Chapter 6

The clinical prognostic value of molecular intrinsic tumor subtypes in older breast cancer patients: A FOCUS study analysis

Charla C. Engels, Mandy Kiderlen, Esther Bastiaannet, Antien L. Mooyaart, Ronald L.P. van Vlierberghe, Vincent T.H.B.M. Smit, Peter J.K. Kuppen, Cornelis J.H. van de Velde, Gerrit Jan Liefers

Mol Oncol. 2016 Apr;10(4):594-600

ABSTRACT

Introduction

It was recently proposed that the molecular breast tumor subtypes are differently distributed in the elderly breast cancer patients, and also lack prognostic value. Given the limited number of elderly patients in previous studies, the aim of this study was to determine the prognostic effect of the molecular intrinsic subtypes in a large older breast cancer population.

Material and method

Older breast cancer patients with invasive, non-metastatic breast cancer with tumor material available for immunohistochemical determination of Ki67, EGFR, CK5/6 and HER-2 were included. ER and PR expression was retrieved from the pathology report. Molecular subtypes were: Luminal A, Luminal B, ERBB2, Basal-like and Unclassified. Primary endpoint was Relapse Free Period (RFP), taking into account the competing risk of mortality, and adjusted for the most important patient, tumor and treatment characteristics. Secondary endpoint was Relative Survival (RS).

Results

Overall, 1,362 patients were included. Patients with a Luminal A subtype had the lowest risk of recurrence (11% at 5 yrs). Patients with a Basal (24% at 5yrs) or ERBB2 (34% at 5yrs) molecular breast tumor subtype had the highest risk of recurrence. The ERBB2 subtype had the worst prognosis in terms of RFP (SHR 2.07, 95% CI 1.35-3.20; p=0.001). The worst RS was again observed for the ERBB2 subtype (48% at 10 yrs). In multivariable analyses, the relative excess risk of death for all molecular subtypes was significantly worse compared to the Luminal A subtype.

Conclusion

Molecular intrinsic breast tumor subtypes have significant prognostic value in the elderly population, even after taking competing mortality into account.

INTRODUCTION

Due to a lack of presentation in clinical trials and translational studies, our knowledge on the effects and associations of prognostic and predictive biomarkers in the elderly breast cancer population is limited. Current breast cancer treatment guidelines are based on studies performed in relatively young or fit elderly populations [1-3]. This observation is alarming for the clinical care of older breast cancer patients, given the fact that breast cancer is increasingly becoming a disease affecting the aged population [4]. It has been shown that older breast cancer patients tend to present more frequently with hormone receptor (HR) positive tumors, less human epidermal growth factor (HER-2) receptor overexpression and with lower proliferation rates than their younger counterparts [5;6]. Although the tumor characteristics may seem more favorable, recent studies have shown an inferior breast cancer specific prognosis for older breast cancer patients [7;8]. Current treatment guidelines are based on studies performed in younger breast cancer patients, herewith increasing the chance of suboptimal treatment in the elderly breast cancer patients. Lately therefore, the demand for prognostic and predictive research focusing on the elderly breast cancer population has greatly increased.

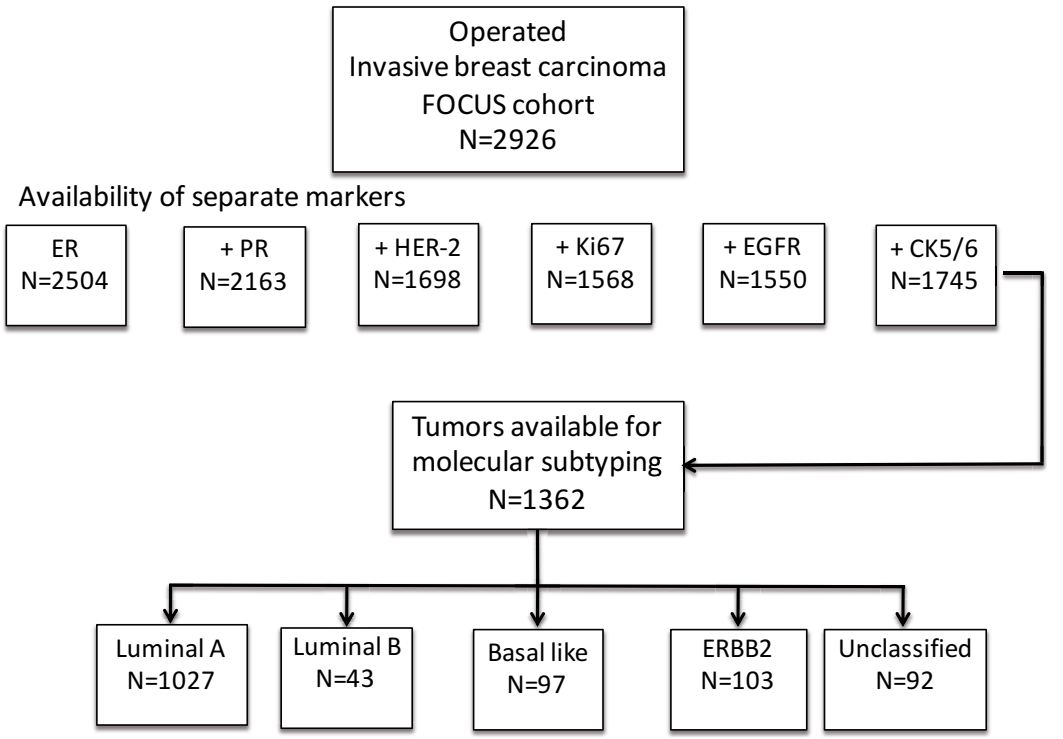
A modern-day genetic array showing promising prognostic results is the intrinsic breast tumor classification, also known as the molecular breast cancer subtypes [9-11]. The intrinsic classification proposes four different classes of breast tumors: Luminal A and B, which are mostly hormone receptor positive and express high amounts of genes related to the luminal epithelial cell layer [9-11]. Compared to Luminal A tumors, Luminal B tumors tend to have more cellular proliferation. Furthermore, the intrinsic subtypes also include two tumor subtypes which do not express hormonal receptors, namely: the Basal like tumors, which are triple negative tumors (estrogen receptor (ER) negative, progesterone receptor (PR) negative and Human Epidermal Growth Factor Receptor-2 (HER-2) negative) combined with expression of genes characteristic of the basal epithelial layer such as cytokeratin (CK) 5 and 6; and the ERBB2 tumor subtype, which clusters near the basal-like subtypes, but expresses high HER-2 on the tumor surface. Previous studies showed that Luminal A tumors have the most indolent character, closely followed by Luminal B. ERBB2 and Basal-like tumors are both characterized by more aggressive phenotypes, resulting in unfavorable patient outcome [10].

Recently, de Kruijf *et al.* showed that the molecular intrinsic breast cancer subtypes have a different distribution in elderly breast cancer compared to their younger counterparts [12]. However, in this study no prognostic effect of the intrinsic breast tumor subtypes was shown within the older breast cancer patients. However, this study only included 189 breast cancer patients above the age of 65. Therefore, the aim of our current study was to determine the prognostic effect of the intrinsic molecular breast cancer subtypes in a large population of elderly breast cancer patients.

MATERIAL AND METHODS

Patients and tumors

For this study, all patients with invasive, non-metastatic breast cancer from the FOCUS cohort (Female breast cancer in the elderly, Optimizing Clinical guidelines USing clinic-pathological and molecular data) who received surgery and had formalin fixed paraffin embedded (FFPE) intra-operative tumor samples available with successful staining of HER-2, Ki67, EGFR and CK5/6 were included (CONSORT diagram). ER and PR status was retrieved from the pathology report of each patient. The FOCUS cohort has been described extensively in previous publications [13]. In brief, the cohort consists of all women aged ≥ 65 years at time of diagnosis, with invasive and *in situ* breast cancer, diagnosed between 1997 and 2004 in the South Western region of The Netherlands. Follow-up on survival status was available until the 1st of January 2013. All tumor samples were handled in a coded fashion, according to national ethical guidelines ("Code for Proper Secondary Use of Human Tissue", Dutch Federation of Medical Scientific Societies).



CONSORT diagram

Immunohistochemistry

Tissue sections of 4µm were cut from intra-operatively derived FFPE tumor material of the FOCUS cohort processed into a tissue microarray (TMA). Mouse anti-Ki67 (cline

MIB-1, Dako, NL), anti-epidermal growth factor receptor (EGFR) (NLC-EGFR, Novocastra, UK), anti-CK5/6 (clone D5/16 B4, Dako, NL) and rabbit anti-c-erbB-2 Oncoprotein (A0485, Dako, Denmark) were used for immunohistochemical staining. Immunohistochemical staining was performed according to previously described standard protocol [12]. Briefly, tissue sections were deparafinized and antigen retrieval was performed using a Pre Treatment (PT) module (PT link, DAKO, Denmark). Endogenous peroxidase activity was blocked with hydrogen peroxidase 0.3% in PBS for 30 minutes. Sections were incubated with the primary antibody at room temperature overnight or for 20 minutes (only for c-erbB2 antibody). Subsequently, all TMA slides were incubated with Envision anti-mouse (DAKO, Denmark, Cytomation K4002) or anti-rabbit (DAKO, Denmark, Cytomation K4003) for 30 minutes at room temperature. DAB was used for visualization of positively stained breast tumor tissue on the TMA and counterstained with haematoxylin, dehydrated and finally mounted with pertex. Per staining, all slides were stained simultaneously to avoid inter-assay variation. Negative controls were slides that underwent the entire staining protocol without primary antibody.

Evaluation of immunostaining and molecular subtype determination

Microscopic quantification of positive tumor cells for Ki67, EGFR, CK5/6 and c-erbB-2 protein was performed by two independent observers. Ki67 staining was considered negative if less than 10% of the tumor cells had visible staining and positive if Ki67 was immunohistochemically present in ≥10% of the tumor cells. Cut-offs for low versus high expression of EGFR and CK5/6 were based on the median expression level, which was 0% for both stainings. HER-2 staining was scored as follows: 0 for no staining at all or incomplete or faint/barely perceptible membrane staining in <10% of the invasive tumor cells; 1+ for a faint/barely perceptible partial membrane staining in >10% of the tumor cells; 2+ for weak to moderate complete membrane staining in >10% of the tumor cells; and 3+ for strong to complete membrane staining in >30% of the tumor cells. For all patients, the highest score out of the three punches of the same tumor was used for statistical analysis. If one or more punches were missing, the highest score of the remaining punch(es) was included for analyses. Immunohistochemical HER-2 scores 0, 1+ and 2+ were considered HER-2 negative and a HER-2 3+ score was considered HER-2 positive.

Immunohistochemical profiles have been previously developed and validated by combinations of the following immunohistochemically determined markers: ER, PR, HER-2, Ki67, EGFR, and CK5/6. Based on these papers, we defined the immunohistochemical molecular intrinsic breast tumor subtypes as follows: *Luminal A*: ER+ and/or PR+, HER-2- and Ki67-; *Luminal B*: ER+ and/or PR+, HER-2- and Ki67+; *ERBB2*: HER-2+; *Basal-like*: ER-, PR-, HER-2- and EGFR+ and/or CK5/6+; *Unclassified*: ER-, PR-, HER-2-, EGFR-, and CK5/6- [9;10;12].

Statistical analyses

Statistical analyses were performed using the statistical packages SPSS (version 20.0 for Windows, IBM SPSS statistics) and Stata SE 12.0.

Cohen's kappa coefficient was used to assess the inter-observer agreement in quantification of HER-2, Ki67, EGFR and CK5/6 tumor expression. The χ^2 test was used to evaluate associations between various clinicopathological parameters and molecular intrinsic tumor subtypes.

The primary endpoint examined was Relapse-Free Period (RFP), defined as the time from date of diagnosis until any recurrence (any registered relapse of breast cancer, either locoregional recurrence, distant recurrence or contralateral breast cancer, whichever came first). The Cumulative Incidence Competing Risks method was used for plotting of the cumulative incidence of recurrence, taking into account the competing risk of death [14]. Fine & Gray competing risks regression analyses were used for univariable and multivariable analysis for RFP, taking into account the competing risk of death of any cause [15]. Multivariable analyses were adjusted for patient (age), tumor (TNM stage, grade) and treatment factors (type of breast surgery, axillary surgery, radiotherapy, endocrine therapy and chemotherapy).

The secondary endpoint was relative survival, calculated as the ratio between the observed survival in the cohort and the expected survival as calculated from the age-, sex- and year-matched background population [16]**.** Assuming that other factors influencing mortality risk are the same in the cohort and background population, this means that the excess risk of death, as measured in the cohort, can be attributed to breast cancer. Therefore, the excess mortality can be interpreted as cancer-specific mortality.

Patients with missing data on the determinant of interest due to material handling (which is considered to happen randomly, so we assume these data to be missing at random) were excluded from the statistical analyses. To test for the robustness of results, due to the relatively large proportion of missing values, as a sensitivity analysis we used multiple imputation to impute the six markers ER, PR, HER-2, EGFR, CK5/6 and Ki67. Therefore we used 25 replications of the original dataset, with the following variables as predicting variables: age, morphology, grade T-stage, N-stage, screen-detected, type of breast surgery, type of axillary surgery, radiotherapy, endocrine therapy, and chemotherapy. We also accounted for the outcomes: time to first recurrence, recurrence status, time to death or last follow-up and vital status. We analyzed the primary endpoint (RFP with competing risks regression) using the imputed dataset. We were not able to analyze the secondary endpoint (relative survival) with the imputed dataset, because the statistical package does not support this analysis with imputed data.

RESULTS

Patient and tumor characteristics

Overall, 1,362 patients were included in all analyses.

Median age of patients in the cohort was 75 years (range 65-98 years). The majority of the patients had an early stage tumor of ductal morphology. Molecular tumor subtypes were associated with different tumor stage, showing more early stage tumors in the luminal tumor subtypes, and more stage III tumors in the ERBB2, basal and unclassified subtypes (p=0.014 (Table 1)). In addition, molecular subtypes were significantly associated with tumor grade and morphology. Regarding therapies, patients with an ERBB-2, basal and unclassified subtype received more chemotherapy, whereas the patients with a luminal A or B subtype received more adjuvant endocrine therapy (both therapies p<0.001) (Table 1). The Cohen's kappa coefficient for inter-observer agreement for HER-2, Ki67, EGFR and CK5/6 were all >0.6.

Relapse Free Period for molecular intrinsic breast tumor subtypes

Median follow-up time for the endpoint of relapse free period (RFP) was 5.2 years (range 0-13.4). Patients with a luminal A subtype had the lowest risk of recurrence (11% at 5 years), followed by luminal B and unclassified (both 18%). Patients with a Basal or ERBB2 molecular breast tumor subtype had the highest risk of recurrence (24% and 34% at 5 years, respectively). Cumulative incidences of recurrence are depicted in Figure 1. Table 2 shows the results of the crude and adjusted Fine & Gray competing risks regression analyses, where patients with Luminal A were taken as reference group. Patients with an ERBB2 subtype had, after taking into account the competing risk of mortality and after adjustment for the most important clinical factors, the worst prognosis in terms of RFP (adjusted sub-distribution hazard ratio (SHR) 2.07, 95% confidence interval (CI) 1.35-3.20; p=0.001). Patients with a Basal subtype also had a significantly higher risk of recurrence (adjusted SHR 1.80, 95% CI 1.14-2.85; p=0.012). Patients with a Luminal B or unclassified subtype had no statistically significant different RFP than patients with a Luminal A subtype.

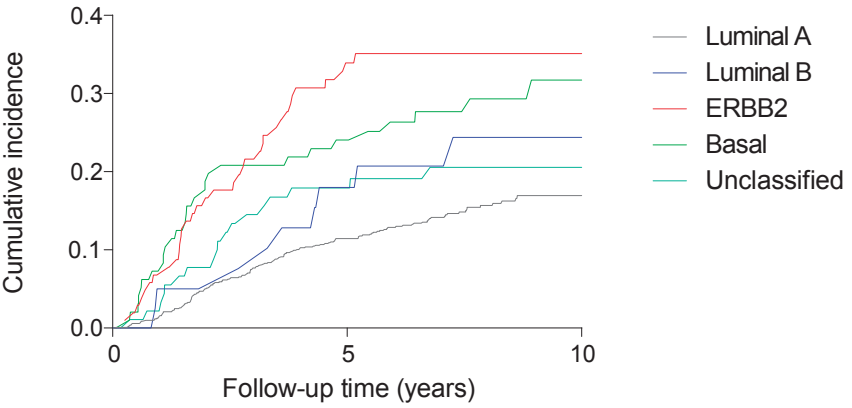
Results of the sensitivity analysis, using multiple imputation of missing values is shown in web-table 1, confirming the higher risk of recurrence for patients with an ERBB-2 and Basal breast cancer subtype.

When separate analyses were performed for loco-regional and distant relapse, no significant association was seen for loco-regional relapse and molecular breast tumor subtypes (web-table 2). Contrarily, the same significant association was seen for the molecular breast tumor subtypes and distant metastases as with RFP (web-table 3). These results could be explained by the greater number of events for distant metastases compared to loco-regional relapse in this patient set. Implying that the significant association seen in our study for RFP and the molecular breast tumor subtypes is largely dependent on the distant metastases.

Table 1: Patient, tumor and treatment characteristics

	Luminal A		Luminal B		ERBB2		Basal		Unclassified		*P**
	N=1027		N=43		N=103		N=97		N=92		
	N	%	N	%	N	%	N	%	N	%	
Age in years (mean, SD)	76.3 (7.2)		75.2 (7.2)		74.9 (6.9)		75.9 (6.8)		76.4 (7.8)		0.392
Number of comorbidities											0.712
0-1	492	47.9	20	46.5	51	49.5	49	50.5	41	44.6	
2-4	443	43.1	20	46.5	39	37.9	39	40.2	46	50.0	
5 or more	92	9.0	3	7.0	13	12.6	9	9.3	5	4.5	
TNM stage											**0.014**
I	355	34.6	14	32.6	21	20.4	25	25.8	27	29.3	
II	528	51.4	28	65.1	66	64.1	57	58.8	45	48.9	
III	124	12.1	0	0.0	14	13.6	14	14.4	15	16.3	
Missing	20	1.9	1	2.3	2	1.9	1	1.0	5	5.4	
Grade											**<0.001**
1	164	16.0	2	4.7	4	3.9	2	2.1	5	5.4	
2	374	36.4	9	20.9	16	15.5	11	11.3	20	21.7	
3	218	21.2	17	39.5	62	60.2	56	57.7	45	48.9	
Missing	271	26.4	15	34.9	21	20.4	28	28.9	22	23.9	
Morphology											**0.002**
Ductal	780	75.9	33	76.7	91	88.3	71	73.2	75	81.5	
Lobular	118	11.5	8	18.6	4	3.9	5	5.2	8	8.7	
Other/missing	129	12.6	2	4.7	8	7.8	21	21.6	9	9.8	
Breast surgery*											0.052
BCS	389	37.6	18	41.9	24	23.3	32	33.0	35	38.0	
Mastectomy	641	62.4	25	58.1	79	76.7	65	67.0	57	62.0	
Axillary surgery*											0.4
No axillary surgery	121	11.8	3	7.0	16	15.5	11	11.3	15	16.3	
Sentinel node	258	25.1	12	27.9	16	15.5	21	21.6	22	23.9	
ALND	648	63.1	28	65.1	71	68.9	65	67.0	55	59.8	
Adjuvant radiotherapy											0.944
No	522	50.9	21	48.8	54	52.4	49	50.5	43	46.7	
Yes	505	49.2	22	51.2	49	47.6	48	49.5	49	53.3	
Adjuvant endocrine therapy											**<0.001**
No	470	45.8	13	30.2	66	64.1	82	84.5	74	80.4	
Yes	557	54.2	30	69.8	37	35.9	15	15.5	18	19.6	
Adjuvant chemotherapy											**<0.001**
No	981	95.5	42	97.7	89	86.4	88	90.7	75	81.5	
Yes	46	4.5	1	2.3	14	13.6	9	9.3	17	18.5	

*calculated by one-way ANOVA test for continuous variables and a Chi-square test for categorical variables. P-values in bold indicate a statistical significant difference between the molecular subtypes at the p-level of 0.05.



Numbers at risk:

Luminal A	1025	579	48
Luminal B	43	22	5
ERBB2	103	33	4
Basal	97	49	11
Unclassified	92	46	6

Figure 1: Cumulative incidence of recurrence by molecular subtype.

Table 2: Relapse free period (Fine & Gray regression)

	N	N of events	Cumulative incidence of recurrence at 5 years (%)	95% CI			
				SHR	lower	upper	*P*
Luminal A	1027	112	11%	1 (reference)			
Luminal B	43	7	18%	1.56	0.81	3.02	0.184
ERBB2	103	34	34%	2.78	1.91	4.04	**<0.001**
Basal	97	23	24%	2.19	1.44	3.31	**<0.001**
Unclassified	92	16	18%	1.49	0.92	2.41	0.106

SHR*	95% CI		*P*
	lower	upper	
1 (reference)			
1.31	0.69	2.48	0.407
2.07	1.35	3.20	**0.001**
1.80	1.14	2.85	**0.012**
1.27	0.75	2.15	0.372

*Adjusted for age, morphology, grade, tumor stage, type of breast surgery, type of axillary surgery, radiotherapy, endocrine therapy, chemotherapy

Relative survival for molecular intrinsic breast tumor subtypes

Median follow-up time was 8.6 years (range 0-17.0 years). Relative survival, calculated as the observed survival in the cohort, divided by the expected survival in the age-, year and sex matched general population, was highest for the patients with a Luminal A subtype (88%). All other subtypes had a worse relative survival at 10 years. The worst clinical outcome was again observed for patients with an ERBB2 subtype, showing a relative survival at 10 years of 48%. In multivariable analyses, the relative excess risk (RER) of death for all molecular breast cancer subtypes was significantly worse than patients with a Luminal A subtype (Table 3).

Table 3: Relative survival

	N of observed deaths / N of Expected deaths	Relative survival at 10 years (%)	95% CI				95% CI			
			RER	lower	upper	*P*	RER*	lower	upper	*P*
Luminal A	533/430	88%	1 (reference)				1 (reference)			
Luminal B	26/16	67%	2.59	0.97	6.93	0.058	2.88	1.26	6.57	**0.012**
ERBB2	69/23	48%	5.79	3.53	9.51	**<0.001**	4.28	2.51	7.30	**<0.001**
Basal	59/32	68%	3.34	1.74	6.41	**<0.001**	3.11	1.74	5.55	**<0.001**
Unclassified	59/33	63%	2.84	1.46	5.53	**0.002**	2.22	1.13	4.35	**0.020**

*Adjusted for age, morphology, grade, tumor stage, type of breast surgery, type of axillary surgery, radiotherapy, endocrine therapy, chemotherapy

DISCUSSION

In this study it was shown that molecular intrinsic breast tumor subtypes are of significant prognostic value in the older (≥65years) breast cancer population. Our results indicate that the ERBB2 and Basal molecular breast tumor subtypes are associated with worse Relapse Free Period. Moreover, all molecular subtypes hold a poor prognosis in terms of Relative Survival, compared to the Luminal A breast tumor subtype in the elderly breast cancer patients.

These results are in contrast with the results of a recent study performed by de Kruijf *et al.*, in which it was proposed that intrinsic breast tumor subtyping is of limited prognostic value in the older breast cancer population. However, the study from the Kruijf *et al.* only included 189 patients aged 65 years or older, resulting in small molecular subtype subgroups with minimal discriminative capacity. The current study contained 1,362 breast cancer patients aged 65 years or older, resulting in much more statistical power and thus more reliable, clinically translatable outcome.

In addition to the results of our current study, evidence is accumulating about the prognostic role of tumor biology, even in the presence of high competing risk of mortality. For instance, Mook *et al.* showed that usage of the 70-gene prognosis signature is

able to accurately select postmenopausal breast cancer patients, between 55 and 70 years of age, who are at low risk of breast cancer-related death within 5 years of diagnosis [17]. These results may help to more adequately select patients for adjuvant systemic treatment.

In our study, the distribution of the molecular intrinsic breast tumor subtypes in this older breast cancer population showed a higher prevalence of the assumed more indolent Luminal A tumor and a relatively low prevalence of the more aggressive Basal molecular tumor subtype compared to the studies performed in a younger breast cancer population [18]. Noteworthy is the same prevalence, of around 7%, of the ERBB2 intrinsic molecular breast tumor subtype in both the younger and the older breast cancer population. These results imply that the chance of getting a more aggressive molecular tumor subtype decreases with increasing age, which is in accordance with the observation of milder tumor characteristics in the older breast cancer population. However, our results confirm the prognostic value of the more aggressive tumor subtypes (Basal and ERBB2) in the older breast cancer population, which is reflected in a worse relapse free period as well as a worse relative survival. These results imply that older breast cancer patients with aggressive tumor types could potentially benefit from a more aggressive (systemic) treatment, irrespective of their advanced age.

The major strength of our study is that we used the largest consecutive series of older breast cancer patients from a population-based cohort, from which tumor material was available. Therefore, our study is not affected by selection bias. A limitation of the study is that there was no tumor material available for all patients from the original cohort. This was mostly due to logistical reasons and tissue loss during experimental procedures. After statistical imputation of the missing data, our results did not change. Second, by using TMA for immunohistochemical stainings one does not have an overview of the exact number of positively stained cells on the histological slide. Therefore, some degree of underscoring cannot be ruled out. Third, no confirmatory microarray genetic analysis was performed. However, immunohistochemical surrogates, like those used in this study, have been validated with good agreement in previous studies [12].

In conclusion, the molecular intrinsic classification and its impact on clinical outcome have been extensively investigated in breast cancer. So far, molecular breast cancer studies identified breast cancer as a heterogeneous disease, emphasizing the need for different systemic treatment approaches. However, as is the case with most translational studies and clinical trials, these studies mostly include relatively young or fit elderly patients. Our present study is the first study performed in a large unselected population of older breast cancer patients, showing significant prognostic value of the molecular breast tumor subtypes, even after taking the risk of competing mortality into account.

Therefore, the result of this study supports the use of molecular subtyping in the older breast cancer patients, even when dealing with the older, more fragile breast cancer population for prognostication and consequently, therapy allocation.

REFERENCE LIST

(1) Anderson WF, Jatoi I, Sherman ME. Qualitative age interactions in breast cancer studies: mind the gap. *J Clin Oncol* 2009;27:5308-5311.

(2) Beadle BM, Woodward WA, Buchholz TA. The impact of age on outcome in early-stage breast cancer. *Semin Radiat Oncol* 2011;21:26-34.

(3) Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thurlimann B, Senn HJ. Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. *Ann Oncol* 2007;18:1133-1144.

(4) Petrakis IE, Paraskakis S. Breast cancer in the elderly. *Arch Gerontol Geriatr* 2010;50:179-184.

(5) Daidone MG, Coradini D, Martelli G, Veneroni S. Primary breast cancer in elderly women: biological profile and relation with clinical outcome. *Crit Rev Oncol Hematol* 2003;45:313-325.

(6) Diab SG, Elledge RM, Clark GM. Tumor characteristics and clinical outcome of elderly women with breast cancer. *J Natl Cancer Inst* 2000;92:550-556.

(7) Bastiaannet E, Liefers GJ, de Craen AJ *et al.* Breast cancer in elderly compared to younger patients in the Netherlands: stage at diagnosis, treatment and survival in 127,805 unselected patients. *Breast Cancer Res Treat* 2010;124:801-807.

(8) van de Water W, Markopoulos C, van de Velde CJ *et al.* Association between age at diagnosis and disease-specific mortality among postmenopausal women with hormone receptor-positive breast cancer. *JAMA* 2012;307:590-597.

(9) Perou CM, Sorlie T, Eisen MB *et al.* Molecular portraits of human breast tumours. *Nature* 2000;406:747-752.

(10) Sorlie T, Perou CM, Tibshirani R *et al.* Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001;98:10869-10874.

(11) Sorlie T, Tibshirani R, Parker J *et al.* Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A* 2003;100:8418-8423.

(12) de Kruijf EM, Bastiaannet E, Ruberta F *et al.* Comparison of frequencies and prognostic effect of molecular subtypes between young and elderly breast cancer patients. *Mol Oncol* 2014;8:1014-1025.

(13) de Glas NA, Kiderlen M, Bastiaannet E *et al.* Postoperative complications and survival of elderly breast cancer patients: a FOCUS study analysis. *Breast Cancer Res Treat* 2013;138:561-569.

(14) Verduijn M, Grootendorst DC, Dekker FW, Jager KJ, le CS. The analysis of competing events like cause-specific mortality--beware of the Kaplan-Meier method. *Nephrol Dial Transplant* 2011;26:56-61.

(15) Putter H, Fiocco M, Geskus RB. Tutorial in biostatistics: competing risks and multi-state models. *Stat Med* 2007;26:2389-2430.

(16) Hakulinen T, Seppa K, Lambert PC. Choosing the relative survival method for cancer survival estimation. *Eur J Cancer* 2011;47:2202-2210.

(17) Mook S, Schmidt MK, Weigelt B *et al.* The 70-gene prognosis signature predicts early metastasis in breast cancer patients between 55 and 70 years of age. *Ann Oncol* 2010;21:717-722.

(18) Carey LA, Perou CM, Livasy CA *et al.* Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* 2006;295:2492-2502.

SUPPLEMENTARY TABLES

Webtable 1: Relapse free period (Fine & Gray regression) - sensitivity analysis using multiple imputation for missing values

		95% CI				95% CI		
	SHR	**lower**	**upper**	***P***	**SHR***	**lower**	**upper**	***P***
Luminal A	1 (reference)				1 (reference)			
Luminal B	1.35	0.80	2.30	0.259	1.39	0.81	2.39	0.233
ERBB2	2.65	1.81	3.90	**<0.001**	2.33	1.55	3.52	**<0.001**
Basal	1.72	1.21	2.44	**0.003**	1.66	1.15	2.39	**0.006**
Unclassified	1.31	0.91	1.88	0.14	1.34	0.90	1.99	0.142

*Adjusted for age, morphology, grade, tumor stage, type of breast surgery, type of axillary surgery, radiotherapy, endocrine therapy, chemotherapy

Web-table 2: Locoregional relapse free period (Fine & Gray regression)

	N of events	**Cumulative incidence of recurrence at 5 years (%)**	**SHR**	**95% CI**		***P***
				lower	**upper**	
Luminal A	23	2%	1 (reference)			
Luminal B	2	5%	1.50	0.35	6.31	0.584
ERBB2	5	5%	1.85	0.78	4.41	0.164
Basal	4	4%	1.92	0.80	4.60	0.143
Unclassified	4	4%	1.36	0.48	3.88	0.562

*Adjusted for age, morphology, grade, tumor stage, type of breast surgery, type of axillary surgery, radiotherapy, endocrine therapy, chemotherapy

	95% CI		
SHR*	**lower**	**upper**	***P***
1 (reference)			
1.36	0.32	5.76	0.676
1.42	0.57	3.50	0.451
1.33	0.52	3.37	0.548
1.09	0.38	3.10	0.877

Web-table 3: Distant metastasis free period (Fine & Gray regression)

	N of events	**Cumulative incidence of recurrence at 5 years (%)**		**95% CI**		***P***
				lower	**upper**	
Luminal A	88	9%	1 (reference)			
Luminal B	5	12%	1.53	0.73	3.21	0.263
ERBB2	29	29%	2.95	1.95	4.47	**<0.001**
Basal	19	20%	2.26	1.41	3.60	**0.001**

Unclassified	10	11%	1.32	0.74	2.34	0.346

*Adjusted for age, morphology, grade, tumor stage, type of breast surgery, type of axillary surgery, radiotherapy, endocrine therapy, chemotherapy

	95% CI		
SHR*	**lower**	**upper**	***P***
1 (reference)			
1.28	0.62	2.64	0.504
2.18	1.33	3.55	**0.002**
1.96	1.16	3.32	**0.012**
1.14	0.61	2.14	0.681