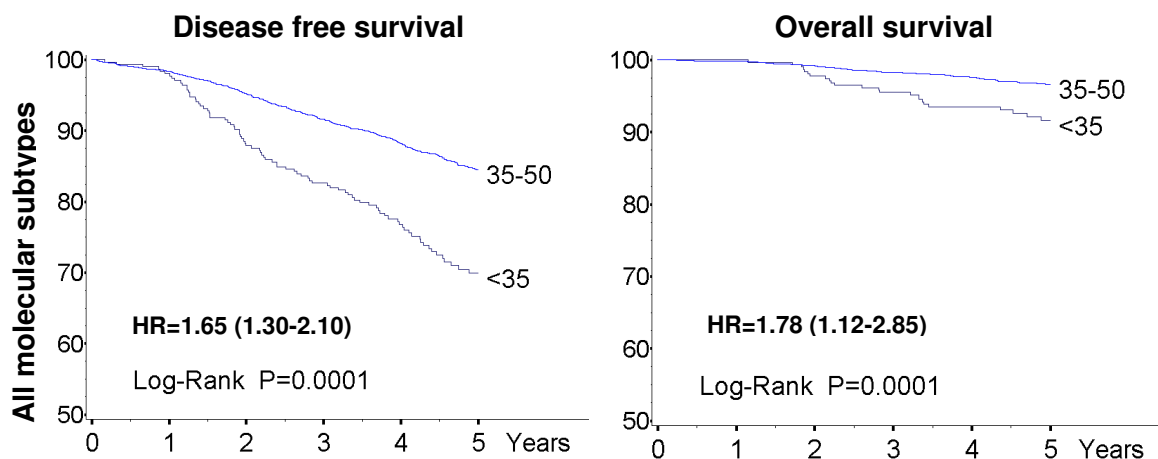
Figure 3: Outcome of breast cancer patients according to age at diagnosis and molecular classification.

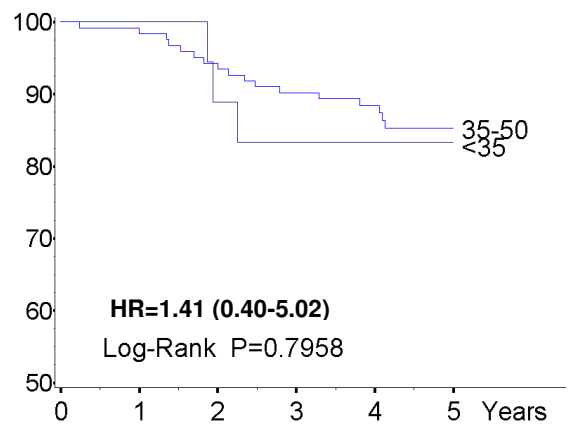
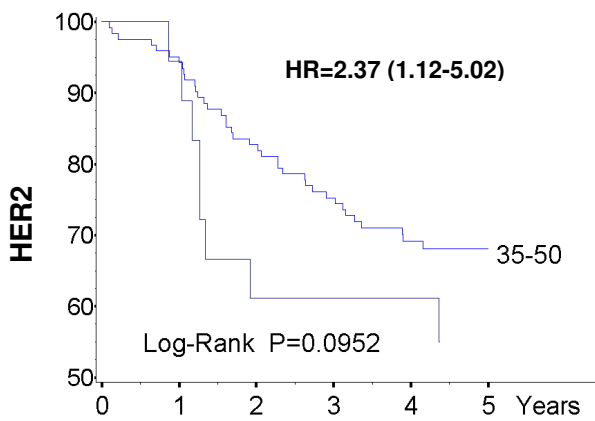
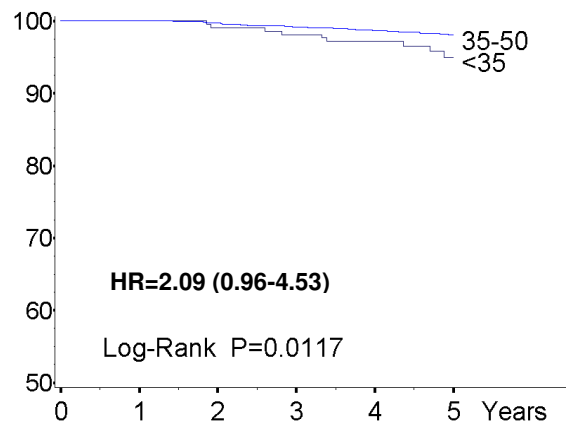
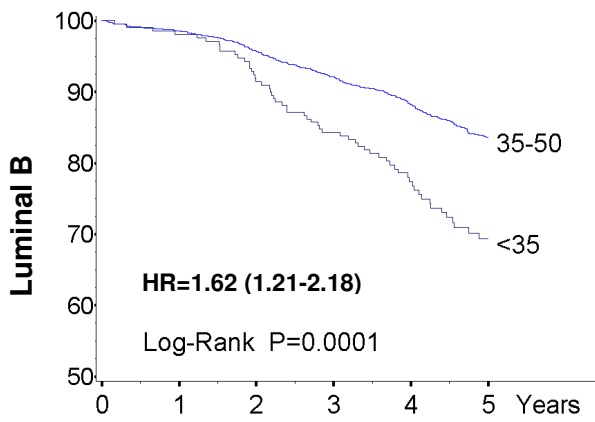
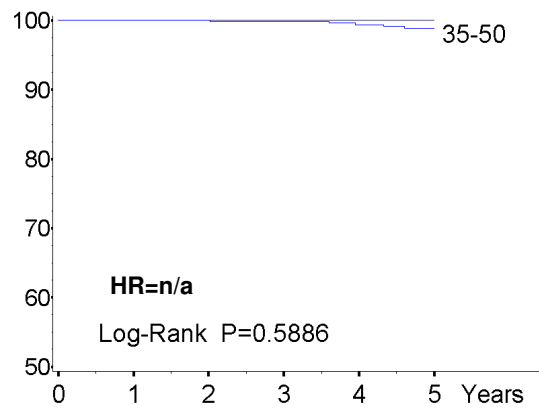
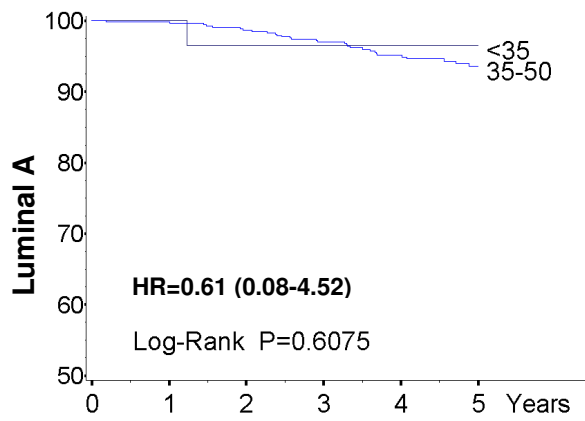


In the Figure 4 is shown the comparison of survival outcomes between very young and older patients. Analysis for all biological subtypes showed a statistically significant difference of DFS and OS ($p=0.0001$) between patients below 35 years and patients aged 35-50. At multivariate analysis, age < 35 years was a risk factor for increased recurrence (HR=1.65, 1.30-2.10) and death (HR=1.78, 1.12-2.85).

Very young patients with tumors classified as Luminal B, HER2 and Triple Negative were at increased risk of recurrence (HR=1.62, 1.21-2.18, HR=2.37, 1.12-5.02, HR=2.04, 1.11-3.72, respectively) compared with older patients. Very young patients in HER2 subtype were at increased risk of death, but with no statistically significant difference; while, in the Luminal B and Triple negative subtypes, patients below 35 years had a 2-fold higher risk of death compared with older patients (HR=2.09, 95%CI 0.96-4.53; HR=2.20, 95%CI 1.10-4.41, respectively)

Figure 4: Disease free survival and overall survival in breast cancer patients according to age at diagnosis and molecular class





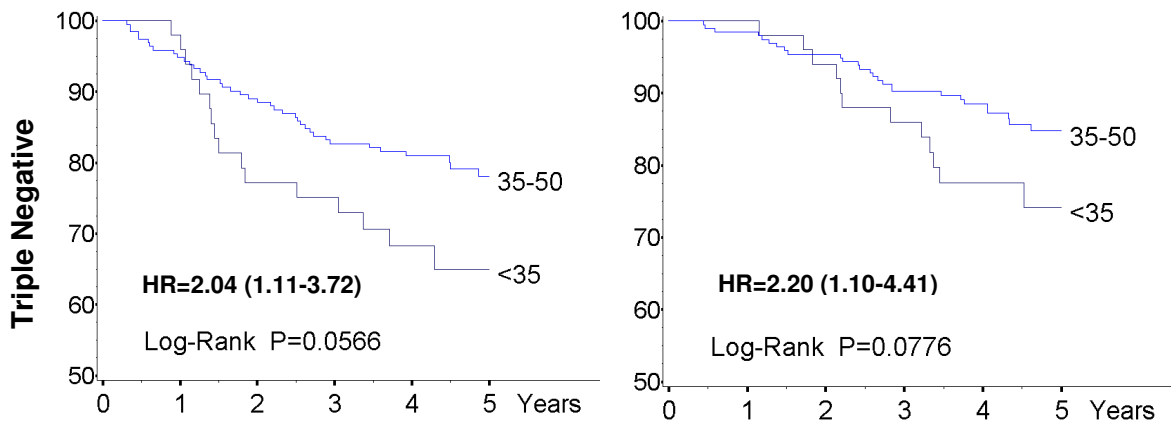


Figure 4 (cont')

Legend : Hazards Ratio (HR) and 95% confidence intervals obtained from multivariable Cox proportional hazards regression model adjusted for hormonal receptor status, proliferative index (ki-67), peritumoural vascular invasion, tumour size, nodal status and Her2Neu overexpression, chemotherapy (none/ CMF/Anthracycline containing therapy, other regimen) and hormonotherapy (none, LHRH or Tamoxifen alone, LHRH+Tamoxifen, other regimen).

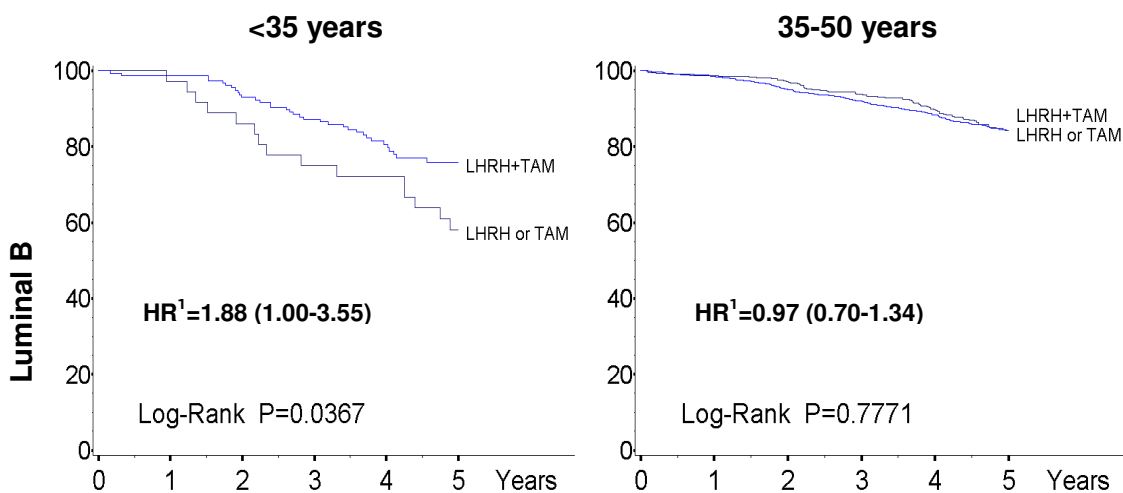
3.2.3 Clinical outcomes according to hormonal therapy

An analysis was conducted to evaluate the impact of different treatments on disease-free survival in two groups of patients, very young patients (below 35 years) and older patients (between 35 and 50 years).

A statistically significant reduced DFS was showed in the young patients with tumors defined as Luminal B who received tamoxifen or LHRH analogue alone versus the combination of the two drugs ($p=0.0367$)(Figure 5). This result was confirmed at multivariate analysis, with an increased risk of recurrence for patients who received LHRH agonist or Tamoxifen alone versus the combination of the same drugs ($HR=1.88, 1.00-3.55$) (Figure 5);

In the group of older patients with age more than 35 years no difference was found between the different hormonal therapies in terms of disease-free survival ($p= 0.7$; $HR=0.97, 0.70-1.34$) (Figure 5)

Figure 5: Disease free survival according to selected adjuvant therapies in breast cancer patients according to age at diagnosis and molecular classification



Hazards Ratios and 95% confidence intervals (CI) for LHRH or Tamoxifen alone vs. LHRH+Tamoxifen (HR^1) and for CMF vs. anthracycline containing therapy (HR^2) obtained from multivariable Cox proportional hazards regression model adjusted for proliferative index (ki-67), peritumoural vascular invasion, tumour size, nodal status and Her2Neu overexpression.

4 Discussion

The present study provides useful insights into the treatment of breast cancer because it is based on a large population of very young patients with breast cancer evaluated within the context of a central pathology analysis using modern classification according to IHC defined subtypes

In fact, as recently showed, an IHC profile based on the degree of expression of ER, PgR, Ki-67 and HER2 might identify subgroups of breast cancer patients who will respond to different systemic adjuvant treatments (17). However, limited information is available in the adjuvant setting on the outcome and responsiveness to therapy in the very young population, according to molecular or immunohistochemical (IHC) classification.

Data from Carolina Breast Cancer Study were analyzed to determine population-based distributions and clinical associations for breast cancer subtypes with IHC surrogates applied to 496 incident cases of invasive breast cancer; study showed that the IHC subtypes differed significantly by age ($P<.001$) and menopausal status ($P=.008$) so that patients with luminal A and B tumours were older than the other patients; moreover this study showed that breast cancer-specific survival differed by subtype ($P<0.001$), with shortest survival among HER2+/ER- and basal-like subtypes.(31).

Study of Bauer et al, evaluated features of 6370 triple-negative breast cancers compared with 44,704 other breast cancers. One of the study results was that women with triple-negative breast cancers were significantly more likely to be under age 40 (odds ratio 1.53) (32).

Our study showed that in the group of patients aged < 35 years there were less tumors defined as Luminal A (9.2% versus 21.2%) and more triple negative tumors (16.2% versus 7.5%, $P<0.0001$) than older patients . Moreover our data confirmed other studies results, for both the two groups of

young patients (<35 and 35-50 years) that is, the HER2 and Triple negative are the subtypes with the worst prognosis(31, 17).

More interesting, beside the feature of a more aggressive disease presentation which reflects on patients outcome, the results of the present study led to the identification of immunohistochemically defined subtypes within the group of very young patient which require adjuvant tailored therapies. In fact, our study showed that very young patients with tumors classified as Luminal B, HER2 and Triple Negative were at increased risk of LRR, DM, BCE, recurrence and death, compared with older patients.

Previous analyses evaluated prognosis of young patients focusing on the only well known prognostic and predictive factor, hormone receptor status (33,34). Recent researches permitted to understand the molecular complexity of breast cancer, defining so some different genetic portraits, but analysis of gene expression is not still now a useful tool for the physician. Therefore, also if the IHC classification could be only a surrogate of molecular and genetic definition of breast cancer, it can be much more useful in the clinical practice, allowing a more accurate prognosis and selection of treatment for each predefined subtype in breast cancer patients

Results of the present study support the issue that younger patients require adjuvant tailored therapies.

. The results of this analysis are important for clarifying the role of adjuvant tamoxifen in younger premenopausal patients and provide information on the importance of ovarian function suppression and its impact on the efficacy of tamoxifen..

The effect of chemotherapy-induced amenorrhea have been studied although it remains controversial.

As previously proposed, endocrine effects of chemotherapy are probably insufficient for young women with ER-positive breast cancer. A large analysis on 7,631 patients who were treated with chemotherapy alone showed markedly increased risks of relapse for young patients with ER-positive tumours

compared with older patients (9). In a retrospective analysis of 3,700 premenopausal patients involved in IBCSG trials I, II, V and VI patients treated with adjuvant cyclophosphamide, methotrexate, and fluorouracil chemotherapy alone, the failure to achieve chemotherapy-induced amenorrhea was associated with an increased risk of relapse. (35)

In the study of Ahn et al, hormonal therapy with tamoxifen, after chemotherapy, added significant survival benefit with a hazard ratio of 0.61 for overall survival in patients between 35 and 50 years of age with positive or unknown hormone receptor status, but there was no significant additional benefit from tamoxifen alone in patients younger than 35 years, (34). The Early Breast Cancer Overview group reported a meta-analysis of individual patient data on the use of LHRH agonists. In patients 40 years old or younger, the addition of an LHRH agonist to chemotherapy significantly reduced the risk of recurrence and death (HR 0.74; $p = 0.01$) versus chemotherapy alone. This effect was greatest in the group 35 years old or younger, whereas in the group older than 40 years, the addition of an LHRH agonist did not improve outcome. (10). Similarly, data from the NSABP indicating an increased risk (HR=1.91; 95% CI 1.21 to 3.01; $P = 0.006$) for younger versus older patients with endocrine responsive disease treated with tamoxifen alone (36).

In the present analysis we found that the combination of LH-RH analogue with tamoxifen was significantly correlated with improved DFS for very young patients (aged < 35 years) in the Luminal B subtype, if compared with either tamoxifen or LH-RH analogue alone, thus supporting a role for complete endocrine therapy in the adjuvant treatment of young premenopausal patients. We limited the analysis of the effect of hormonotherapy on DFS to women with Luminal B since there were few events in Luminal A.

The question of whether additional benefit can be obtained from ovarian suppression in premenopausal patients receiving tamoxifen is now being directly addressed by the global Suppression of Ovarian Function Trial coordinated by the IBCSG on behalf of the Breast International Group and the

North American Breast Cancer Intergroup. The Suppression of Ovarian Function Trial compares tamoxifen alone versus ovarian function suppression plus tamoxifen versus ovarian function suppression plus exemestane for patients with steroid hormone receptor-positive tumors who remain premenopausal after adjuvant chemotherapy or for whom tamoxifen alone is considered reasonable treatment option.(37).

5 Conclusions

The present study indicate that the outcome of very young patients with early breast cancer is worse in selected tumor subtypes identified by IHC. Moreover the results herein presented support the hypothesis that the progress in the adjuvant treatment of very young patients requires study of tailored treatments in specific “niches” of patients. It should however be emphasized that the tumor subtypes identified in the present analysis include heterogeneous groups of tumors, and that the identification of further tumor subtypes amenable to targeted treatments represents a research priority.

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