



Characterization of estrogen receptor-low-positive breast cancer

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Abstract

Purpose The biology of breast cancer with a low expression level (1–10%) of estrogen receptor (ER) remains a matter of confusion. The recent American Society of Oncology/College of American Pathologist Guidelines have recommended reporting such tumors as a new “ER-low-positive” category with a recommended comment to emphasize the possible overall benefit of endocrine therapies in these patients. The aim of the study was to analyze the clinicopathologic features and clinical outcomes of ER-low-positive breast cancers.

Methods We characterized the clinicopathologic features and survival outcomes of ER-low-positive breast cancers in our 4179 patients diagnosed from 1998 to 2018.

Results The ER-positive, ER-low-positive, and ER-negative cases in our cohort were 2982 (71.4%), 97 (2.3%), and 1100 (26.3%), respectively. ER-low-positive tumors showed similar clinicopathologic characteristics yet significantly superior prognosis when compared to ER-negative tumors, while demonstrated largely overlapping survival outcomes with ER-positive tumors in the entire cohort. In the subcohort of tumors with a PR-positive phenotype, the prognosis of ER-low-positive tumors was intermediate between that of the ER-positive and ER-negative groups. ER-low-positive/PR-positive tumors had a significantly worse prognosis than ER-positive tumors, and a trend toward favorable survival outcomes when compared to ER-negative tumors, although no significant difference was identified for the latter. In contrast, the ER-positive and ER-low-positive groups showed similar survival outcomes in the subset of tumors with a PR-negative status, both being significantly better than ER-negative tumors.

Conclusions PR status as a surrogate marker of functional ER signaling provides critical information in this regard. These findings suggest that while ER-low-positive tumors are themselves heterogeneous, they often respond to endocrine treatment. Analysis of molecular signatures and standardization of therapeutic strategies are important to understand the biology of ER-low-positive tumors and to enable optimal treatment in the pursuit of individualized medicine.

Keywords Breast cancer · Estrogen receptor · Progesterone receptor · Estrogen receptor-low-positive · Survival

Introduction

Estrogen receptor (ER) signaling plays a crucial role in mammary carcinogenesis and breast cancer progression. ER and its closely related molecule, progesterone receptor (PR), are important prognostic factors and the essential predictive markers for endocrine therapies [1–4]. Approximately 78%

of invasive breast cancers are ER-positive, and this rate is projected to increase 0.75% per year in the United States [5]. ER-targeted therapies have significantly improved the clinical outcomes in patients with ER-positive breast cancers, and the therapeutic effects are correlated with the ER levels in tumor cells [6–8]. Thus, precise assessment of ER status is pivotal to predict whether a patient may benefit from endocrine therapy.

The cutoff value for ER positivity in breast cancer, however, has long been controversial. A 10% cutoff for ER positivity was widely used in early practice, although the cutoffs varied from 1 to 20% among studies from different countries/regions [9]. With advancements in immunohistochemical techniques and the introduction of new highly sensitive antibodies, the 2010 American Society of Oncology

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(ASCO)/College of American Pathologist (CAP) Guidelines recommended that ER status was considered positive if only 1% of tumor cells demonstrated positive nuclear reactivity by immunohistochemistry [10].

With regard to ER pathway activation, however, the biology of breast cancer with low ER expression remained a matter of controversy. Approximately 2–5% of ER-positive cancers reportedly demonstrate only between 1 and 10% of their tumor cells staining for ER [11–14]. A number of studies have shown that these tumors are a heterogeneous group, and at least a subset have pathologic features, molecular characteristics and clinical outcomes more similar to those tumors that lack ER expression [12–17]. Thus, some authorities have suggested that 1% may not be the ideal cutoff for ER positivity in predicting response to endocrine therapy, especially in patients with ER-low-positive breast cancers. To that end, the 2020 ASCO/CAP Guidelines have recommended the reporting of ER, if 1–10% of tumor cell nuclei are immunoreactive, as a new category of ER-low-positive breast cancer with a recommended comment on the limited data currently available on the overall benefit of endocrine therapies in these patients [11]. In this study, we sought to characterize the clinicopathologic features and prognostic outcomes of ER-low-positive breast cancers in comparison to ER-negative and ER-positive (> 10%) tumors in a cohort of more than 4000 cases from a single institution.

Materials and methods

This study was conducted after the approval of the institutional review board of the University of Alabama at Birmingham. A search of the tumor registry at the authors' institution was performed to identify female patients diagnosed with invasive breast cancer between 1998 and 2018. The patients' demographic information (age at diagnosis and race), the pathologic characteristics of the primary tumor (histologic type and grade, ER, PR and HER2 status, pathologic tumor and nodal stages), and clinical outcomes were collected. The patients who did not receive systemic therapy and those who had missing treatment or follow-up data were excluded from the analysis. With regard to endocrine therapy in the 97 patients with ER-low-positive tumors, 29 received endocrine therapy (17 PR-positive and 12 PR-negative) and 63 did not (20 PR-positive and 43 PR-negative). The remaining 5 patients did not receive endocrine therapy at the authors' institution (all PR-negative); it was unclear if they received endocrine therapy at other institutions. The median follow-up time was 4.4 years.

Hormonal receptor testing was performed following the ASCO/CAP Guideline Recommendations. The cutoff value for ER and PR positivity was 10% prior to the 2010 ASCO/CAP Guideline Recommendations and revised to 1%

thereafter [10]. The pathology reports of the cases with a negative ER and/or PR status before 2010 were reviewed to record the percentage and intensity of the staining, and those either not documented or slides inaccessible were excluded from the study. HER2 overexpression or gene amplification status was determined by immunohistochemistry or in situ hybridization following the ASCO/CAP Guideline Recommendations [18, 19].

The categorical data obtained were analyzed by Chi-square testing, while continuous data were evaluated by using an independent *t*-test. Distant relapse-free survival (RFS; from the date of diagnosis to the date of distant recurrence) and disease-specific survival (DSS; from the date of diagnosis to the date of death from the disease) were calculated by Kaplan–Meier analysis. Patients who survived or were lost to follow-up were considered as censored data in the analysis. The Cox proportional hazards regression model was used to identify significant factors for RFS and DSS. A *P* value of less than 0.05 was considered statistically significant. All data were analyzed using IBM SPSS Statistics (Version 26) predictive analytics software.

Results

Clinicopathologic characteristics and prognostic outcomes of breast cancer patients stratified by ER status in the entire cohort

There were a total of 4179 patients meeting the inclusion criteria in this cohort. The mean and median ages at diagnosis were 57.8 and 58 years, respectively (range 18–99 years). The majority of the patients were Caucasians ($N = 3073$; 73.5%), followed by African Americans ($N = 1034$; 24.8%) and “others” ($N = 72$; 1.7%). The ER-positive, ER-low-positive and ER-negative cases were 2982 (71.4%), 97 (2.3%) and 1100 (26.3%), respectively. The clinical and pathologic features stratified by ER status are summarized in Table 1.

When compared to the patients with ER-positive cancers, those with ER-low-positive tumors were significantly younger (mean age 59.1 vs. 56.4 years, $P = 0.039$) and more likely to be African American (77.4% vs. 61.9%, $P = 0.001$). Further, the ER-low-positive tumors were more likely of a ductal phenotype (83.5% vs. 71.4%, $P = 0.005$), and higher histologic grades (Grade II/III 94.8% vs. 74.0%, $P < 0.001$). Furthermore, these tumors were significantly larger (mean size 27.6 mm vs. 21.7 mm, $P = 0.003$). Thus, it is not surprising that ER-low-positive tumors more frequently presented at advanced clinical stages (Stage II/III/IV 69.1% vs. 48.8%, $P = 0.001$). Moreover, these tumors were also less likely to express PR (84.0% vs. 38.1%, $P < 0.0001$) and more likely to be HER2-positive (28.9% vs 11.2%, $P < 0.0001$).

Table 1 Clinicopathologic characteristics of breast cancer cases stratified by ER status in the entire cohort

Clinicopathologic factor	ER status (N)			Total (n = 4179)	P value		
	ER-positive (n = 2982)	ER-low-positive (n = 97)	ER-negative (n = 1100)		ER-positive vs. ER-low-positive	ER-low-positive vs. ER-negative	ER-positive vs. ER-negative
Age							
Mean/median (range)	59.1/60 (18–99)	56.4/58 (31–90)	54.5/54.5 (22–95)	57.8/58 (18–99)	0.039	0.164	< 0.0001
Race							
Caucasian	2307	60	706	3073	0.001	0.809	< 0.0001
African American	621	34	379	1034			
Other	54	3	15	72			
Tumor type							
Ductal	2130	81	974	3185	0.005	0.242	< 0.0001
Lobular	817	14	118	949			
Other	35	2	8	45			
Histologic grade							
Grade I	744	4	16	764	< 0.0001	0.001	< 0.0001
Grade II	1553	24	198	1775			
Grade III	654	68	886	1608			
Unknown	31	1	0	32			
Mean tumor size (mm) (range)	21.7 (0.4–275)	27.6 (3–120)	31.0 (1–205)	24.3 (0.4–275)	0.003	0.234	< 0.0001
Pathologic nodal stage							
N0	1962	63	630	2655	0.683	0.205	< 0.0001
N1	645	25	270	940			
N2	153	4	69	226			
N3	69	1	54	124			
Unknown	153	4	77	234			
PR status							
Positive	2504	37	144	2685	< 0.0001	< 0.0001	< 0.0001
Negative	478	60	956	1494			
HER2 status							
Positive	335	28	281	644	< 0.0001	0.376	< 0.0001
Negative	2567	65	804	3436			
Equivocal	40	1	10	51			
Unknown	40	3	5	48			
Clinical stage							
I	1506	29	333	1868	0.001	0.279	< 0.0001
II	1014	50	483	1547			
III	271	11	159	441			
IV	169	6	122	297			
Unknown	22	1	3	26			

We next compared ER-low-positive and ER-negative tumors. To that end, the former was less likely to be of high histologic grade (Grade III 80.5% vs. 70.1%, $P = 0.001$) and more likely to be PR-positive (38.1% vs. 13.1%, $P < 0.0001$). Overall, ER-low-positive tumors demonstrated distinct clinical and pathologic characteristics when compared

to ER-positive cases, while the former exhibited largely overlapping features with ER-negative tumors that were drastically different from the ER-positive cancers.

To attest to the prognostic significance of breast cancers with low ER expression, we next analyzed the patients' survival outcomes based on the ER status. As expected,

ER-positive breast cancers were associated with significantly better RFS [hazard ratio (HR) 0.365 (0.307–0.434), $P < 0.0001$] and DSS [HR 0.192 (0.156–0.236), $P < 0.0001$] when compared to ER-negative tumors. The very same observations were also obtained when comparing ER-low-positive and ER-negative tumors [RFS: HR 0.407 (0.216–0.766), $P = 0.005$; DSS: HR 0.361 (0.185–0.703),

$P = 0.003$], as illustrated in Fig. 1. No significant difference for RFS or DSS was observed between ER-positive and ER-low-positive groups.

Multivariate analysis was next performed to identify significant prognostic factors in the entire cohort. As expected, high histologic grade, larger tumor size and positive lymph node status were independently associated with both RFS and DSS, as shown in Table 2. A lobular phenotype and HER2 positivity were associated with an inferior RFS, but were not independent factors for DSS. Importantly, ER-low-positive tumors had a significantly better RFS and DSS when compared to ER-negative tumors despite overlapping clinicopathologic features between the two groups. Interestingly, while race and PR status were not significant factors for RFS, they were both independently associated with DSS.

Clinical and pathological analyses of PR-positive breast cancers

Given that PR status was an independent prognostic factor for DSS, we next analyzed the clinicopathologic features of breast cancers further stratified by PR status. The clinicopathologic characteristics of the 2685 PR-positive cases are summarized in Table 3. When compared to ER-positive tumors in this subcohort, ER-low-positive tumors more frequently affected younger patients (mean age 59.0 vs. 54.5 years, $P = 0.034$), were more likely to be of higher histologic grades (Grade II/III 91.9% vs. 73.4%, $P < 0.0001$) and were HER2-positive (37.8% vs. 9.7%, $P < 0.0001$). Consequently, they more frequently presented at higher clinical stages (Stage II/III/IV 70.3% vs. 47.8%, $P = 0.049$).

When compared to ER-negative tumors, however, the ER-low-positive cases were more frequently of low histologic grade (Grade I 8.1% vs. 2.1%, $P = 0.017$). Overall, ER-low-positive tumors exhibited distinct clinicopathologic features

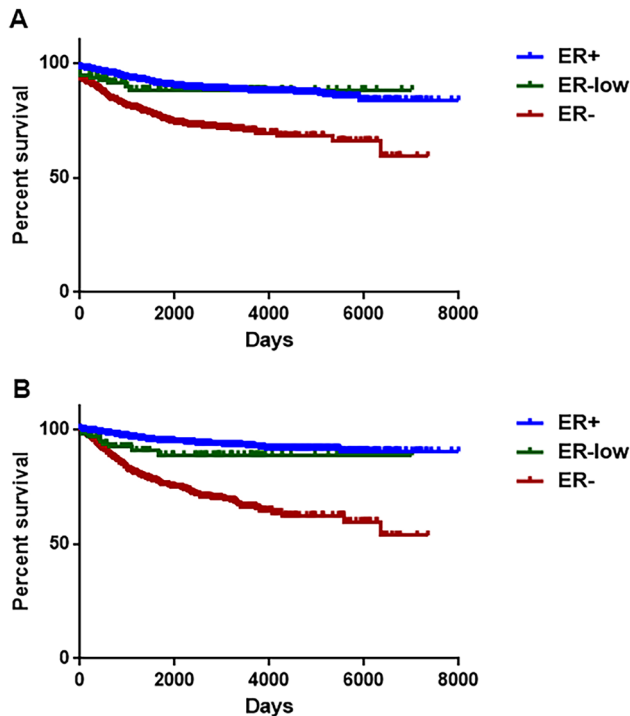


Fig. 1 Relapse-free survival (A, Chi-square 151.0, $P < 0.0001$) and disease-specific survival (B, Chi-square 304.8, $P < 0.0001$) of breast cancers in the entire cohort stratified by ER status. ER+ ER-positive, ER-low ER-low-positive, ER- ER-negative

Table 2 Multivariate analysis of clinicopathologic factors-associated prognostic outcomes in the entire cohort

Clinicopathologic factor	Relapse-free survival		Disease specific survival	
	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value
Age (> 58 vs. ≤ 58)	0.868 (0.723–1.042)	0.130	1.172 (0.948–1.449)	0.143
Race (Caucasian vs. African American)	0.926 (0.767–1.119)	0.426	0.688 (0.557–0.851)	0.001
Tumor type (lobular vs. ductal)	1.427 (1.142–1.784)	0.002	1.270 (0.957–1.686)	0.098
Histologic grade (III vs. I/II)	1.489 (1.211–1.832)	< 0.0001	1.870 (1.447–2.417)	< 0.0001
Tumor size (2.0–5.0 cm vs. ≤ 2.0 cm)	2.534 (1.917–3.350)	< 0.0001	1.749 (1.326–2.306)	< 0.0001
Tumor size (> 5.0 cm vs. ≤ 2.0 cm)	4.599 (3.387–6.245)	< 0.0001	2.532 (1.755–3.653)	< 0.0001
Nodal status (positive vs. negative)	4.716 (3.673–6.055)	< 0.0001	4.908 (3.714–6.487)	< 0.0001
ER-positive vs. ER-negative	0.609 (0.470–0.788)	< 0.0001	0.367 (0.270–0.497)	< 0.0001
ER-low-positive vs. ER-negative	0.424 (0.218–0.827)	0.012	0.487 (0.246–0.964)	0.039
PR (positive vs. negative)	0.839 (0.660–1.066)	0.150	0.691 (0.521–0.916)	0.01
HER2 (positive vs. negative)	1.372 (1.123–1.678)	0.002	1.010 (0.797–2.306)	0.936

HR hazard ration, CI confidence interval

Table 3 Clinicopathologic characteristics of PR-positive breast cancer cases stratified by ER status

Clinicopathologic factor	ER status (<i>N</i>)			Total (<i>n</i> = 2685)	<i>P</i> value		
	ER-positive (<i>n</i> = 2504)	ER-low-positive (<i>n</i> = 37)	ER-negative (<i>n</i> = 144)		ER-positive vs. ER-low-positive	ER-low-positive vs. ER-negative	ER-positive vs. ER-negative
Age							
Mean/median (range)	59/60 (18–99)	54.5/57 (31–87)	54.3/54.5 (28–93)	58.68/59 (18–99)	0.034	0.99	< 0.0001
Race							
Caucasian	1956	28	86	2070	0.836	0.142	< 0.0001
African American	514	8	55	577			
Other	34	1	3	38			
Tumor type							
Ductal	1786	30	121	1937	0.395	0.767	0.004
Lobular	695	7	22	724			
Other	23	0	1	24			
Histologic grade							
Grade I	652	3	3	658	< 0.0001	0.017	< 0.0001
Grade II	1312	13	31	1356			
Grade III	527	21	110	658			
Unknown	13	0	0	13			
Mean tumor size (mm) (range)	21.3 (1–160)	26.5 (4–73)	34.5 (3–150)	22.1 (1–160)	0.094	0.127	< 0.0001
Pathologic nodal stage							
N0	1664	24	79	1767	0.929	0.701	< 0.0001
N1	545	11	42	598			
N2	117	2	7	126			
N3	54	0	10	64			
Unknown	124	0	6	130			
HER2 status							
Positive	242	14	38	294	< 0.0001	0.163	< 0.0001
Negative	2231	23	103	2357			
Equivocal	29	0	3	32			
Unknown	2	0	0	2			
Clinical stage							
I	1298	11	40	1349	0.049	0.941	< 0.0001
II	838	19	69	926			
III	217	5	24	246			
IV	142	2	11	155			
Unknown	9	0	0	9			

from ER-positive cases but had similar characteristics when compared to ER-negative tumors in the subset of PR-positive cases, similar to the aforementioned observations in the entire cohort.

Survival analyses of this subcohort revealed a significantly superior DSS associated with ER-positive tumors when compared to ER-low-positive cases [HR 0.332 (0.122–0.901), $P = 0.031$], but not RFS [HR 0.525 (0.216–1.275), $P = 0.154$]. No significant difference for RFS [HR 1.401 (0.543–3.614), $P = 0.486$] or DSS [HR 1.582

(0.553–4.525), $P = 0.393$] was identified when comparing ER-negative and ER-low-positive groups, while a trend of inferior DSS in the former group was observed (Fig. 2).

However, multivariate analyses demonstrated significantly better survival outcomes for ER-positive tumors when compared to ER-low-positive cases for both RFS and DSS (Table 4). Again, no significant difference for RFS or DSS was identified between ER-negative and ER-low-positive tumors. High histologic grade, lobular phenotype, larger tumor size, positive nodal status and HER2

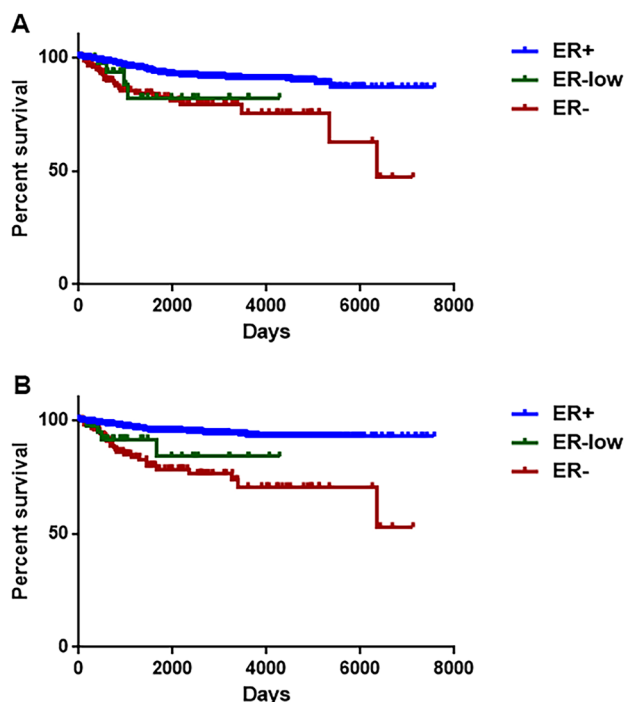


Fig. 2 Relapse-free survival (A, Chi-square 31.66, $P < 0.0001$) and disease-specific survival (B, Chi-square 65.59, $P < 0.0001$) of in the subset of patients with PR-positive breast cancers. ER+ ER-positive, ER-low ER-low-positive, ER- ER-negative

positivity were each independently associated with RFS, whereas only histologic grade, tumor size, and nodal status remained significant for DSS.

When comparing the clinical outcomes of ER-low-positive/PR-positive tumors, those with endocrine therapy were associated with a better RFS [HR 0.111 (0.015–0.800), $P = 0.03$] and DSS [HR 0.093 (0.012–0.696), $P = 0.02$] in this limited subset of cases, although a higher level of ER

was found in those receiving endocrine therapy (mean 9%, range 5–10% vs. mean 5%, range 1–10%; $P = 0.0001$).

Analyses of the subcohort of cases with a PR-negative phenotype

In the subcohort of 1494 cases with a PR-negative phenotype, ER-low-positive cancers affected African Americans more often than ER-positive tumors (55.2% vs. 23.4%, $P = 0.001$), and were more likely to be of higher histologic grades (Grade II/III 96.7% vs 77.0%, $P < 0.0001$). No significantly different clinicopathologic features were identified between ER-low-positive and ER-negative cases. Once again, the latter two groups showed similar clinicopathologic characteristics (Table 5).

When compared to those lacking ER expression, ER-positive tumors were associated with a significantly superior RFS [HR 0.509 (0.385–0.674), $P < 0.0001$] and DSS [HR 0.663 (0.463–0.951), $P = 0.025$], as expected (Fig. 3). ER-low-positive tumors also demonstrated a significantly better RFS when compared to the former [HR 0.311 (0.128–0.756), $P = 0.01$], while no significant difference was found for DSS [HR 0.649 (0.358–1.177), $P = 0.155$]. No substantial difference for RFS or DSS was identified between ER-positive and ER-low-positive cases.

Multivariate analyses revealed a significantly superior DSS associated with both ER-positive and ER-low-positive cancers when compared to ER-negative tumors, while the ER status was not associated with RFS (Table 6). HER2 status, pathologic tumor and nodal stages were each independently correlated with RFS, whereas race, histologic grade, and pathologic tumor and nodal stages were significant prognostic factors for DSS.

When comparing the clinical outcomes of the small subset of patients with ER-low-positive/PR-negative tumors who did or did not receive endocrine therapy, no significant

Table 4 Multivariate analysis of clinicopathologic factors-associated prognostic outcomes in PR-positive breast cancers

Clinicopathologic factor	Relapse-free survival		Disease specific survival	
	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value
Age (> 59 vs. ≤ 59)	0.984 (0.754–1.286)	0.908	1.077 (0.751–1.545)	0.687
Race (Caucasian vs. African American)	0.857 (0.643–1.142)	0.293	0.737 (0.507–1.073)	0.111
Tumor type (lobular vs. ductal)	1.871 (1.394–2.509)	< 0.0001	1.395 (0.925–2.105)	0.112
Histologic grade (III vs. I/II)	1.533 (1.154–2.037)	0.003	1.911 (1.295–2.820)	0.001
Tumor size (2.0–5.0 cm vs. ≤ 2.0 cm)	1.896 (1.323–2.718)	< 0.0001	2.055 (1.282–3.295)	0.003
Tumor size (> 5.0 cm vs. ≤ 2.0 cm)	2.666 (1.749–4.063)	< 0.0001	1.800 (0.978–3.315)	0.059
Nodal status (positive vs. negative)	4.817 (3.238–7.164)	< 0.0001	4.299 (2.588–7.140)	< 0.0001
ER-positive vs. ER-low-positive	0.365 (0.147–0.906)	0.030	0.299 (0.107–0.839)	0.022
ER-negative vs. ER-low-positive	0.752 (0.288–1.966)	0.561	1.043 (0.361–3.019)	0.938
HER2 (positive vs. negative)	1.565 (1.122–2.183)	0.008	1.223 (0.781–1.914)	0.379

HR hazard ration, CI confidence interval

Table 5 Clinicopathologic characteristics of PR-negative breast cancer cases stratified by ER status

Clinicopathologic factor	ER status (<i>N</i>)			Total (<i>n</i> = 1494)	<i>P</i> value		
	ER-positive (<i>n</i> = 478)	ER-low-positive (<i>n</i> = 60)	ER-negative (<i>n</i> = 956)		ER-positive vs. ER-low-positive	ER-low-positive vs. ER-negative	ER-positive vs. ER-negative
Age							
Mean/median (range)	59.7/60 (20–98)	57.5/58.5 (33–90)	54.5/54 (22–95)	56.3/57 (20–95)	0.217	0.928	0.596
Race							
Caucasian	351	32	620	1003	< 0.0001	0.103	< 0.0001
African American	107	26	324	457			
Other	20	2	12	34			
Tumor type							
Ductal	344	51	853	1248	0.06	0.102	< 0.0001
Lobular	122	7	96	225			
Other	12	2	7	21			
Histologic grade							
Grade I	92	1	13	106	< 0.0001	0.949	< 0.0001
Grade II	241	11	167	419			
Grade III	127	47	776	950			
Unknown	18	1	0	19			
Mean tumor size (mm), range	23.5 (0.4–275)	28.3 (3–120)	30.5 (1–205)	28.2 (0.4–205)	0.128	0.087	0.004
Pathologic nodal stage							
N0	298	39	551	888	0.587	0.475	0.215
N1	100	14	228	342			
N2	36	2	62	100			
N3	15	1	44	60			
Unknown	29	4	71	104			
HER2 status							
Positive	93	14	243	350	0.803	0.697	0.007
Negative	336	42	701	1079			
Equivocal	11	1	7	19			
Unknown	38	3	5	46			
Clinical stage							
I	208	18	293	519	0.139	0.416	< 0.0001
II	176	31	414	621			
III	54	6	135	195			
IV	27	4	111	142			
Unknown	13	1	3	17			

difference was identified in RFS [HR 0.408 (0.037–2.657), $P = 0.3$] or DSS [HR 0.437 (0.045–2.998), $P = 0.4$]. The levels of ER expression were similar between the two groups (mean 7%, range 3–10% vs. mean 6%, range 1–10%; $P = 0.3$).

We next compared the prognostic outcomes of patients with Grade III, ER-low-positive/PR-negative/HER2-negative tumors ($N = 36$, including 6 receiving endocrine

therapy) and those having triple-negative breast cancers ($N = 701$), given that these tumors would frequently be treated similarly in clinical practice. To that end, a significantly better RFS was associated with the former [HR 0.336 (0.147–0.770), $P = 0.01$] in the patients without distant metastasis at diagnosis ($N = 33$ and 660, respectively), while a trend of favorable DSS with marginal significance was seen for DSS [HR 0.391 (0.274–1.002), $P = 0.053$].

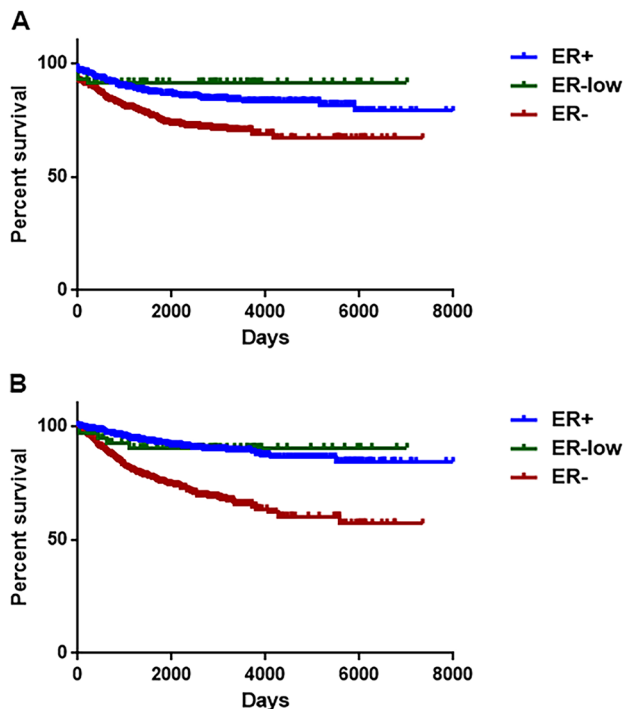


Fig. 3 Relapse-free survival (**A**, Chi-square 28.47, $P < 0.0001$) and disease-specific survival (**B**, Chi-square 65.38, $P < 0.0001$) in the subcohort of PR-negative breast cancers. *ER+* ER-positive, *ER-low* ER-low-positive, *ER-* ER-negative

Discussion

ER-low-positive breast cancers are defined as those with 1–10% of tumor nuclei staining positive for ER by the ASCO/CAP Guideline Recommendations [11], although 1–9% has also been used in some studies [12, 13]. These borderline ER-positive tumors are rare, with a reported

incidence of 2–5% of all breast cancer cases in large series [11–14], although some smaller cohorts reported higher incidence rates [15, 16]. This terminology has been used owing to the 2010 ASCO/CAP Guideline Recommendations that mandated tumors with $\geq 1\%$ ER nuclear immunoreactivity as ER-positive [10]. However, a range of arbitrary thresholds has been used in clinical practice for endocrine therapy, including 1%, 5–10% and 20% [20], of which 10% is commonly utilized by treating physicians [12]. In this cohort, we found 2.3% of all of our breast cancer cases falling into the category of ER-low-positive tumors, thus in keeping with the reported incidence range. It is interesting to note that all ER-low-positive cases in the present study showed weak (1+) nuclear staining, and no case with 1–10% ER expression demonstrated moderate or strong intensity.

A number of early studies have analyzed clinical and pathologic characteristics of ER-low-positive breast cancers. In conformity with previous observations, our findings also demonstrated that ER-low-positive tumors more commonly occurred in younger-aged women, more frequently affected African Americans, were associated with a higher histologic grade, negative PR and positive HER2 status, and more advanced pathologic and clinical stages, thus similar to ER-negative carcinomas [12, 14, 15]. Furthermore, a significant proportion of ER-low-positive tumors have been shown to possess ER-negative molecular characteristics based on quantitative estimation of ER mRNA and ER-related gene transcripts [15, 16].

There have been limited studies with regard to the survival outcomes in ER-low tumors. A respective cohort consisting of 250 ER-low-positive breast cancers showed a worse RFS and diminished overall survival when compared to ER-positive tumors [12]. Further, pathologic complete response rates for ER-low-positive/HER2-negative tumors were similar to those of triple-negative breast cancers [17,

Table 6 Multivariate analysis of clinicopathologic factors-associated prognostic outcomes in PR-negative breast cancers

Clinicopathologic factor	Relapse-free survival		Disease specific survival	
	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value
Age (> 57 vs. ≤ 57)	0.792 (0.614–1.020)	0.071	1.242 (0.954–1.617)	0.107
Race (Caucasian vs. African American)	1.008 (0.781–1.300)	0.953	0.669 (0.516–0.869)	0.003
Tumor type (lobular vs. ductal)	1.048 (0.724–1.516)	0.804	1.143 (0.767–1.704)	0.512
Histologic grade (III vs. I/II)	1.323 (0.976–1.793)	0.072	1.626 (1.158–2.285)	0.005
Tumor size (2.0–5.0 cm vs. ≤ 2.0 cm)	1.801 (1.22–2.510)	0.001	1.582 (1.125–2.224)	0.008
Tumor size (> 5.0 cm vs. ≤ 2.0 cm)	2.486 (1.707–3.620)	< 0.0001	2.609 (1.786–3.812)	< 0.0001
Nodal status (positive vs. negative)	4.497 (3.259–6.207)	< 0.0001	4.926 (3.542–6.852)	< 0.0001
ER-positive vs. ER-negative	0.788 (0.570–1.090)	0.150	0.375 (0.151–0.930)	< 0.0001
ER-low-positive vs. ER-negative	0.439 (0.175–1.100)	0.079	0.430 (0.295–0.626)	0.034
HER2 (positive vs. negative)	1.309 (1.015–1.688)	0.038	0.940 (0.710–1.244)	0.665

HR hazard ration, CI confidence interval

21]. Furthermore, ER-low-positive tumors showed an intermediate disease-free survival between that of the ER-positive and ER-negative groups in a cohort of 3596 patients [22]. Moreover, a meta-analysis of 6 studies consisting of 16,606 patients revealed that ER-low-positive tumors had a poorer endocrine responsiveness when compared to their ER-positive counterparts. Yet, patients with ER-low-positive tumors manifested an overall better prognosis than those with ER-negative cancers regardless of treatment regimen [13]. These findings are concordant, in least in part, with the observations of the present study in which the ER-low-positive tumors had a better RFS and DSS than the ER-negative carcinomas in spite of largely overlapping clinical and pathologic characteristics between the two groups. The lack of significant difference for RFS or DSS between ER-positive and ER-low-positive groups in the current study supports using 1% as the optimal cutoff for ER positivity. The collective findings thus far have suggested that ER-low-positive carcinomas may represent a heterogeneous group with diverse clinical outcomes. In this regard, an early study observed a tendency for survival advantages in patients with ER/PR 6–10% breast cancers when compared to the ER/PR < 1% group, while an ER/PR 1–5% did not appear to have any significant impact on survival outcomes, thus further indicating the heterogeneity of ER-low-positive carcinomas [23].

The largely overlapping prognostic outcomes between ER-positive and ER-low-positive groups in the entire cohort are of substantial interest. While the discrepancies between some early studies and ours might be due to the limited sample size thus requires further validation, the significance of PR status in predicting survival outcomes could provide potential biologic mechanisms for the observed heterogeneity in the subset of ER-low-positive tumors. As an ER-dependent gene product, PR is positive in the vast majority of ER-expressing tumors (82.5% in this cohort), and co-expression of the two molecules is indicative of functional ER signal transduction. It has been generally accepted that PR status added to ER status significantly improves the accuracy of predicting endocrine responsiveness of the primary tumor [24]. Therefore, it is plausible that with intact ER signaling (i.e., ER+/PR+), carcinomas with a lower ER expression are associated with a poorer prognosis, as exemplified by the worse DSS demonstrated in the subcohort of patients with PR-positive tumors in this study. In addition, endocrine treatment of ER-low-positive/PR-positive tumors was associated with a favorable prognostic outcome in the present cohort, further suggesting possible benefits of such therapy, although those with endocrine therapy had higher ER levels and the number of cases was minimal. These observations also provide additional evidence supporting the recommended reporting comments for a low level (1–10%) of ER expression, to emphasize the possible benefit but

limited data on the overall benefit of endocrine therapies in these patients [11].

On the other hand, an ER+/PR– phenotype suggests a blockade of the functional ER signaling cascade. These patients are typically managed similarly to those with ER+/PR+ breast cancers, yet usually have a worse prognosis [25, 26]. Thus, the lack of a significant difference in survival outcomes between ER-positive and ER-low-positive tumors may be due to a generally poorer responsiveness to endocrine therapies in the absence of PR expression. Moreover, endocrine therapy did not provide survival benefit for patients with ER-low-positive/PR-negative tumors in this study, thus further supporting this notion. Interestingly, the Grade III, ER-low-positive/PR-negative/HER2-negative tumors, albeit a small subset of cases, were associated favorable prognostic outcomes when compared to triple-negative breast cancers. This observation requires further confirmation in a larger cohort, while the tumor biology and ER signaling in these tumors merit further investigation. The inferior survival outcomes associated with the ER-negative phenotype in this group are undoubtedly ascribable to their ER-/PR-/HER2+ or triple-negative receptor profiles, which are known to be aggressive subtypes associated with poor prognosis.

Some limitations exist in the current study. First, ER-low-positive tumors are rare thus the limited sample size precludes further detailed analysis, an inherited nature of all similar studies. Second, the cutoff changes for ER and PR from 10 to 1% in the study period likely had an impact in the treatment decision-making in the patients with ER-low-positive tumors. Third, arbitrary thresholds for endocrine therapy used in practice for ER-low-positive tumors might have influenced clinical outcomes. Furthermore, the advancement in chemotherapeutic regimens and HER2-targeted therapy might have had an impact on the course of disease progression in ER-low-positive tumors. Nonetheless, all patients received standard of care treatment at the time of diagnosis.

In summary, we found that ER-low-positive breast cancers had overall superior prognostic outcomes when compared to ER-negative tumors in this cohort, despite having largely overlapping clinicopathologic characteristics between the two groups. PR status as a surrogate marker of functional ER pathway provides critical information in this regard. With intact ER signaling, the prognosis of ER-low-positive tumors was intermediate between that of the ER-positive and ER-negative groups, although no significant difference was found between ER-low-positive and ER-negative tumors. In case of ER cascade blockade (as indicated by PR-negativity), the ER-positive and ER-low-positive groups showed similar survival outcomes, both significantly better than ER-negative tumors. These findings support that ER-low-positive tumors are eligible for endocrine treatment. The utility of PR in assisting in prognostication is also evident in

its inclusion in the recently established breast cancer prognostic stage groups [9, 27]. Analysis of molecular signatures, standardization of therapeutic strategies, validation with a larger sample size and longer follow-up are all important to understand the biology of this group of heterogeneous tumors and to enable optimal treatment in the pursuit of individualized medicine.

Compliance with ethical standards

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

References

- Bartlett JM, Brookes CL, Robson T, van de Velde CJ, Billingham LJ, Campbell FM, Grant M, Hasenburger A, Hille ET, Kay C et al (2011) Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial. *J Clin Oncol* 29(12):1531–1538
- Cianfrocca M, Goldstein LJ (2004) Prognostic and predictive factors in early-stage breast cancer. *Oncologist* 9(6):606–616
- Harvey JM, Clark GM, Osborne CK, Allred DC (1999) Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol* 17(5):1474–1481
- Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, Ruby SG, O'Malley F, Simpson JF, Connolly JL et al (2000) Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med* 124(7):966–978
- Anderson WF, Katki HA, Rosenberg PS (2011) Incidence of breast cancer in the United States: current and future trends. *J Natl Cancer Inst* 103(18):1397–1402
- Burstein HJ, Temin S, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, Giordano SH, Hudis CA, Rowden D, Solky AJ et al (2014) Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: american society of clinical oncology clinical practice guideline focused update. *J Clin Oncol* 32(21):2255–2269
- Bouchard-Fortier A, Provencher L, Blanchette C, Diorio C (2017) Prognostic and predictive value of low estrogen receptor expression in breast cancer. *Curr Oncol* 24(2):e106–e114
- Early Breast Cancer Trialists' Collaborative Group, Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, McGale P, Pan HC, Taylor C et al (2011) Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* 378(9793):771–784
- Aldrees R, Gao X, Zhang K, Siegal GP, Wei S (2020) Validation of the revised 8th AJCC breast cancer clinical prognostic staging system: analysis of 5321 cases from a single institution. *Mod Pathol* 34:291–299
- Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M et al (2010) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol* 28(16):2784–2795
- Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J et al (2020) Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med* 144(5):545–563
- Yi M, Huo L, Koenig KB, Mittendorf EA, Meric-Bernstam F, Kuerer HM, Bedrosian I, Buzdar AU, Symmans WF, Crow JR et al (2014) Which threshold for ER positivity? A retrospective study based on 9639 patients. *Ann Oncol* 25(5):1004–1011
- Chen T, Zhang N, Moran MS, Su P, Haffty BG, Yang Q (2018) Borderline ER-positive primary breast cancer gains no significant survival benefit from endocrine therapy: a systematic review and meta-analysis. *Clin Breast Cancer* 18(1):1–8
- Poon IK, Tsang JY, Li J, Chan SK, Shea KH, Tse GM (2020) The significance of highlighting the oestrogen receptor low category in breast cancer. *Br J Cancer* 123:1223–1227
- Iwamoto T, Booser D, Valero V, Murray JL, Koenig K, Esteva FJ, Ueno NT, Zhang J, Shi W, Qi Y et al (2012) Estrogen receptor (ER) mRNA and ER-related gene expression in breast cancers that are 1% to 10% ER-positive by immunohistochemistry. *J Clin Oncol* 30(7):729–734
- Prabhu JS, Korlimarla A, Desai K, Alexander A, Raghavan R, Anupama C, Dendukuri N, Manjunath S, Correa M, Raman N et al (2014) A majority of low (1–10%) ER positive breast cancers behave like hormone receptor negative tumors. *J Cancer* 5(2):156–165
- Landmann A, Farrugia DJ, Zhu L, Diego EJ, Johnson RR, Soran A, Dabbs DJ, Clark BZ, Puhalla SL, Jankowitz RC et al (2018) Low estrogen receptor (ER)-positive breast cancer and neoadjuvant systemic chemotherapy: is response similar to typical ER-positive or ER-negative disease? *Am J Clin Pathol* 150(1):34–42
- Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A et al (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 25(1):118–145
- Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P et al (2013) Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol* 31(31):3997–4013
- Layfield LJ, Gupta D, Mooney EE (2000) Assessment of tissue estrogen and progesterone receptor levels: a survey of current practice, techniques, and quantitation methods. *Breast J* 6(3):189–196
- Fujii T, Kogawa T, Dong W, Sahin AA, Moulder S, Litton JK, Tripathy D, Iwamoto T, Hunt KK, Pusztai L et al (2017) Revisiting the definition of estrogen receptor positivity in HER2-negative primary breast cancer. *Ann Oncol* 28(10):2420–2428
- Viale G, Regan MM, Maiorano E, Mastropasqua MG, Dell'Orto P, Rasmussen BB, Raffoul J, Neven P, Orosz Z, Braye S et al (2007) Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1–98. *J Clin Oncol* 25(25):3846–3852
- Raghav KP, Hernandez-Aya LF, Lei X, Chavez-Macgregor M, Meric-Bernstam F, Buchholz TA, Sahin A, Do KA, Hortobagyi GN, Gonzalez-Angulo AM (2012) Impact of low estrogen/progesterone receptor expression on survival outcomes in breast cancers previously classified as triple negative breast cancers. *Cancer* 118(6):1498–1506
- Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM (2003) Progesterone receptor status significantly improves

- outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J Clin Oncol* 21(10):1973–1979
25. Dunnwald LK, Rossing MA, Li CI (2007) Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. *Breast Cancer Res* 9(1):R6
 26. Shen T, Brandwein-Gensler M, Hameed O, Siegal GP, Wei S (2015) Characterization of estrogen receptor-negative/progesterone receptor-positive breast cancer. *Hum Pathol* 46(11):1776–1784
 27. Hortobagyi GN, Connolly JL, D’Orsi CJ, Edge SB, Mittendorf EA, Rugo HS, Solin LJ, Weaver DL, Winchester DJ, Giuliano AE (2017) Breast. In: Amin MA (ed) *AJCC cancer staging manual*, 8th edn. New York, Springer, pp 587–628

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