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Original article

Differential prognostic relevance of patho-anatomical factors among different tumor-biological subsets of breast cancer: Results from the adjuvant SUCCESS A study

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ABSTRACT

Objectives: In breast cancer, large tumor size, positive nodal stage and a triple-negative tumor subtype are associated with reduced survival, but the interactions between these prognostic factors are not well understood.

Material and methods: Here we re-evaluated the impact of tumor size, nodal stage and tumor subtype on disease-free survival (DFS), overall survival (OS), distant disease-free survival (DDFS) and breast cancer specific survival (BCSS) in a retrospective analysis using data from the adjuvant SUCCESS A trial. Subgroup analyses were conducted to assess whether the effect of tumor size and nodal stage on survival depended on tumor subtype.

Results: Increasing tumor size, higher nodal stage and triple negative breast cancer (TNBC) were associated with unfavorable prognosis (all $p < 0.001$). There was no significant interaction between tumor subtype and tumor size ($p > 0.5$ for all four survival endpoints), but we found significant interactions between tumor subtype and nodal stage ($p < 0.05$ for all four survival endpoints), with no differences in survival among tumor subtypes for patients with pN0 tumors (all $p > 0.05$) and pronounced differences in survival among tumor subtypes for patients with positive nodal stage (all $p < 0.001$).

Conclusions: This analysis confirms tumor size, nodal stage and tumor subtype as independent prognostic factors in high-risk early breast cancer. Nodal-positive patients with TNBC had a considerably worse outcome compared to nodal-positive patients with another tumor subtype. This underlines the importance for early detection particularly for patients with TNBC.

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1. Introduction

Prognostic markers are used to define patients risk for recurrence and thereby define high-risk breast cancer [1]. Additionally to classical prognostic factors like young age, large tumor size and positive nodal stage, molecular classification of breast cancer gained of importance. The molecular subtype is distinguished by gene expression profiling into defined intrinsic subtypes - Luminal A, Luminal B, HER2 type and Triple-negative [2]. Results of gene expression profiles correspond to characterization of tumors based on hormone- and HER2 status and proliferation markers or histological grade [3], and classification by immunohistochemistry (IHC) is recommended by St. Gallen Expert Consensus to determine molecular subtype in clinical routine [4].

Because of increasing importance of the molecular subtype for therapeutic decisions, we re-evaluated the impact of prognostic factors with a special emphasis on molecular subtypes in a retrospective analysis using data from the adjuvant SUCCESS A trial, in which 3754 patients with high-risk early breast cancer were randomized to anthracycline- and taxane-based chemotherapy with or without gemcitabine. More specifically, we first analyzed the effect of tumor subtype, tumor size and nodal stage on survival outcome, followed by a detailed analysis of differential prognostic relevance of tumor size and nodal stage among different tumor subtypes of breast cancer.

2. Methods

2.1. Study design and patients

In the open-label, phase III clinical trial SUCCESS A (EudraCT 2005-000490-21), breast cancer patients with high-risk disease were included (for definition, see supplementary material A1). Participants were randomized to adjuvant chemotherapy treatment with three cycles of epirubicin, fluorouracil and cyclophosphamide followed by either three cycles of docetaxel or three cycles of docetaxel and gemcitabine. Based on a second randomization, the patients received either 2 or 5 years of zoledronate. Patients with hormone receptor-positive breast cancer received an endocrine therapy after finishing chemotherapy. Neoadjuvant chemotherapy was not part of study treatment and none of the patients in the SUCCESS A study received any neoadjuvant therapy. Inclusion and exclusion criteria as well as therapeutic details are published by Schröder et al. [5]; the main inclusion and exclusion criteria are listed in the supplementary material A1. 37 German ethical boards and the German Federal Institute for Drugs and Medical Devices (BfArM) approved the SUCCESS A Study (lead ethical board: Ludwig-Maximilians-University Munich, project number: 076–05) and the study was performed in accordance with the Declaration of Helsinki. Informed consent was given in written form from all patients.

2.2. Immunohistochemistry

Primary tumor stages at diagnosis were classified according to the criteria defined by the revised American Joint Committee on Cancer and International Union against Cancer TNM classification system [6]. Histologic grading was determined according to the Elston–Ellis modification of the Scarff–Bloom–Richardson grading system [7]. Tumors were defined as hormone receptor positive if the percentage of cells with immunohistochemical nuclear staining for estrogen, progesterone, or both was 10% or higher. Tumors with strong (3+) immunohistochemical membranous staining were defined as HER2-positive; tumors with moderate (2+) membranous staining were classified as HER2-positive only if an additional FISH analysis yielded a positive result. Due to missing values of Ki67, tumor subtypes were defined as follows: luminal A like: hormone receptor positive, human epidermal growth factor receptor 2 (HER2) negative,G1/G2; luminal B like: hormone receptor positive, HER2 negative, G3; HER2 positive: all HER2-positive tumors; triple negative: hormone receptor negative, HER2 negative tumors [8–10]. As our classification of subtypes is not based on molecular/genetic data, we use the term biological subtype throughout. Biological subtypes could be determined only for 3667 of the 3754 randomized patients because of missing values for hormone receptor status, HER2 status and/or histological grading.

2.3. Data analysis

Descriptive statistics for the categorical data are provided in terms of absolute and relative frequencies. The non-normally distributed continuous variables age and body mass index (BMI) are described by medians and ranges. Associations between biological subtypes and patient or tumor characteristics were evaluated using Kruskal-Wallis H tests for age and chi-square tests for all other categorical variables. Patient outcomes were analyzed in terms of four different survival endpoints defined according to the Standardized Definitions for Efficacy End Points (STEEP) criteria [11]. Disease-free survival (DFS) included local, contralateral and distant disease recurrence as well as secondary primary tumors and death from any cause as event; all non-invasive *in situ* cancer events were excluded. Distant disease-free survival (DDFS) included only distant recurrence (metastasis and second primary tumors) and death from any cause as events, while ipsilateral or regional disease recurrences and contralateral breast cancers were excluded. Overall survival (OS) included death from any cause as event, and for the calculation of breast cancer–specific survival (BCSS) only death due to breast cancer–related causes (e.g., metastasis-dependent organ failure or breast cancer progression) was regarded as event. Time-to-event data (median follow up 65 months, range 1–96 months) were analyzed using the Kaplan-Meier method and summarized using medians, and 95% confidence intervals, and survival curves were compared using log-rank tests. All time-to-event intervals were measured from time of primary diagnosis to date of the event; if no endpoint was reached, data were censored at date of last adequate follow-up. Because of low number of pT4 tumors, tumor stages pT3 and pT4 were combined to one group (pT3/pT4) for all survival analyses. To be able to evaluate whether tumor size, nodal stage and/or biological subtype show independent prognostic effects on survival, we first tested the proportional hazard assumption for a multivariable Cox proportional hazards regression model adjusted for age (continuous), menopausal status (premenopausal, postmenopausal), histological type (ductal, lobular, other), type of surgery (breast conserving, mastectomy), chemotherapy treatment (FEC-Doc, FEC-DocG), and zoledronate treatment (2 years, 5 years) by incorporating a time-by-variable interaction using a time-

dependent covariate. We found no significant time-by-tumor size interaction ($p > 0.12$ for all four survival endpoints) and no significant time-by-nodal stage interaction ($p > 0.15$ for all four survival endpoints). However, we found a significant time-by-biological subtype interaction ($p < 0.01$ for all four survival endpoints), indicating that the effect of biological subtypes on survival was not constant over time and that thus assumptions for a multivariable Cox proportional hazards regression model were not met. To account for this non-proportionality, we performed all Cox regressions including the variable biological subtype with the time-by-biological subtype interaction, which allows the ratio of hazard rates to vary over time. To investigate whether the effect of biological subtype on survival differed according to tumor size or nodal stage, we ran cox regressions with the two main effects together with the corresponding two-way interaction term (i.e., a cox regression with the main effects of biological subtype and tumor size together with the two-way interaction term between biological subtype and tumor size, and a cox regression with the main effects of biological subtype and nodal stage together with the two-way interaction term between biological subtype and nodal stage) for all four survival endpoints. Statistical analyses were performed using IBM® SPSS® Statistics, version 22 (IBM Corp., Armonk, NY, USA). All statistical tests were two-sided, and p values of less than 0.05 were considered significant (no adjustment of significance levels for multiple testing).

3. Results

3.1. Baseline patient and tumor characteristics

Between September 2005 and March 2007, 3754 patients at 271 study centers were randomized for the SUCCESS A trial. Baseline patient and tumor characteristics are shown in Table 1.

Table 1
Baseline patient and tumor characteristics of all 3754 patients randomized in the SUCCESS A study.

Age (years)	median	53.0
	range	21–86
Menopausal status	premenopausal	1565 (41.7%)
	postmenopausal	2189 (58.3%)
Tumor size	pT1	1552 (41.3%)
	pT2	1929 (51.4%)
	pT3	198 (5.3%)
	pT4	52 (1.4%)
	unknown	23 (0.6%)
Nodal stage	pN0	1273 (33.9%)
	pN1	1705 (45.4%)
	pN2	511 (13.6%)
	pN3	236 (6.3%)
	unknown	29 (0.8%)
Histological grading	G1	176 (4.7%)
	G2	1783 (47.5%)
	G3	1773 (47.2%)
	unknown	22 (0.6%)
Histological type	invasive ductal	3060 (81.5%)
	invasive lobular	419 (11.2%)
	other	253 (6.7%)
	unknown	22 (0.6%)
Hormone receptor status	negative	1100 (29.3%)
	positive	2633 (70.1%)
	unknown	21 (0.6%)
HER2 status	negative	2787 (74.2%)
	positive	883 (23.5%)
	unknown	84 (2.2%)
Biological subtype	luminal A like	1423 (37.9%)
	luminal B like	619 (16.5%)
	HER2 type	883 (23.5%)
	triple negative	742 (19.8%)
	unknown	87 (2.3%)
Type of surgery	breast conserving	2638 (70.3%)
	mastectomy	1097 (29.2%)
	unknown	19 (0.5%)
Chemotherapy	FEC-DocG	1856 (49.4%)
	FEC-Doc	1898 (50.6%)
Adjuvant endocrine therapy	yes	2677 (71.3%)
	no	1048 (27.9%)
	unknown	29 (0.8%)
Adjuvant HER2-targeted therapy	yes	754 (20.1%)
	no	2971 (79.1%)
	unknown	29 (0.8%)
Zoledronate treatment	2 years	1847 (49.2%)
	5 years	1907 (50.8%)

3.2. Prognostic value of tumor size, nodal stage and biological subtype

Fig. 1 shows survival curves according to tumor size, pT and nodal stage, pN. Tumor size was a significant prognostic factor for all survival endpoints (Log rank test, all $p < 0.0001$), and all pairwise comparisons revealed significant differences between tumor sizes pT1, pT2 and pT3/pT4 (Log rank test, all $p < 0.001$). Similarly, nodal stage was a significant prognostic factor for all survival endpoints (Log rank test, all $p < 0.0001$). Pairwise comparisons showed no differences between pN0 and pN1 tumors for any survival endpoint (Log rank test, all $p > 0.05$), while all other pairwise comparisons between nodal stages yielded significant differences (Log rank test, all $p < 0.0001$).

Baseline patient and tumor characteristics according to biological subtype are shown in Table 2. There were significant differences in survival among biological subtypes for all survival endpoints (Log rank test, all $p < 0.0001$; Fig. 2). 5-year DFS rates were 91.4%, 84.9%, 87.3% and 78.9% for patients with luminal A like, luminal B like, HER2 type and triple negative tumors, respectively. Corresponding values were 95.3%, 92.7%, 93.8% and 86.3%for 5-year OS rates, 94.0%, 89.0%, 89.4% and 82.8% for 5-year DDFS rates, and 96.5%, 94.0%, 94.6% and 87.6% for 5-year BCSS rates. Pairwise comparisons for all survival endpoints showed that patients with triple-negative tumors had significantly worse survival than patients with luminal A like tumors (Log rank test, all $p < 0.0001$), patients with luminal B like tumors (Log rank test, all $p < 0.03$) and patients with HER2 type tumors (Log rank test, all $p < 0.002$). Furthermore, pairwise comparisons showed that patients with luminal A like tumors had significantly better DFS, OS, DDFS and BCSS than patients with tumors of any other subtype (Log rank test, all $p < 0.02$); the only exception was pairwise comparison between patients with luminal A like and HER2 type tumors with regard to OS, which revealed only a tendency for better survival in the luminal A like group (Log rank test, $p = 0.055$). No significant difference was found in pairwise comparisons between patients with luminal B like and HER2 type tumors for any survival endpoint (Log rank test, all $p > 0.1$).

Multivariable cox regressions with tumor size, nodal stage and biological subtype adjusted for other well-known prognostic factors (see methods) confirmed significant independent effects of tumor size, nodal stage and biological subtype on DFS, OS, DDFS, and BCSS (Table 3). However, it has to be noted that for OS and BCSS the difference between patients with luminal A like tumors and both patients with luminal B like tumors and patients with HER2 type tumors did not reach statistical significance (see Table 3).

3.3. Prognostic value of biological subtype according to tumor size

Fig. 3 shows the effect of biological subtype on DFS separately for tumor sizes pT1, pT2, and pT3/pT4. For each tumor size,

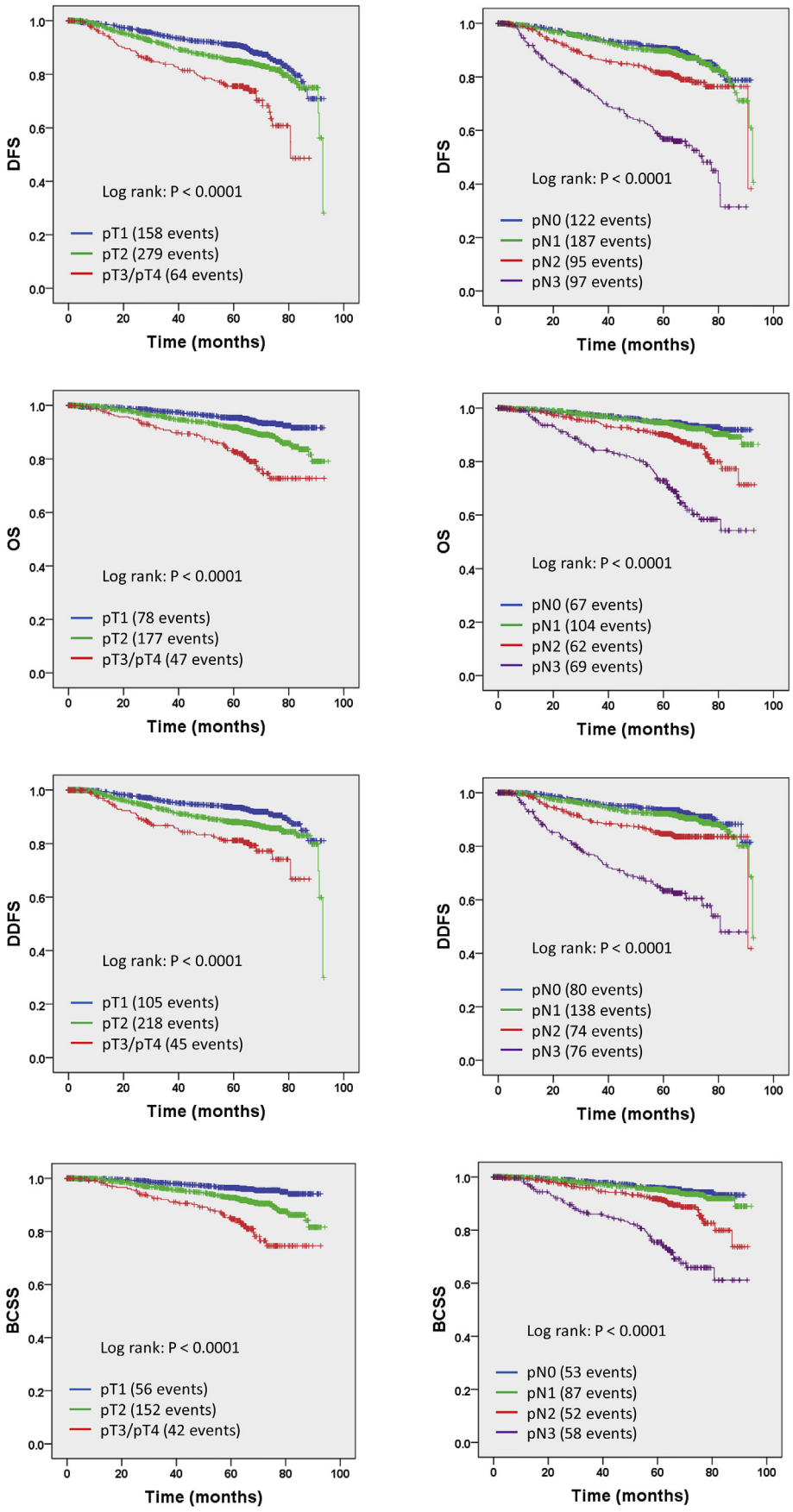

Fig. 1. Kaplan–Meier plots of survival according to tumor size pT (left panel) and nodal stage pN (right panel). DFS: disease-free survival; OS: overall survival; DDFS: distant disease-free survival; BCSS: breast cancer specific survival. P values refer to log-rank tests. For numbers of patients in the different tumor size and nodal stage categories see Table 1.

Table 2
Baseline patient and tumor characteristics by biological subtype (n = 3667).

		Biological subtype				*p*-value[a]
		Luminal A like	Luminal B like	HER2 type	Triple negative	
		(n = 1423)	(n = 619)	(n = 883)	(n = 742)	
Age (years)	median	54.0	55.0	53.0	51.0	p < 0.0001[b]
	range	21–85	22–79	26–77	26–86	
Menopausal status	premenopausal	589 (41.4%)	242 (39.1%)	359 (40.7%)	342 (46.1%)	p = 0.045[c]
	postmenopausal	834 (58.6%)	377 (60.9%)	524 (59.3%)	400 (53.9%)	
Tumor size	pT1	583 (41.0%)	224 (36.2%)	376 (42.6%)	339 (45.7%)	p = 0.001[c]
	pT2	728 (51.2%)	346 (55.9%)	451 (51.1%)	368 (49.6%)	
	pT3	96 (6.7%)	33 (5.3%)	41 (4.6%)	27 (3.6%)	
	pT4	15 (1.1%)	15 (2.4%)	15 (1.7%)	7 (0.9%)	
	unknown	1 (0.1%)	1 (0.2%)	0 (0.0%)	1 (0.1%)	
Nodal stage	pN0	238 (16.7%)	227 (36.7%)	337 (38.2%)	450 (60.6%)	p < 0.0001[c]
	pN1	874 (61.4%)	236 (38.1%)	357 (40.4%)	202 (27.2%)	
	pN2	218 (15.3%)	101 (16.3%)	124 (14.0%)	61 (8.2%)	
	pN3	90 (6.3%)	53 (8.6%)	64 (7.2%)	26 (3.5%)	
	unknown	3 (0.2%)	2 (0.3%)	1 (0.1%)	3 (0.4%)	
Histological grading	G1	155 (10.9%)	0 (0.0%)	10 (1.1%)	8 (1.1%)	p < 0.0001[c]
	G2	1268 (89.1%)	0 (0.0%)	350 (39.6%)	137 (18.5%)	
	G3	0 (0.0%)	619 (100.0%)	523 (59.2%)	597 (80.5%)	
Histological type	invasive ductal	1050 (73.8%)	523 (84.5%)	801 (90.7%)	630 (84.9%)	p < 0.0001[c]
	invasive lobular	290 (20.4%)	55 (8.9%)	45 (5.1%)	21 (2.8%)	
	other	82 (5.8%)	41 (6.6%)	37 (4.2%)	91 (12.3%)	
	unknown	1 (0.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Type of surgery	breast conserving	957 (67.3%)	435 (70.3%)	578 (65.5%)	617 (83.2%)	p < 0.0001[c]
	mastectomy	466 (32.7%)	184 (29.7%)	305 (34.5%)	125 (16.8%)	
Chemotherapy	FEC-DocG	702 (49.3%)	296 (47.8%)	443 (50.2%)	377 (50.8%)	p = 0.712[c]
	FEC-Doc	721 (50.7%)	323 (52.2%)	440 (49.8%)	365 (49.2%)	
Zoledronate treatment	2 years	693 (48.7%)	295 (47.7%)	439 (49.7%)	376 (50.7%)	p = 0.689[c]
	5 years	730 (51.3%)	324 (52.3%)	444 (50.3%)	366 (49.3%)	

[a] All tests without category "unknown".
[b] Kruskal Wallis test.
[c] Chi-square test.

biological subtype was a prognostic factor for DFS (Log rank test, all p < 0.005). Pairwise comparisons between biological subtypes for each tumor size indicated no difference in DFS between luminal A like and luminal B like subtypes in patients with pT3/pT4 tumors (Log rank test, p = 0.567), while there were significant differences with regard to DFS between luminal A like and luminal B like subtypes in patients with pT1 or pT2 tumors (Log rank test, both p < 0.01). In addition, in patients with pT3/pT4 tumors the triple negative subtype seems to have especially worse DFS compared to the other three biological subtypes, while in patients with pT1 or pT2 tumors the differences in DFS among the biological subtypes appear less pronounced (Fig. 3). However, as the interaction term between biological subtype and tumor size was not significant (p = 0.580), there is no clear evidence that the effect of biological subtype on DFS differed depending on tumor size (see supplementary material A2 for the similar results regarding the interaction effect between biological subtype and tumor size on OS, DDFS and BCSS).

3.4. Prognostic value of biological subtype according to nodal stage

The effect of biological subtype on DFS for nodal stages pN0, pN1, pN2 and pN3 is shown in Fig. 4. While survival curves for the four biological subtypes were close together for pN0 tumors, they separated with increasing nodal stage. Accordingly, there was no significant difference in DFS among biological subtypes for patients with pN0 tumors (Log rank test, p = 0.228). In contrast, biological subtype significantly predicted DFS in patients with pN1, pN2 and pN3 tumors (Log rank test, all p < 0.0001).Pairwise comparisons revealed that triple negative tumors were associated with worse DFS compared to other biological subtypes in patients with nodal stage pN1, pN2 or pN3 (Log rank test, all p < 0.04). For patients with pN3 tumors, no significant differences in DFS among luminal A or B like and HER2 type tumors were found (Log rank test, all p > 0.7). The interaction term between biological subtype and nodal stage was significant (p = 0.003), confirming that the effect of biological subtype on DFS depended on nodal stage (see supplementary material A3 for the similar results regarding the interaction effect between biological subtype and nodal stage on OS, DDFS and BCSS).

4. Discussion

We reevaluated the prognostic impact of patho-anatomical factors and biological subtype on survival in early breast cancer within our large multicenter prospective phase III study. The 5-year OS for all patients was 92.7% and 5- year DFS was 86.9%. Therefore, overall prognosis was excellent after treatment with anthracycline and taxane containing chemotherapy; nevertheless, subsets of patients with less favorable outcome were identified.

Tumor size and nodal stage are well established prognostic factors in early breast cancer [12–14]. Accordingly, we found that tumor size was a significant prognostic factor for all four survival endpoints with significant differences between tumor sizes pT1, pT2 and pT3/pT4. With regard to nodal stage, our univariable analyses revealed no significant difference in survival rates between patients with pN0 and pN1 tumors, whereas a higher nodal stage (pN2, pN3) was associated with decreased survival. However, in adjusted multivariable analyses, survival of patients with pN1 tumors was significantly worse compared to patients with pN0 tumors and survival decreased significantly with increasing nodal stage in accordance with findings in other studies [15,16].

Our results concerning biological subtypes and their impact on survival are in accordance to other studies [17,18]. Patients with Luminal A like tumors had a very favorable outcome with a 5-year

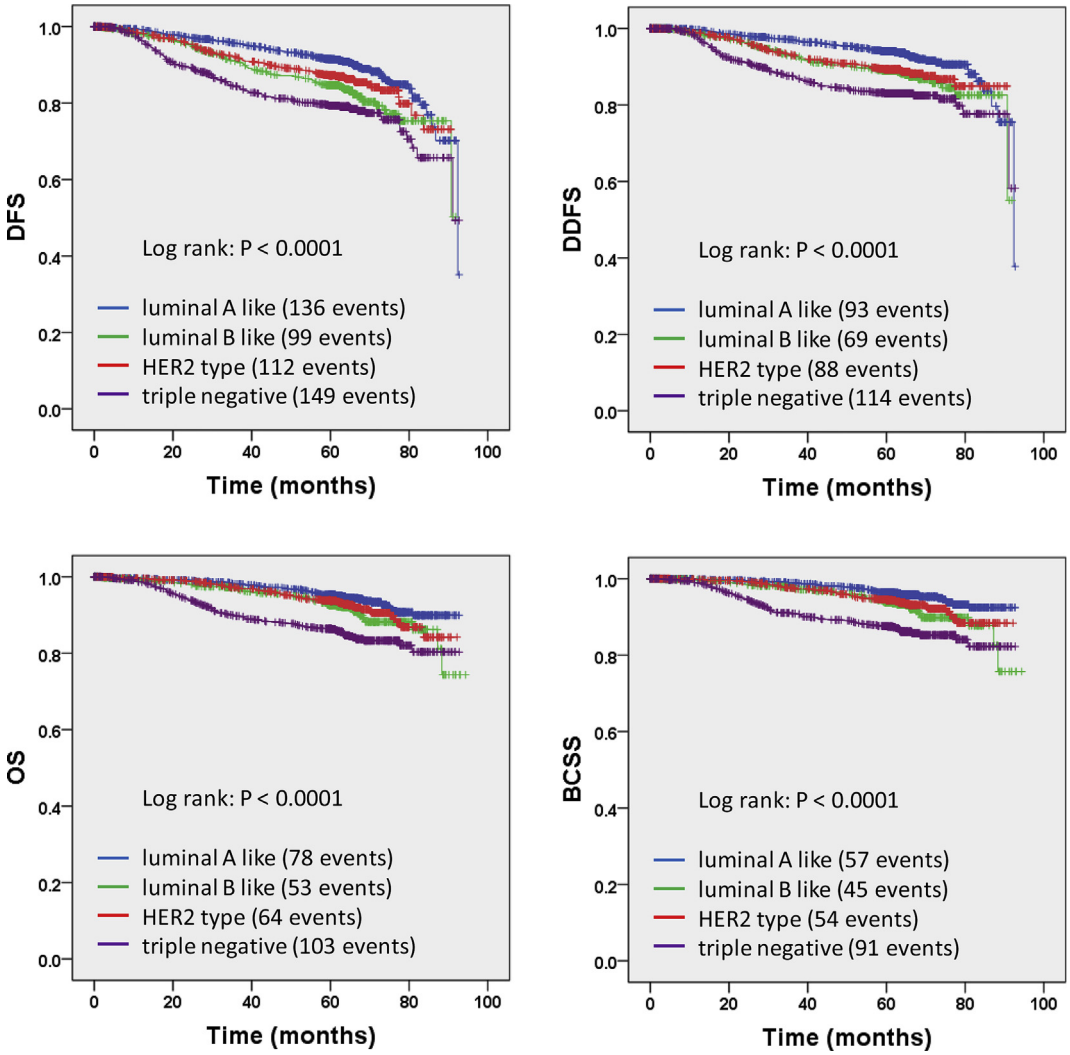

Fig. 2. Kaplan–Meier plots of survival according to biological subtype. DFS: disease-free survival; OS: overall survival; DDFS: distant disease-free survival; BCSS: breast cancer specific survival. P values refer to log-rank tests. For numbers of patients in the different biological subtype categories see Table 1.

DFS rate of 91.4%, while a worse prognosis was found in Luminal B like tumors and HER2 positive tumors with comparable 5-year DFS rates of 84.9% and 87.3%. The worst outcome was present in patients with TNBC with a 5-year DFS rate of 78.9%. In this context, it must be mentioned that not all patients with a HER2 positive tumor received an adjuvant anti HER2 therapy so that survival rates of the HER2 subgroup may differ from those in more recent investigations. Overall, we confirmed biological subtype as an independent prognostic parameter in addition to other established clinic pathologic variables [17,19,20].

In clinical routine, it often remains unclear whether molecular subtype or patho-anatomical factors should be regarded as more important prognostic factors, and there are studies speculating that molecular subtype overrides the prognostic relevance of TNM staging in cases with discordance between stage and biological subtype [21,22]. Our study showed that the negative effect of advanced tumor stage (pT3/pT4) on survival was especially pronounced in patients with TNBC; however, the impact of biological subtype on survival was not statistically significantly dependent on tumor size (as there was no significant interaction term). In contrast, the interaction term between biological subtype and nodal stage was significant, confirming that the impact of nodal stage on survival was strongly dependent on biological subtype. We found that particularly patients with pN0 tumors had a good prognosis when receiving an anthracycline and taxane containing chemotherapy, even if they had prognostically unfavorable TNBC. Thus, in patients with pN0 tumors, biological subtype seems to be less important. On the other hand, in patients with nodal positive tumors the outcome depended to a large extent on biological subtype, with advanced stages (pN2 and pN3) in TNBC being associated with a particularly unfavorable prognosis. There are data demonstrating that TNBC is less likely to be node-positive than either HER2 positive or HR+/HER2 negative tumors, especially above tumor sizes greater than 2 cm [23], but that prognosis is worse, when TNBC are diagnosed in advanced stages [24]. This finding and our data therefore suggest that it is important to detect TNBC in early stage with no axillary involvement.

One strength of our study is the homogenous patient sample from a large prospective randomized clinical trial, which reduces the potentially confounding effects of heterogeneous patient samples and different treatment regimens. However, given the homogeneity of the patient population, our results may not be extrapolated to all early breast cancer patients, particularly if other adjuvant chemotherapy regimen are used. In addition, this is the first study in which the interaction effects between tumor subtypes and both tumor size and nodal stage on survival in breast cancer

Table 3
Effects of tumor size (reference group pT1), nodal stage (reference group pN0) and biological subtype (reference group luminal A like) on disease-free survival (DFS), overall survival (OS), distant disease-free survival (DDFS) and breast cancer specific survival (BCSS) in fully adjusted multivariable Cox regressions that included the time-by-biological subtype interaction.

Survival endpoint		Hazard ratio	95% CI	P- value
DFS	Tumor size			**0.029**
	pT2 vs. pT1	1.28	1.05–1.56	0.016
	pT3/pT4 vs. pT1	1.43	1.02–1.99	0.036
	Nodal stage			<**0.0001**
	pN1 vs. pN0	1.57	1.23–2.00	<0.001
	pN2 vs. pN0	2.29	1.71–3.05	<0.0001
	pN3 vs. pN0	6.06	4.49–8.18	<0.0001
	Biological subtype			<**0.0001**
	luminal B like vs. luminal A like	2.63	1.52–4.55	0.001
	HER2 type vs. luminal A like	2.49	1.46–4.26	0.001
	triple negative vs. luminal A like	9.59	5.86–15.69	<0.0001
OS	Tumor size			**0.003**
	pT2 vs. pT1	1.57	1.20–2.07	0.001
	pT3/pT4 vs. pT1	1.78	1.17–2.70	0.007
	Nodal stage			<**0.0001**
	pN1 vs. pN0	1.69	1.22–2.34	0.001
	pN2 vs. pN0	2.62	1.81–3.80	<0.0001
	pN3 vs. pN0	7.48	5.11–10.97	<0.0001
	Biological subtype			<**0.0001**
	luminal B like vs. luminal A like	1.73	0.73–4.09	0.213
	HER2 type vs. luminal A like	1.56	0.68–3.57	0.293
	triple negative vs. luminal A like	15.63	7.76–31.49	<0.0001
DDFS	Tumor size			**0.004**
	pT2 vs. pT1	1.49	1.17–1.89	0.001
	pT3/pT4 vs. pT1	1.49	1.00–2.21	0.048
	Nodal stage			<**0.0001**
	pN1 vs. pN0	1.87	1.40–2.50	<0.0001
	pN2 vs. pN0	2.85	2.03–4.00	<0.0001
	pN3 vs. pN0	8.29	5.84–11.76	<0.0001
	Biological subtype			<**0.0001**
	luminal B like vs. luminal A like	3.31	1.68–6.52	0.034
	HER2 type vs. luminal A like	3.57	1.87–6.83	0.012
	triple negative vs. luminal A like	15.97	8.66–29.45	<0.0001
BCSS	Tumor size			<**0.0001**
	pT2 vs. pT1	1.93	1.41–2.64	<0.0001
	pT3/pT4 vs. pT1	2.39	1.54–3.79	<0.001
	Nodal stage			<**0.0001**
	pN1 vs. pN0	1.92	1.35–2.75	<0.001
	pN2 vs. pN0	3.00	1.99–4.52	<0.0001
	pN3 vs. pN0	8.57	5.61–13.07	<0.0001
	Biological subtype			<**0.0001**
	luminal B like vs. luminal A like	1.94	0.67–5.61	0.220
	HER2 type vs. luminal A like	2.34	0.86–6.39	0.096
	triple negative vs. luminal A like	31.65	13.19–75.93	<0.0001

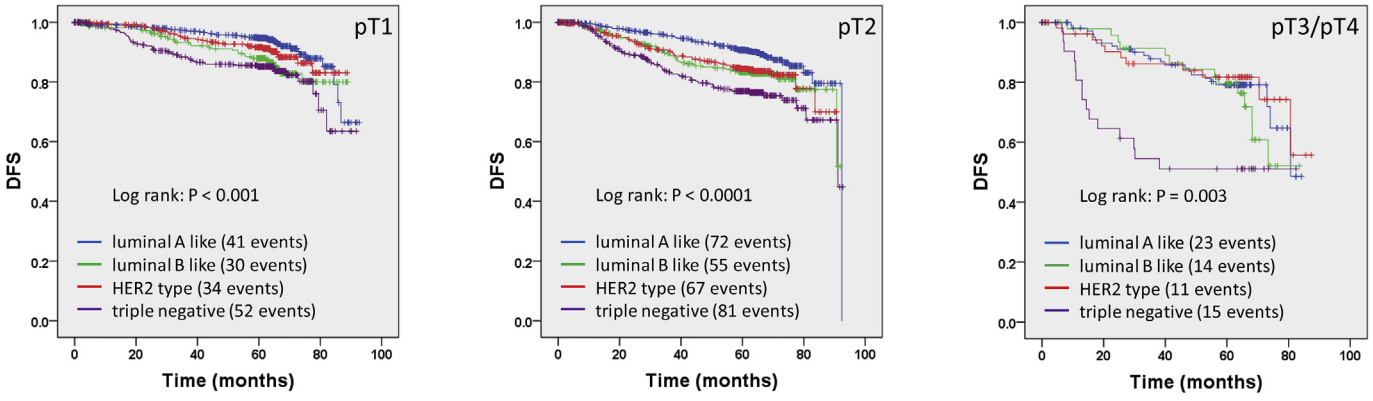

Fig. 3. Kaplan–Meier plots of disease-free survival (DFS) according to biological subtype for tumor size pT1, pT2, and pT3/pT4. P values refer to log-rank tests. For numbers of patients in the different biological subtype by tumor size categories see Table 2. There was no significant interaction between biological subtype and tumor size (p = 0.580).

patients were comprehensively evaluated. Limitations of our study are the fact that histopathology and immunohistochemistry were not reviewed centrally, the lack of information on Ki-67 and the inconsistent treatment of HER2 positive patients with trastuzumab or lapatinib. Furthermore, we did not validate our clinicopathological classification of biological subtypes with a gene expression

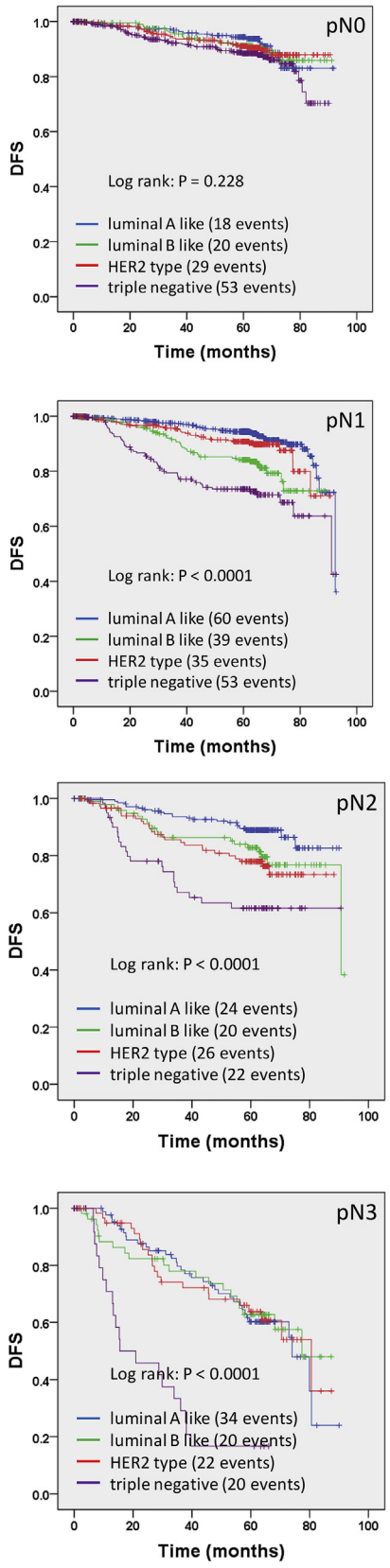

Fig. 4. Kaplan–Meier plots of disease-free survival (DFS) according to biological subtype for nodal stage pN0, pN1, pN2, and pN3. P values refer to log-rank tests. For numbers of patients in the different biological subtype by nodal stage categories see Table 2. There was a significant interaction between biological subtype and nodal stage ($p = 0.003$).

test. However, recent studies showed considerable discordance with regard to risk classification of individual patients among different gene expression tests [25]. Recently, the Panel of the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer in 2017 acknowledged that classification of tumor subtypes based on routine histopathology is clinically valuable and could be used to inform adjuvant treatment decisions [26]. Thus, we are confident that our clinicopathological classification of biological subtypes into luminal A like, luminal B like, HER2 type and triple negative tumors is valid and clinically justified.

5. Conclusions

In conclusion, this single-study analysis of 3754 patients demonstrated that in this high-risk collective of breast cancer patients those with a TNBC and a positive nodal stage had by far the worst prognosis. On the other hand, nodal negative TNBC patients had the same favorable outcome as nodal-negative patients with tumors of other biological subtypes. This underscores both the importance of early detection particularly in TNBC patients and the urgent need for more research to better understand the biological features of this unfavorable subtype in order to develop targeted therapeutic options.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2018.12.008.

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