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## Original Article

Prognosis in women with small (T1mic,T1a,T1b) node-negative operable breast cancer by immunohistochemically selected subtypes.

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## Summary

*Background:* Knowledge is limited about prognostic significance of breast cancer subtypes among women with small invasive node-negative breast tumours.

*Patients and Methods:* We explored patterns of recurrence in 1691 women with pT1mic/T1a/T1b, pN0 and M0 breast cancer according to four immunohistochemically-defined tumour subtypes: (i) Luminal A (ER-positive, PgR-positive, HER2-negative and Ki-67<14%); (ii) Luminal B (ER-positive and/or PgR-positive, HER2-positive and/or Ki-67≥14%); (iii) HER2-positive, both endocrine receptors absent; and (iv) Triple Negative.

*Results:* At multivariate analysis, women with the Triple Negative breast cancer subtype had an increased risk of loco-regional relapse (Hazards Ratio (HR) 3.58; 95%CI: 1.40-9.13) and breast cancer related events (HR 2.18; 95%CI: 1.04-4.57). Overall, Luminal B subtype was not associated with a statistically significant increased risk of recurrence compared with Luminal A, while patients with Luminal B subtype tumours overexpressing HER2 had a 2 fold risk of reduced breast cancer related survival , but not an increased risk of loco-regional relapse and distant metastases

Women with HER2 breast cancer subtype had a statistically significant increased risk of loco-regional relapse (HR 4.53; 95%CI: 1.56-13.1), distant metastases and reduced breast cancer related survival (HR 3.22; 95%CI: 1.44-7.18) and overall survival (HR 2.87; 95%CI: 1.05-7.89) when compared with the Luminal A subtype, at multivariate analysis.

*Conclusion:* Women with small size, node-negative, breast cancer are at higher risk of relapse if with HER2-positive endocrine receptor absent or Triple Negative disease.

Key words: breast cancer, prognosis, tumour size, immunohistochemistry, subtypes

## Introduction

In the last decades, the use of screening mammography increased the incidence of small size invasive tumours, including tumours with diameters less than 1 cm [1,2].

Uncertainties exist concerning the biology, prognosis and management of early breast cancers with size less than 1 cm and node-negative status (or pT1mic,a,bN0M0).

Several classical risk factors might have a prognostic role within the subgroup of patients with small tumours and node negative disease [3]. Features which might indicate increased risk of relapse in such patients include peritumoural vascular invasion if extensive [4]. Also the presence of high grade and/or high proliferation indexes might select a subgroup of patients with higher risk of relapse [5-7]. Amplification or overexpression of HER2 should each be considered to elevate risk category [8-9].

Finally, young age should also be taken into consideration due to its prognostic role and its related different responsiveness to endocrine therapy if compared with older premenopausal women with breast cancer [5, 6].

However, some of these studies suffered from small sample size, information on biological features were based on old methodologies and there have been few clinical studies with large number of patients followed long enough for an adequate evaluation of the prognosis.

Care for patients with breast cancer tend to be more selective interventions to minimize acute and late toxicity without compromising efficacy. In particular, for the subgroup of patients classified as pT1a,b N0 appropriate adjuvant systemic therapy involves choosing treatments tailored to individual patients.

Breast cancer is in fact a heterogeneous disease and gene expression studies have identified molecularly distinct subtypes with prognostic implications across multiple treatment settings [10-12]. These subtypes include Oestrogen receptor (ER)-positive – Luminal A (Luminal A), ER-positive – Luminal B (Luminal B), HER2- enriched (i.e. tumours that overexpress HER2-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 [10,13,14].

Immunohistochemical evaluation of ER, progesterone receptor (PgR), Ki-67 and HER2 may be considered a surrogate means for identifying the molecular subtypes of breast cancer [15]. The classification according to four subtypes (Luminal A, Luminal B, HER2 and Triple Negative) appeared useful to define different prognostic subgroups with different relationship with adjuvant treatment received [16,17].

Whether the use of this classification for tumours less than 1 cm might be useful in clinical practice in order to accurately predict outcome is an hypothesis that has not been tested.

The aim of this study was to evaluate the overall outcome of women with lymph node negative invasive carcinoma of the breast of  $\leq 1$ cm in size, including microinvasive carcinoma (T1mic) according to immunohistochemically defined subtypes.

## Patients and methods

We collected information on all consecutive breast cancer patients operated 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, haematological 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 110–200mm intervals) and from all surgical resections performed elsewhere were reviewed. Tumours were classified histologically according to the World Health Organization Histological Classification of Breast Tumours, as modified by Rosen and Obermann [18]. Tumour grading was assessed according to Elston and Ellis [19]. We looked for peritumoural vascular invasion as recommended by Rosen and Obermann [18]. Microinvasive breast cancer was diagnosed according to the TNM classification and following the criteria of Rosen and Obermann [18]. ER, PgR status, Ki-67 labelling index determined with the MIB1 monoclonal antibody, and HER2/neu overexpression were evaluated immunohistochemically as previously reported [20]. In particular, HER2/neu overexpression was evaluated using a 1/100 dilution of a polyclonal antiserum (Dako, Glostrup, Denmark) and considering only complete and intense membrane staining of at least 10% neoplastic cells as evidence of overexpression (3+). For evaluation of ER and PgR status and Ki-67 labelling index, the percentage of cells exhibiting definite nuclear staining over up to 2000 neoplastic cells examined at 400x 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% [20]. Histological grade and biological features were evaluated on the invasive component of the tumour.

**Statistics:** The Fisher 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 endpoints were the incidence of loco-regional relapse (LRR), distant metastasis (DM), breast cancer related survival (BCS) and overall survival (OS).

Loco-Regional Relapse (LRR) included ipsilateral breast cancer, breast cancer recurrence in the axilla, regional lymph nodes, chest wall, skin of ipsilateral breast. Distant Metastasis (DM) included all sites of recurrence except loco-regional relapses and contralateral breast cancer as first of subsequent event. Breast cancer related Survival (BCS) included loco-regional relapses, distant metastases, contralateral breast cancer or death from breast cancer. Overall Survival 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. The log-rank test was used to assess the 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 tumour on event free or overall survival. Factors included in multiple regression analyses included age, tumour size (pT), multifocality, peritumoural vascular invasion and immunohistochemical classification as follows:

- Luminal A (ER>0 or PgR>0) and (Ki-67<14%) and (HER2 0/+/+),
- Luminal B (ER>0 or PgR>0) and ((Ki-67≥14%) or (HER2 +++)),
- HER2- positive (ER=0 and PgR=0) and (HER2 +++), and
- Triple Negative (ER=0 and PgR=0) and (HER2 0/+/+).

Other factors, including menopausal status, presence of in-situ component, ER and PgR expression. Ki-67 labelling index, HER2 over expression, tumour grade and histotype were not included into the final multivariate model, due to lack of association at univariate analysis or presence of collinearity with immunohistochemical classification.

All analyses were performed with the SAS software, version 8.2 (Cary, NC).

## Results

We included in the analysis 1691 women with pT1mic/pT1a/pT1b N0M0 breast cancer treated at the European Institute of Oncology during 1997-2005. Male patients and females with previous cancer, non-invasive breast cancers or bilateral tumours were excluded.

The characteristics of the evaluable patients are given in **Table 1**.

A higher proportion of pT1mic had the HER2 and triple negative immunohistochemical subtypes compared with pT1a or pT1b breast cancers.

HER2 and Triple Negative subtypes were more frequent in young patients (aged less than 35 years) than in older patients. Poor differentiation was strictly correlated to the presence of HER2 and Triple Negative subtype.

As shown in **Table 2**, a higher proportion of patients with small tumours classified as HER2 subtype were treated with mastectomy without radiotherapy. Moreover, about the 99% of patients with Luminal A tumours didn't receive adjuvant chemotherapy, while about 70% of patients with Triple Negative tumours received chemotherapy. Finally, only 8% and 6.5% of patients with Luminal A and Luminal B tumours, respectively, did not receive any hormonal treatment.

In our study the median follow-up was 6.3 years for events and 7 years for overall survival. Figure 1 shows the incidence of locoregional relapse and distant metastases and survival curves according to immunohistochemical classification.

We found a statistical significant difference about LRR, BCS, OS and a borderline difference about the incidence of DM between the four subtypes. Increased rate of LRR and DM and a reduced BCS and OS are observed in the HER2 and Triple-negative subtypes (**Figure 1**).

We performed a multivariate analysis including significant clinical and biological features at univariate analysis (data not shown); moreover we analysed data subdividing Luminal B subtype according to HER2 overexpression status, too.



The presence of peritumoural vascular invasion was associated with a significant risk of DM and reduced OS.

Very young patients (aged<35 years) were at increased risk of LRR plus DM and had a statistically significant reduced BCS and OS compared to older patients.

pT1a and pT1b tumours had an increased risk of LRR, DM, breast cancer-events and death compared with pT1mic, though these results were not statistically significant at multivariate analyses (**Table 3**).

HER2 subtype was the only subtype associated with a statistical significant increased risk of LRR, DM and a reduced BCS and OS compared with Luminal A subtype, at multivariate analysis (**Table 3a**), while patients with Triple Negative subtype had an increased risk of LRR (Hazards Ratio (HR) 3.58; 95%CI: 1.40-9.13) and breast cancer related events (HR 2.18; 95%CI: 1.04-4.57) (**Table 3a**).

No difference was found between Luminal B and Luminal A subtypes for any of the outcomes analysed (Table 3a). When we analysed outcomes of patients with Luminal B according HER2 status (101 patients with Luminal B and HER2 overexpression), we found a reduced BCS (HR 2.15; 95%CI: 1.02-4.53) for Luminal B HER2-positive and a borderline increased risk of LRR (HR 2.70; 95%CI: 0.96-7.55) compared to Luminal A.

We reported number and rate of events and mortality in a Supplementary table (on line Supplementary Table)

## Discussion

Treatment of small tumours still represents an area of controversy. In fact, although several reports focused on this issue in the past years, limited information are still available on their prognosis.

In this study, performed on a large group of patients submitted to surgery in the recent years and with biological features evaluated with new techniques and by the same team of pathologists, a limited but not negligible risk of recurrence was detected confirming the importance of the identification of subgroup of patients at higher risk of recurrence.

An immunohistochemical profile, based on the degree of expression of ER, PgR, Ki-67 and HER2, was used to identify subgroups of breast cancer patients with different outcome and who will respond to different systemic adjuvant treatments [16,17].

In our study the Triple Negative subtype was correlated with a significantly increased risk of LRR and breast cancer related events if compared with the Luminal A subtype. These results are in line with those already presented in two previous studies [8,21]

In a Korean analysis, patients with Triple Negative disease had a significantly lower Relapse-Free Survival rate compared to hormone receptor-positive and/or HER2-positive patients ( $p=0.016$ ); in the multivariate analysis, triple negativity was an independent prognostic factors of recurrence (HR 4.96; 95% CI: 1.00-23.1;  $p=0.018$ ) [21].

In the study by Gonzalez-Angulo et al., patients who had Triple Negative breast cancer had 3.89 times (95% CI: 2.56-10.14;  $P .0001$ ) the risk of recurrence and 2.84 times (95% CI: 0.99-8.14;  $P .053$ ) the risk of distant recurrence if compared with patients who had hormone receptor-positive breast cancer [8].

Although the number of events was low in the present experience related to the fact that we are focusing on a population with a lower intrinsic metastatic potential, the observation of a worse

breast cancer survival indicated that new adjuvant strategies should be explored in this subgroup of patients.

In the present study the HER2 subtype (HER2 positive and steroid hormone receptor negative disease) was associated with worse outcome and increased incidence of LRR and DM. Approximately 6%–10% of small node-negative tumours are HER2 positive and there is increasing evidence from some retrospective analyses for a worse outcome of patients with HER2-positive tumours.

In a study that analysed 965 T1a,bN0M0 breast cancers, patients with HER2-positive disease (including both hormone receptor positive and hormone receptor negative tumour) had higher risks of recurrence (HR, 2.68; 95% CI: 1.44-5.00; P=0.002) and distant recurrence (HR, 5.30; 95% CI, 2.23-12.62; P=0.001) than those with HER2-negative tumours [8].

In a second study, conducted at European Institute of Oncology in Milan, after a median follow-up of 4.6 years, in the hormone receptor-positive group, 5-year DFS rates were 99% for HER2-negative disease and 92% for HER2-positive disease (P=0.013), while no difference was found in the group of hormone receptor-negative tumours [9].

Our results showed that HER2 is an independent prognostic factor, mainly when associated with negative hormone receptors (HER2 subtype). However, in the Luminal B, HER2 positive subset (ER positive and HER2 positive disease) a significantly worse BCS was observed (HR, 2.18; 95% CI: 1.03-4.60) when compared with Luminal A. The different follow-up, the different study design as well as the limited number of events registered in the subgroup of patients with tumour less than 1 cm in size might explained the partially different results observed.

A limited number of studies that evaluated age as prognostic factor in patients with small size breast cancer is available in the literature. A recently published study evaluated the outcome of 378 Korean women with small breast cancers. After a median follow-up of 60 months, authors showed that age younger than 35 years was significantly associated with a higher rate of recurrence HR 4.91; 95%CI=1.01-23.76; P=0.048) [21]. In our previous published study we explored patterns of

recurrence by age according to four immunohistochemically-defined breast cancer subtypes; very young patients (age less than 35) with Triple Negative, Luminal B or HER2-positive breast cancer had a worse prognosis when compared with older patients with similar characteristics of disease [17].

Current study adds important information about the prognosis of very young women with breast cancer; also when tumour size is less than 1 centimetre age less than 35 is a significant risk factor for a reduced BCS and overall survival compared to older patients.

In conclusion, the present study confirms that the prognosis of patients with node negative small breast cancer depends on variable features. We show that risk of relapse significantly correlates with tumour subtypes identified by immunohistochemistry as well as classical prognostic features such as young age. Patients whose tumours are classified as pT1a,b pN0 include a spectrum from patients at very low risk for whom there is little evidence supporting the use of adjuvant therapy, to those with higher risk disease where an approach including chemotherapy plus targeted therapy appears clearly justified.

It should however be emphasised that the tumour subtypes identified in the current study still include heterogeneous groups of tumours, and that the identification of further tumour subtypes amenable to targeted treatments represents a research priority.

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**Table 1:** Patients characteristics

Characteristics	All N	Luminal A N (%)	Luminal B N (%)	HER2 N (%)	Triple Negative N (%)	P-value
<b>All</b>	1691	881 (100)	633 (100)	82 (100)	95 (100)	
<b>Tumour size</b>						
pT1mic	68	17 ( 1.9)	22 ( 3.5)	22 (26.8)	7 ( 7.4)	
pT1a	362	197 (22.4)	114 (18.0)	30 (36.6)	21 (22.1)	
pT1b	1261	667 (75.7)	497 (78.5)	30 (36.6)	67 (70.5)	<.0001
<b>Age</b>						
<35	27	7 ( 0.8)	10 ( 1.6)	3 ( 3.7)	7 ( 7.4)	
35-49	507	239 (27.1)	219 (34.6)	22 (26.8)	27 (28.4)	
50-69	1023	576 (65.4)	342 (54.0)	50 (61.0)	55 (57.9)	
70+	134	59 ( 6.7)	62 ( 9.8)	7 ( 8.5)	6 ( 6.3)	<.0001
<b>Menopausal status</b>						
Pre-menopausal	581	283 (32.1)	243 (38.4)	23 (28.0)	32 (33.7)	
Peri-menopausal	1040	562 (63.8)	363 (57.3)	56 (68.3)	59 (62.1)	
Post-menopausal	70	36 ( 4.1)	27 ( 4.3)	3 ( 3.7)	4 ( 4.2)	0.21
<b>Histology</b>						
Ductal	1276	596 (67.7)	523 (82.6)	77 (93.9)	80 (84.2)	
Lobular	164	102 (11.6)	59 ( 9.3)	0 ( 0.0)	3 ( 3.2)	
Ductal+Lobular	214	162 (18.4)	35 ( 5.5)	5 ( 6.1)	12 (12.6)	
Other	37	21 ( 2.4)	16 ( 2.5)	0 ( 0.0)	0 ( 0.0)	<.0001
<b>Multifocality</b>						
Monofocal	1517	794 (90.1)	564 (89.1)	73 (89.0)	86 (90.5)	
Multifocal	174	87 ( 9.9)	69 (10.9)	9 (11.0)	9 ( 9.5)	0.91
<b>In-situ component</b>						
Absent	1231	685 (77.8)	451 (71.2)	33 (40.2)	62 (65.3)	
Present	460	196 (22.2)	182 (28.8)	49 (59.8)	33 (34.7)	<.0001
<b>Tumour Grade</b>						
G1	688	568 (64.5)	113 (17.9)	1 ( 1.2)	6 ( 6.3)	
G2	739	279 (31.7)	406 (64.1)	29 (35.4)	25 (26.3)	
G3	205	6 ( 0.7)	90 (14.2)	48 (58.5)	61 (64.2)	<.0001
Unknown	83	28 ( 3.2)	24 ( 3.8)	4 ( 4.9)	3 ( 3.2)	
<b>Peritumoural vascular invasion</b>						
Absent	1611	853 (96.8)	590 (93.2)	79 (96.3)	89 (93.7)	
Present	80	28 ( 3.2)	43 ( 6.8)	3 ( 3.7)	6 ( 6.3)	0.008



**Table 2:** Treatments

Characteristics	Luminal A		Luminal B		HER2		Triple Negative		P-value
Type of surgery									
Quadrantectomy	824	(93.5%)	572	(90.4%)	52	(63.4%)	87	(91.6%)	<.0001
Mastectomy	57	( 6.5%)	61	( 9.6%)	30	(36.6%)	8	( 8.4%)	
Sentinel lymph node biopsy									
No	63	( 7.1%)	54	( 8.5%)	12	(14.6%)	18	(19.0%)	0.003
Yes	818	(92.9%)	579	(91.5%)	70	(85.4%)	77	(81.0%)	
Chemotherapy									
Antracycline	4	( 0.5%)	16	( 2.5%)	20	(24.4%)	4	( 4.2%)	<.0001
CMF	4	( 0.5%)	31	( 4.9%)	11	(13.4%)	60	(63.2%)	
Other/NOS	3	( 0.3%)	2	( 0.3%)	5	( 6.1%)	2	( 2.1%)	
NONE	870	(98.8%)	584	(92.3%)	46	(56.1%)	29	(30.5%)	
Endocrine therapy									
LHRH	25	( 2.8%)	16	( 2.5%)	0		0		<.0001
TAM	515	(58.5%)	322	(50.9%)	1	( 1.2%)	2	( 2.1%)	
LHRH+TAM	177	(20.1%)	169	(26.7%)	0		1	( 1.1%)	
Other/NOS	95	(10.8%)	86	(13.6%)	0		0		
NONE	69	( 7.8%)	40	( 6.3%)	81	(98.8%)	92	(96.8%)	
Radiotherapy									
NONE	61	( 6.9%)	68	(10.7%)	28	(34.2%)	9	(9.5%)	<.0001
ELIOT	203	(23.0%)	125	(19.8%)	8	( 9.8%)	15	(15.8%)	
External RT	617	(70.0%)	440	(69.5%)	46	(56.1%)	71	(74.7%)	

**Table 3a** Multivariate analysis

		Loco-regional relapse	Distant metastasis	Local + distant metastasis	Breast cancer survival	Overall survival
<b>Age</b>	<35	1.60 (0.35-7.23)	3.34 (0.68-16.4)	2.78 (0.93-8.34)	2.94 (1.11-7.81)	4.72 (0.92-24.2)
	35-49	1.00	1.00	1.00	1.00	1.00
	50-69	0.73 (0.39-1.36)	0.90 (0.39-2.11)	0.89 (0.53-1.51)	0.79 (0.50-1.26)	2.74 (1.12-6.70)
	70+	0.26 (0.04-1.97)	1.04 (0.22-4.84)	0.59 (0.18-1.97)	1.05 (0.46-2.40)	4.86 (1.55-15.2)
<b>pT</b>	pT1mic	1.00	1.00	1.00	1.00	1.00
	pT1a	1.37 (0.29-6.42)	0.58 (0.10-3.31)	1.37 (0.38-4.89)	1.83 (0.53-6.26)	2.39 (0.29-19.6)
	pT1b	1.29 (0.29-5.77)	0.80 (0.17-3.81)	1.44 (0.42-4.91)	1.58 (0.47-5.24)	2.34 (0.30-18.2)
<b>Multifocality</b>	Monofocal	1.00	1.00	1.00	1.00	1.00
	Multifocal	2.64 (1.35-5.16)	0.95 (0.28-3.18)	1.95 (1.07-3.53)	1.56 (0.90-2.70)	1.03 (0.40-2.65)
<b>Peritumoural vascular invasion</b>	Absent	1.00	1.00	1.00	1.00	1.00
	Present	1.68 (0.58-4.84)	3.73 (1.35-10.3)	1.92 (0.86-4.28)	1.67 (0.79-3.53)	3.53 (1.43-8.71)
<b>Immunohistochemical subtype</b>	Luminal A	1.00	1.00	1.00	1.00	1.00
	Luminal B	1.59 (0.80-3.18)	1.99 (0.81-4.85)	1.78 (1.02-3.12)	1.45 (0.90-2.33)	0.74 (0.36-1.50)
	HER2+++	4.53 (1.56-13.1)	4.37 (1.06-17.9)	5.15 (2.20-12.1)	3.22 (1.44-7.18)	2.87 (1.05-7.89)
	Triple Negative	3.58 (1.40-9.13)	2.46 (0.61-9.92)	3.04 (1.35-6.84)	2.18 (1.04-4.57)	1.94 (0.71-5.31)

Hazards ratio (HR) and 95% confidence intervals (CI) derived from univariate Cox proportional hazard regression model

NOTE: Tumour grade was not included in the final multivariate model due to strong colinearity with immunohistochemical subtype.

**Table 3b:** Multivariate analysis

		Loco-regional relapse	Distant metastasis	Local + distant metastasis	Breast cancer survival	Overall survival
<b>Age</b>	<35	1.69 (0.37-7.69)	3.80 (0.76-19.1)	2.93 (0.97-8.82)	3.06 (1.14-8.14)	6.71 (1.22-36.8)
	35-49	1.00	1.00	1.00	1.00	1.00
	50-69	0.75 (0.40-1.41)	0.98 (0.41-2.37)	0.92 (0.54-1.56)	0.80 (0.51-1.28)	3.66 (1.33-10.0)
	70+	0.28 (0.04-2.09)	1.12 (0.24-5.32)	0.62 (0.18-2.06)	1.08 (0.47-2.46)	6.65 (1.93-22.9)
<b>pT</b>	pT1mic	1.00	1.00	1.00	1.00	1.00
	pT1a	1.31 (0.28-6.17)	0.56 (0.10-3.22)	1.32 (0.37-4.73)	1.80 (0.52-6.17)	2.27 (0.28-18.6)
	pT1b	1.36 (0.30-6.09)	0.84 (0.17-4.01)	1.52 (0.44-5.19)	1.65 (0.49-5.48)	2.44 (0.31-19.1)
<b>Multifocality</b>	Monofocal	1.00	1.00	1.00	1.00	1.00
	Multifocal	2.56 (1.30-5.07)	0.87 (0.25-2.97)	1.92 (1.05-3.50)	1.55 (0.89-2.68)	0.91 (0.34-2.39)
<b>Peritumoural vascular invasion</b>	Absent	1.00	1.00	1.00	1.00	1.00
	Present	1.48 (0.49-4.48)	3.25 (1.12-9.41)	1.80 (0.79-4.14)	1.60 (0.74-3.44)	2.88 (1.10-7.59)
<b>Immunohistochemical subtype</b>	Luminal A	1.00	1.00	1.00	1.00	1.00
	Luminal B (HER2 -/+/+)	1.31 (0.61-2.82)	1.69 (0.65-4.42)	1.49 (0.81-2.75)	1.30 (0.78-2.16)	0.52 (0.22-1.22)
	Luminal B (HER2 +++)	2.70 (0.96-7.55)	3.02 (0.78-11.7)	3.12 (1.37-7.09)	2.15 (1.02-4.53)	1.30 (0.38-4.38)
	HER2+++	4.64 (1.60-13.4)	4.41 (1.08-18.1)	5.27 (2.25-12.3)	3.27 (1.47-7.30)	2.95 (1.07-8.10)
	Triple Negative	3.62 (1.42-9.24)	2.50 (0.62-10.1)	3.05 (1.35-6.86)	2.18 (1.04-4.57)	2.01 (0.73-5.49)

Hazards ratio (HR) and 95% confidence intervals (CI) derived from univariate Cox proportional hazard regression model

NOTE: Tumour grade was not included in the final multivariate model due to strong colinearity with immunohistochemical subtype.

Figure 1: Breast cancer outcome according to immunohistochemical subtype



