

## 4-IHC classification of breast cancer subtypes in a large cohort of a clinical cancer registry: use in clinical routine for therapeutic decisions and its effect on survival

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**Abstract** The aim of the present study was to evaluate to what extent the combination of standard histopathological parameters determines the biology of breast cancer and the effect on therapy and prognosis. The Clinical Cancer Registry Regensburg (Bavaria, Germany) included  $n = 4,480$  female patients with primary, non-metastatic (M0) invasive breast cancer diagnosed between 2000 and 2012. Immuno-histochemical analyses, i.e., estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 (4-IHC), defined the tumor biological subtypes Luminal A, Luminal B, HER2-like, and Basal-like. Subtype-related differences in therapies and overall survival (OS) were analyzed using multivariable statistical methods. 4344 patients (97.0 %) could be classified into the four common tumor biological subtypes. The two most frequent entities were Luminal A (48.4 %), Luminal B (24.8 %), HER2-like (17.8 %), and Basal-like subtype (9.0 %). A multivariable Cox regression model showed that the best 7-year OS was seen in Luminal A patients and that OS of Luminal B and HER2-like patients was comparable (HR = 1.59,  $P < 0.001$  versus HR = 1.51,  $P = 0.03$ ). Lowest OS was seen in patients with Basal-like tumors (HR = 2.18,  $P < 0.001$ ). In conclusion, the classification of tumor

biological subtypes by the ER, PR, HER2, and Ki-67 biomarkers is practical in routine clinical work. Providing that quality assurance of these markers is ensured, this classification is useful for making therapy decisions in the routine clinical management of breast cancer patients.

**Keywords** 4-IHC · Tumor biological subtypes · Breast cancer · Cancer registry · Overall survival

### Introduction

Based on the identification of the human genome and gene expression analyses in breast cancer [1, 2], more detailed information about the biology of tumors has been detected in the last 15 years. The respective molecular taxonomy describes breast cancer subtypes whose clinical usefulness is critically discussed. Even though gene expression profiling is commercially available to analyze tumor characteristics, this method is not likely to be widely adopted into routine diagnostics at present because of high costs and lack of evidence from prospective trials.

In the recent years, a number of multigene tests for risk assessment in early breast cancer have been developed including different proliferation-related genes to optimize treatment and avoid unnecessary chemotherapy (CHT). Two large ongoing prospective randomized multicenter studies, called TAILORx (Trial for Assigning Individualized Options for Treatment Rx) [3] using Oncotype DX<sup>®</sup> [4] and MINDACT (Microarray in Node-Negative Disease May Avoid ChemoTherapy) [5] using Mammaprint<sup>®</sup> [6] address the clinical importance of these multigene expression assays. The overall objective is to reveal the benefit of CHT in addition to endocrine therapy (ET) in node-negative early

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breast cancer patients [7]. However, data from these prospective randomized studies are not available yet. Nevertheless, tumor biological factors like estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 are routinely assessed with appropriate quality control. Therefore, the use of these molecular parameters in combination with grading is proposed in order to achieve an equivalent classification. In the original work published by Perou et al. in 2000, he revealed that histopathological parameters correlate with the respective genetic profile [8].

Recent studies were able to demonstrate that a prognostic model—the 4-IHC score—using ER, PR, HER2, and Ki-67 provides similar prognostic information to that in the 21-gene Genomic Health recurrence score [9].

The aim of the present study was to evaluate to what extent well-established histopathological parameters approximately reflect the biology of breast cancer in routine care. By means of immuno-histochemical analyses, i.e., ER, PR, HER2, and Ki-67 (4-IHC), we investigated if the common tumor biological subtypes Luminal A, Luminal B, HER2-like, and Basal-like can be defined. Moreover, we analyzed the subtype-related overall survival (OS) in a large cohort of a clinical cancer registry.

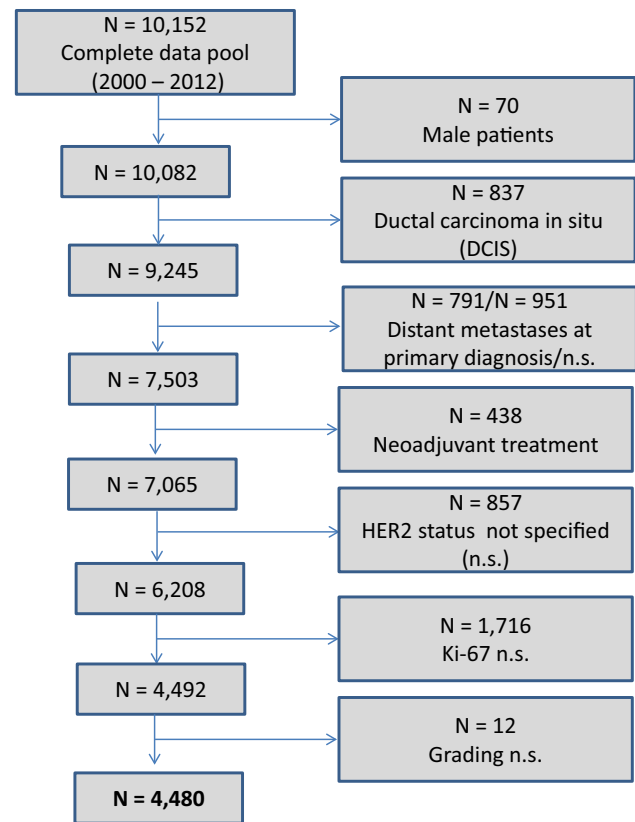
## Materials and methods

### Database

In the current study data from the Tumor Centre Regensburg (Bavaria, Germany) were analyzed. This high-quality population-based regional cancer registry was founded in 1991 and covers a population of more than 2.2 million people of Upper Palatinate and Lower Bavaria. Currently, the follow-up data of 241,250 patients are available. Following a stringent protocol, this cancer registry obtains a cross-sectorial documentation of all breast cancer patients in the area. Information about diagnosis, course of disease, therapies, and long-term follow-up are documented. Patient data originate from the University Hospital Regensburg, 53 regional hospitals, and more than 1000 practicing doctors. On the basis of medical reports, pathology, and follow-up records, the population-based data were routinely documented in each case [10].

### Patients' inclusion and exclusion criteria

The current analysis includes all female patients of the cancer registry with primary, non-metastatic (M0) invasive breast cancer diagnosed between January 2000 and December 2012 (13 years). Exclusion criteria were male



**Fig. 1** Scheme of data extraction

patients, ductal carcinoma in situ (DCIS), and neoadjuvant treatment. Schema of data extraction is presented in Fig. 1.

Immuno-histochemical determination of 4-IHC was performed consistent with defined standards as described in detail in previous studies of our group [10–12].

### Statistical analyses

Continuous data were expressed as means  $\pm$  standard deviations (SD) and categorical data as frequency counts and percentages. OS was calculated from the date of cancer diagnosis to the date of death from any cause. Patients who were not dead or patients without follow-up were classified as censored. The impact of subtypes on OS was assessed by means of a multivariable Cox regression analysis. Hazard ratios (HR) and corresponding 95 % confidence intervals (CI) were calculated and considered statistically significant if CI excluded 1.0. All reported *p* values were two-sided, and a *p* value of 0.05 was considered the threshold of statistical significance. Calculations were made with the software packages SPSS 22 (Chicago, EUA) and R (version 3.0.3).

## Results

### Analysis of patients characteristics

According to the ICD-10 classification, 7065 female patients with invasive, non-metastatic breast cancer (C50) were extracted from the total data pool of breast cancer patients (Fig. 1). In terms of the tumor biological classification, the following histopathological parameters are essential: ER- and PR-status, grading, HER2, and Ki-67 (4-IHC). Therefore, all patients with accordingly missing values of these parameters were excluded from further analysis. Thus, a total of 4480 breast cancer patients were included in the following analyses.

982 patients (21.9 %) were premenopausal and 3498 patients (78.1 %) were postmenopausal. Mean age was 62 years (median: 62 years, range: 25–97 years). More than 55 % had node-negative pT1 tumors. The most common histological type was invasive ductal carcinoma ( $n = 3588/80.1\%$ ). Detailed information of the distribution of the classical histological parameters is given in

Table 1. Moreover, parameters of tumor biological subtypes were further investigated (Table 2). 3896 patients (87.0 %) were ER- and/or PR-positive. 82.7 % ( $n = 3706$ ) were HER2-negative. The most common type of grading was intermediate (G2) both in premenopausal ( $n = 517/52.6\%$ ) and in postmenopausal patients ( $n = 2051/58.6\%$ ). The majority of cases ( $n = 2565/57.3\%$ ) belonged to the first category of Ki-67 (Ki-67 0–15 %).

### Classification of tumor biological subtypes

4344 patients (97.0 %) out of 4480 patients could be designated to the four common tumor biological subtypes. Selection criteria for classification of subtypes are shown in Table 3 according to the 2011 St Gallen Consensus Conference [13] and a modification of the original classification by Perou et al. [8]. The most common subtype was Luminal A ( $n = 2102/48.4\%$ ). Luminal B was the second most frequent entity ( $n = 1078/24.8\%$ ). The distinguishing mark between the hormone receptor (HR) positive Luminal A and Luminal B was the Ki-67 cut-off point of

**Table 1** Classical histopathological parameters

Parameter	Premenopausal ( $n = 982, 21.9\%$ )	Postmenopausal ( $n = 3498, 78.1\%$ )	Total ( $n = 4480, 100\%$ )
Age (year), mean $\pm$ SD	44 $\pm$ 6	66 $\pm$ 10	62 $\pm$ 13
Tumor size, $n$ (%)			
pT1	596 (60.7 %)	1875 (53.6 %)	2471 (55.2 %)
pT2	341 (34.7 %)	1298 (37.1 %)	1639 (36.6 %)
pT3	36 (3.7 %)	130 (3.7 %)	166 (3.7 %)
pT4	6 (0.6 %)	179 (5.1 %)	185 (4.1 %)
Unknown	3 (0.3 %)	16 (0.5 %)	19 (0.4 %)
Nodal status, $n$ (%)			
pN0	594 (60.5 %)	2173 (62.1 %)	2767 (61.8 %)
pN1	247 (25.2 %)	836 (23.9 %)	1083 (24.2 %)
pN2	94 (9.6 %)	231 (6.6 %)	325 (7.3 %)
pN3	40 (4.1 %)	188 (5.4 %)	228 (5.1 %)
Unknown	7 (0.7 %)	70 (2.0 %)	77 (1.7 %)
Lymphatic invasion, $n$ (%)			
Positive	323 (32.9 %)	1040 (29.7 %)	1363 (30.4 %)
Negative	526 (53.6 %)	1954 (55.9 %)	2480 (55.4 %)
Unknown	133 (13.5 %)	504 (14.4 %)	637 (14.2 %)
Vascular invasion, $n$ (%)			
Positive	58 (5.9 %)	203 (5.8 %)	261 (5.8 %)
Negative	767 (78.1 %)	2696 (77.1 %)	3463 (77.3 %)
Unknown	157 (16.0 %)	599 (17.1 %)	756 (16.9 %)
Histology, $n$ (%)			
Ductal	804 (81.9 %)	2784 (79.6 %)	3588 (80.1 %)
Lobular	103 (10.5 %)	468 (13.4 %)	571 (12.7 %)
Other	75 (7.6 %)	246 (7.0 %)	321 (7.2 %)

**Table 2** Parameters for subtypes

Parameter	Premenopausal ( <i>n</i> = 982, 21.9 %)	Postmenopausal ( <i>n</i> = 3498, 78.1 %)	Total ( <i>n</i> = 4480, 100 %)
Estrogen receptor, <i>n</i> (%)			
Positive	802 (81.7 %)	3037 (86.8 %)	3839 (85.7 %)
Negative	180 (18.3 %)	461 (13.2 %)	641 (14.3 %)
Progesterone receptor, <i>n</i> (%)			
Positive	758 (77.2 %)	2690 (76.9 %)	3448 (77.0 %)
Negative	224 (22.8 %)	808 (23.1 %)	1032 (23.0 %)
Receptor status, <i>n</i> (%)			
ER+PR+	742 (75.6 %)	2649 (75.7 %)	3391 (75.7 %)
ER+PR−	60 (6.1 %)	388 (11.1 %)	448 (10.0 %)
ER−PR+	16 (1.6 %)	41 (1.2 %)	57 (1.3 %)
ER−PR−	164 (16.7 %)	420 (12.0 %)	584 (13.0 %)
Grading, <i>n</i> (%)			
G1	159 (16.2 %)	602 (17.2 %)	761 (17.0 %)
G2	517 (52.6 %)	2051 (58.6 %)	2568 (57.3 %)
G3	306 (31.2 %)	845 (24.2 %)	1151 (25.7 %)
HER2 status, <i>n</i> (%)			
Positive	206 (21.0 %)	568 (16.2 %)	774 (17.3 %)
Negative	776 (79.0 %)	2930 (83.8 %)	3706 (82.7 %)
Ki-67 categories (%), <i>n</i> (%)			
0–15	469 (47.8 %)	2096 (59.9 %)	2565 (57.3 %)
16–25	196 (20.0 %)	640 (18.3 %)	836 (18.7 %)
26–35	112 (11.4 %)	332 (9.5 %)	444 (9.9 %)
36–45	59 (6.0 %)	151 (4.3 %)	210 (4.7 %)
>45	146 (14.9 %)	279 (8.0 %)	425 (9.5 %)

**Table 3** Classification of subtypes (*n* = 4344 patients)

Luminal A ( <i>n</i> = 2102, 48.4 %)	Luminal B ( <i>n</i> = 1078, 24.8 %)	HER2-like ( <i>n</i> = 774, 17.8 %)	Basal-like ( <i>n</i> = 390, 9.0 %)
ER+PR+	ER+PR+	ER+PR+	ER−PR−
ER+PR−	ER+PR−	ER+PR−	
ER−PR+	ER−PR+	ER−PR+	
		ER−PR−	
G1	G1	G1	G1
G2	G2	G2	G2
	G3	G3	G3
HER2−	HER2−	HER2+	HER2−
Ki-67 ≤15 %	Ki-67: >15 %	Any Ki-67	Any Ki-67

15 % and the permission of G3 tumors in the Luminal B group. The HER2-like subtype was characterized by positive HER2-status and any HR status, any grading as well as any Ki-67. 774 patients (17.8 %) referred to this subtype. The basal-like subtype was scarce (*n* = 390/9.0 %). It is defined by a triple-negative status, i.e., absence of ER, PR, and HER2 as well as any grading and any Ki-67.

### Histopathological characteristics in different subtypes

The different histopathological characteristics of the four subtypes are shown in Table 4. Luminal A patients were older and had smaller predominantly pT1, node-negative, and low-grade tumors. Patients in the HER2-like and

**Table 4** Histopathological characteristics in different subtypes

	Luminal A ( <i>n</i> = 2102)	Luminal B ( <i>n</i> = 1078)	HER2-like ( <i>n</i> = 774)	Basal-like ( <i>n</i> = 390)
Age (year) mean, median (range)	63, 63 (25–94)	61, 62 (29–97)	60, 60 (25–96)	58, 57 (27–94)
Menopausal state, <i>n</i> (%)				
Premenopausal	382 (18.2 %)	259 (24.0 %)	206 (26.6 %)	110 (28.2 %)
Postmenopausal	1720 (81.8 %)	819 (76.0 %)	568 (73.4 %)	280 (71.8 %)
Tumor size, <i>n</i> (%)				
pT1	1366 (65.0 %)	517 (48.0 %)	360 (46.5 %)	175 (44.9 %)
pT2	594 (28.3 %)	457 (42.4 %)	345 (44.6 %)	182 (46.7 %)
pT3	62 (2.9 %)	46 (4.3 %)	30 (3.9 %)	16 (4.1 %)
pT4	73 (3.5 %)	54 (5.0 %)	34 (4.4 %)	16 (4.1 %)
Unknown	7 (0.3 %)	4 (0.4 %)	5 (0.6 %)	1 (0.3 %)
Nodal status, <i>n</i> (%)				
pN0	1427 (67.9 %)	609 (56.5 %)	405 (52.3 %)	257 (65.9 %)
pN1	480 (22.8 %)	282 (26.2 %)	202 (26.1 %)	81 (20.8 %)
pN2	101 (4.8 %)	103 (9.6 %)	78 (10.1 %)	27 (6.9 %)
pN3	60 (2.9 %)	67 (6.2 %)	73 (9.4 %)	17 (4.4 %)
Unknown	34 (1.6 %)	17 (1.6 %)	16 (2.1 %)	8 (2.1 %)
Grading, <i>n</i> (%)				
G1	625 (29.7 %)	80 (7.4 %)	52 (6.7 %)	4 (1.0 %)
G2	1477 (70.3 %)	635 (58.9 %)	367 (47.4 %)	89 (22.8 %)
G3	–	363 (33.7 %)	355 (45.9 %)	297 (76.2 %)
Lymphatic invasion, <i>n</i> (%)				
Positive	436 (20.7 %)	402 (37.3 %)	332 (42.9 %)	135 (34.6 %)
Negative	1399 (66.6 %)	507 (47.0 %)	315 (40.7 %)	205 (52.6 %)
Unknown	267 (12.7 %)	169 (15.7 %)	127 (16.4 %)	50 (12.8 %)
Vascular invasion, <i>n</i> (%)				
Positive	54 (2.6 %)	82 (7.6 %)	72 (9.3 %)	35 (9.0 %)
Negative	1763 (83.9 %)	781 (72.4 %)	542 (70.0 %)	287 (73.6 %)
Unknown	285 (13.6 %)	215 (19.9 %)	72 (9.3 %)	68 (17.4 %)
Histology, <i>n</i> (%)				
Ductal	1541 (73.3 %)	936 (86.8 %)	670 (86.6 %)	325 (83.3 %)
Lobular	374 (17.8 %)	103 (9.6 %)	62 (8.0 %)	15 (3.8 %)
Other	187 (8.9 %)	39 (3.6 %)	42 (5.4 %)	50 (12.8 %)

Basal-like subgroups tended to be younger with larger, node-positive, and high-grade tumors. In the Luminal B subgroup, there were more pT2 tumors (42.4 %) than in the Luminal A group (28.3 %), a considerable percentage of high-grade tumors (33.7 %), and a lymph node involvement that was comparable to that of the HER2-like and Basal-like groups. Also the lymphatic and vascular invasion was notably higher in Luminal B than in Luminal A tumors.

### Survival analyses of different subtypes

To further analyze the four subtypes we compared their overall survival (OS) rate (Table 5). Premenopausal

patients generally had better survival rates than postmenopausal patients (Tables 6, 7). Best OS was found in Luminal A tumors both in premenopausal and in postmenopausal patients (7-year OS rate of 97.7 % in premenopausal patients versus 85.3 % in postmenopausal patients). OS rates of Luminal B tumors and HER2-like tumors were comparable, whereas premenopausal patients clearly had a survival benefit. Premenopausal Luminal B patients had a 7-year OS rate of 92.4 % compared to 76.3 % of postmenopausal patients. In HER2-like patients, 7-year OS rate of premenopausal patients was 88.8 versus 78.4 % in postmenopausal patients. The lowest OS was found in the Basal-like subtype both in premenopausal and in postmenopausal patients (7-year OS rate of 86.9 % in

**Table 5** Overall survival of patients within different subtypes

	3-y-OS (%)	5-y-OS (%)	6-y-OS (%)	7-y-OS (%)
Luminal A ( <i>n</i> = 2,102, 48.4 %)	95.7	91.8	90.0	87.5
Luminal B ( <i>n</i> = 1,078, 24.8 %)	92.6	86.2	82.7	80.3
HER2-like ( <i>n</i> = 774, 17.8 %)	91.4	86.3	82.2	81.0
Basal-like ( <i>n</i> = 390, 9.0 %)	85.2	81.5	81.5	79.6

**Table 6** Overall survival of premenopausal patients with different subtypes of breast cancer

	3-y-OS (%)	5-y-OS (%)	6-y-OS (%)	7-y-OS (%)
Premenopausal ( <i>n</i> = 957)				
Luminal A ( <i>n</i> = 382, 39.9 %)	98.7	98.7	98.7	97.7
Luminal B ( <i>n</i> = 259, 27.1 %)	98.7	96.4	94.1	92.4
HER2-like ( <i>n</i> = 206, 21.5 %)	95.2	93.5	89.9	88.8
Basal-like ( <i>n</i> = 110, 11.5 %)	88.1	86.9	86.9	86.9

**Table 7** Overall survival of postmenopausal patients with different subtypes of breast cancer

	3-y-OS (%)	5-y-OS (%)	6-y-OS (%)	7-y-OS (%)
Postmenopausal ( <i>n</i> = 3,387)				
Luminal A ( <i>n</i> = 1,720, 50.8 %)	95.0	90.3	88.1	85.3
Luminal B ( <i>n</i> = 819, 24.2 %)	90.6	82.9	79.0	76.3
HER2-like ( <i>n</i> = 568, 16.8 %)	90.1	83.8	79.6	78.4
Basal-like ( <i>n</i> = 280, 8.3 %)	84.0	79.3	79.3	76.6

**Table 8** Overall survival based on subtype and systemic therapies

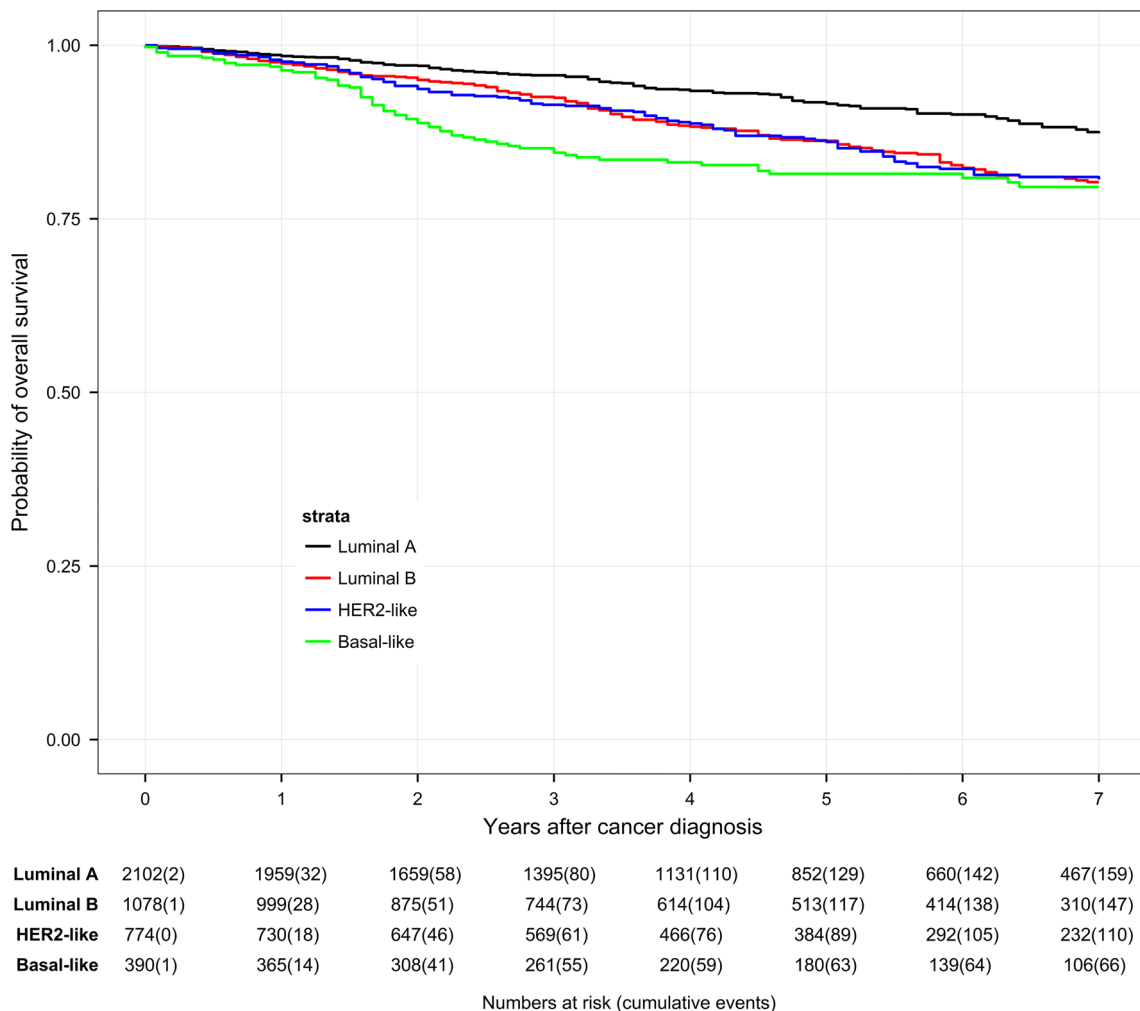
	3-year OS (%)	5-year OS (%)	6-year OS (%)	7-year OS (%)
Luminal A ( <i>n</i> = 2102)				
ET ( <i>n</i> = 1291, 61.4 %) → 97 events	96.7	92.3	90.8	88.3
CHT + ET ( <i>n</i> = 489, 23.3 %) → 28 events	98.7	97.1	96.2	95.1
CHT ( <i>n</i> = 52, 2.5 %) → 5 events	94.2	90.5	86.2	86.2
Other ( <i>n</i> = 270, 12.8 %) → 54 events	82.8	76.1	70.3	61.5
Luminal B ( <i>n</i> = 1078)				
ET ( <i>n</i> = 454, 42.1 %) → 80 events	93.4	85.8	81.9	78.7
CHT + ET ( <i>n</i> = 440, 40.8 %) → 43 events	97.3	92.9	90.9	89.4
CHT ( <i>n</i> = 56, 5.2 %) → 9 events	89.9	82.9	78.3	78.3
Other ( <i>n</i> = 128, 11.9 %) → 44 events	71.7	61.2	51.9	46.8
HER2-like ( <i>n</i> = 774)				
ET + Trastuzumab ( <i>n</i> = 13, 1.7 %) → 0 events	100	100	100	100
ET ( <i>n</i> = 157, 20.3 %) → 26 events	93.3	88.2	82.1	82.1
CHT + ET ( <i>n</i> = 113, 14.6 %) → 14 events	98.2	95.1	89.7	88.6
CHT + ET + Trastuzumab ( <i>n</i> = 193, 24.9 %) → 7 events	97.5	95.4	95.4	93.3
CHT + Trastuzumab ( <i>n</i> = 107, 13.8 %) → 8 events	96.1	94.6	90.7	87.2
CHT ( <i>n</i> = 73, 9.4 %) → 21 events	80.1	71.7	71.7	71.7
Other ( <i>n</i> = 118, 15.2 %) → 43 events	71.9	57.3	48.8	46.5
Basal-like ( <i>n</i> = 390)				
ET ( <i>n</i> = 3, 0.8 %) → 0 events	–	–	–	–
CHT + ET ( <i>n</i> = 13, 3.3 %) → 1 event	92.3	92.3	92.3	92.3
CHT + Trastuzumab ( <i>n</i> = 2, 0.5 %) → 1 event	100	50	–	–
CHT ( <i>n</i> = 270, 69.2 %) → 34 events	90.7	86.5	86.5	85.6
Other ( <i>n</i> = 102, 26.2 %) → 33 events	67.6	65.9	65.9	59.9

premenopausal patients versus 76.6 % in postmenopausal patients).

**Analysis of systemic therapies in different subtypes**

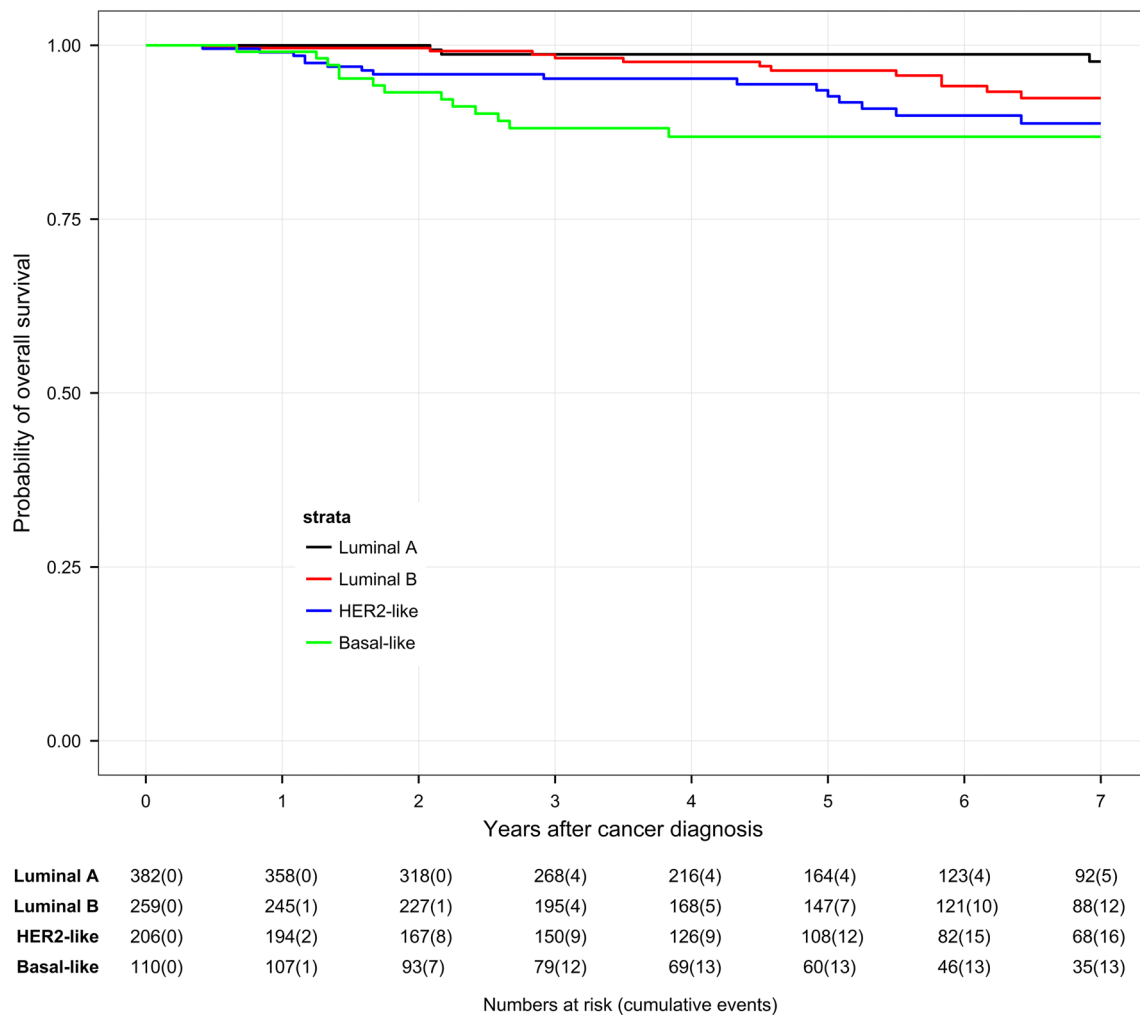
Depending on various systemic therapies, OS rates within the particular subgroups differed remarkably (Table 8). The application of CHT led to improved survival rates in every subgroup. The best OS was found in Luminal A patients receiving CHT plus ET with a 7-year OS rate of 95.1 %. Depriving Luminal A and Luminal B patients ET and only administering CHT caused lower OS rates in both subgroups (7-year OS of 86.2 % in Luminal A patients versus 78.3 % in Luminal B patients). The worse outcome of HER2-like patients was improved by the application of optimal treatment with CHT plus ET plus trastuzumab. 7-year OS rate of HER2-like patients

receiving this therapy was 93.3 %. The lowest OS of patients was found in all subgroups receiving no adjuvant therapy at all and other non-guideline-adherent therapy. The worst OS was analyzed in HER2-like (7-year OS of 46.5 %), Luminal B (7-year OS of 46.8 %), and Basal-like patients (7-year OS of 59.9 %). Even in Basal-like patients which received appropriate treatment with CHT and ET or only CHT led to acceptable 7-year OS rates. However, the low number of events in these groups has to be considered. A Cox regression model (Table 9) provided further evidence that the best OS was seen in Luminal A patients and that OS of Luminal B and HER2-like patients was comparable (HR = 1.59, 95 % CI 1.25–2.02, *P* < 0.001 versus HR = 1.51, 95 % CI 1.15–1.98, *P* = 0.03). The lowest OS was seen in patients with Basal-like tumors (HR = 2.18, 95 % CI 1.55–3.08, *P* < 0.001). Kaplan–Meier plots of OS in years based on subtypes are shown in Figs. 2, 3 and 4.



**Fig. 2** Kaplan-Meier plot of overall survival in years based on subtype





**Fig. 3** Kaplan-Meier plot of overall survival in years of premenopausal patients based on subtype

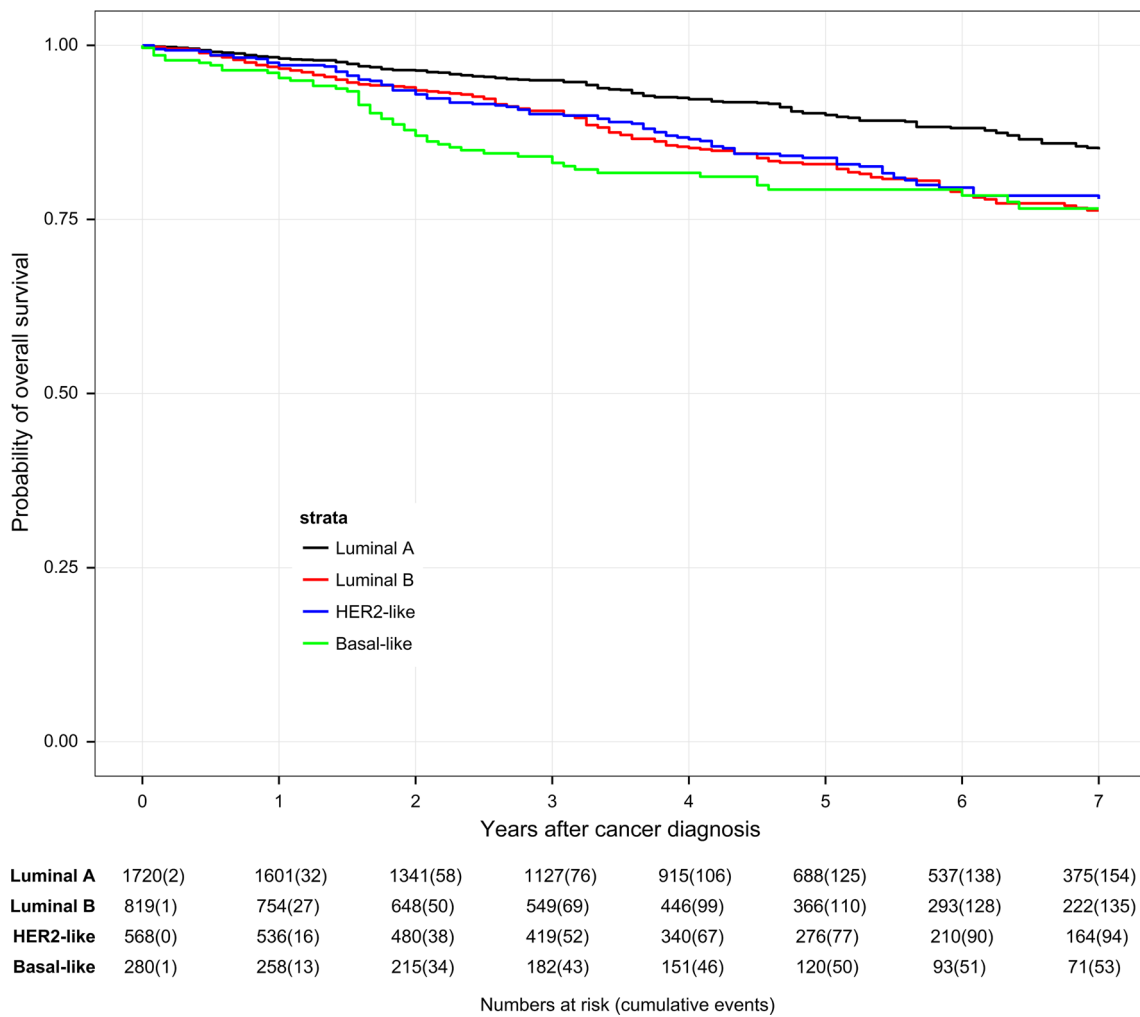
## Discussion

Currently, decisions on adjuvant systemic treatment of breast cancer patients are based mainly on histopathological criteria including tumor size, nodal status, grading, ER, PR, and HER-2 status. These parameters have been recommended both in national [14] and international guidelines [15]. In recent years, several microarray-based gene expression profiling studies have extended our understanding of the heterogeneity and complexity of breast cancer [8, 16, 17]. However, standardized definitions and methodologies for the identification of the molecular subtypes and prospective clinical trials to validate the contribution of these intrinsic subtypes in addition to the common clinical-pathological parameters are still missing [16]. Gene expression profiling is typically used complementarily rather than as a substitute for the traditional clinical-pathological parameters [18]. Thus, until now,

current gene expression signatures do not replace the classical parameters [19].

In the present study, we were able to get a suitable classification of tumor biological subtypes by modification of the original taxonomy suggested by Perou et al. [8] and the classification recommended by the 2011 St Gallen Consensus Conference [13]. This was achieved by analyzing data of a population-based regional cancer registry. By means of subtypes, we were able to classify patient groups that are treated as a function of tumor biology. These are Luminal A, Luminal B, HER2-like, and Basal-like [20]. A previous study of Cuzick et al. further analyzed the cohort of the “Arimidex, Tamoxifen, Alone or in Combination” (ATAC) trial which included 1,125 ER-positive patients who did not receive adjuvant CHT [9]. Distant recurrence was the primary endpoint. The prognostic 4-IHC score was calculated and assessed in a separate cohort of 786 patients. Within this trial, it could be





**Fig. 4** Kaplan-Meier plot of overall survival in years of postmenopausal patients based on subtype

**Table 9** Multivariable Cox proportional hazard model on overall survival

Subtypes	Univariable cox regression (n = 4344)			Multivariable cox regression <sup>a</sup> (n = 4258)		
	HR	95 % CI	P value	HR	95 % CI	P value
Luminal A	1			1		
Luminal B	1.72	1.40–2.11	<0.001	1.59	1.25–2.02	<0.001
HER2-like	1.57	1.25–2.00	<0.001	1.51	1.15–1.98	0.003
Basal-like	1.91	1.45–2.51	<0.001	2.18	1.55–3.08	<0.001

<sup>a</sup> Multivariable model is adjusted for age, menopausal state, tumor size, nodal status, grading, and histology

demonstrated that this 4-IHC score provided independent prognostic information in the presence of classical parameters. Remarkably, the prognostic information of the 4-IHC score was similar to that in the 21-gene Genomic Health recurrence score [9].

In the present study, 4344 (97.0 %) of 4480 patients could be attributed to the four common biological tumor

subtypes. The most frequently subtype was Luminal A with 48.4 % which is in accordance to other studies [21, 22]. However, the differentiation of Luminal A and B tumor subtypes mainly by the definition of the Ki-67 cut-off point is still problematic. Regarding Ki-67, we chose a cut-off point of 15 % which corresponds to the current St Gallen recommendations [15, 23] and to a previous study of our

group [11]. However, an increase of the Ki-67 cut-off to 20 % and above especially for the Luminal B subtype was critically discussed in the 13th St Gallen Consensus Conference [15] and other studies [24, 25] as Ki-67 seems to be a continuous value. Moreover, to date, no standard operating procedure for Ki-67 exists, and therefore, both the interlaboratory and the interstudy comparability of Ki-67 are still limited [11].

We did not include HER2-positive patients to further subdivide the Luminal B group. In the HER2 group, the positive HER2 status was decisively independent of the HR status as described in previous studies [26]. Patients with Luminal A tumors tend to be older, mainly have early stage breast cancer that shows a rather well/moderate differentiation. This observation corresponds with other studies [18, 27]. As expected, the Luminal A breast cancer patients showed the best OS with a 7-year OS of 87.5 % followed by those with Luminal B tumors with a 7-year OS rate of 80.3 %. HER2-like and Basal-like tumors had the poorest prognosis which correlates well with prior analyses [18, 21, 28]. Patients in these subgroups tended to be younger with larger, node-positive, and high-grade tumors. These facts have also been demonstrated by a publication of the American Cancer Society [29]. Remarkably, a long-term survival study of the Early Breast Cancer Trialists' Group (EBCTCG) has notified that the ER status loses its predictive significance and that the long-term outcome of ER-positive and ER-negative tumors is not differential [30]. A study from Hugh et al. showed that Luminal A tumors respond better to ET, while Luminal B tumors are more often resistant to this therapy and may benefit from combined ET and CHT [31]. Our study could confirm that patients with Luminal B tumors benefit from the addition of CHT to ET. Patients with Luminal B tumors receiving CHT and ET had a 7-year OS of 89.4 % compared to those patients receiving ET alone with a 7-year OS rate of 78.7 %. Notably, the effect of ET alone was comparable to the outcome of CHT alone. The 7-year OS rate of patients treated with CHT alone was 78.3 % (Table 8). Other subtypes showed a distinct benefit from the application of CHT as well. Patients with Luminal A tumors receiving CHT plus ET had a 7-year OS of 95.1 %. As discussed above, these patients benefit more from ET than from CHT if only one of these therapies is used. The 7-year OS of patients receiving only ET was 88.3 % compared to 86.2 % of patients receiving only CHT. A 7-year retrospective study by Onitilo et al. investigated 1,134 patients with invasive breast cancer and compared survival rates of the four subtypes [27]. Among the 781 patients with ER-positive/PR-positive/HER2-negative subtype, i.e., Luminal A, 257 patients (32.9 %) received CHT, whereas 524 patients (67.1 %) received no CHT. Those patients treated with CHT had significantly better disease-free survival

(DFS) and OS compared to patients who did not receive CHT [27]. However, a considerable number of patients received no adjuvant therapy at all or other non-guideline adherent treatment. These patients had the worst OS rates in all subtypes in accordance to previous studies. A longitudinal study of breast cancer patients reported to the Metropolitan Detroit and Los Angeles SEER cancer registries showed that of the 743 patients eligible for ET, 10.8 % never initiated therapy and 15.1 % started therapy but discontinued prematurely [32].

In conclusion, the classification of tumor biological subtypes by the ER, PR, HER2, and Ki-67 biomarkers (4-IHC) is practical, simple, quite discriminative, informative, and—most importantly—clinically useful. Hence, a standardized and reproducible assessment of these markers is exceedingly efficient for making a therapy decision in the routine clinical management of breast cancer. As the resources in the worldwide health care system are finite, the search for the best possible criteria of analysis is indispensable to optimize cost-benefit ratio. The present study showed that standard histopathological parameters are able to determine the biology of breast cancer and the effect on therapy and prognosis.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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