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Which threshold for ER positivity? a retrospective study based on 9639 patients

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Background: Guidelines for the use of chemotherapy and endocrine therapy recently recommended that estrogen receptor (ER) status be considered positive if ≥1% of tumor cells demonstrate positive nuclear staining by immunohistochemistry. In clinical practice, a range of thresholds are used; a common one is 10% positivity. Data addressing the optimal threshold with regard to the efficacy of endocrine therapy are lacking. In this study, we compared patient, tumor, treatment and survival differences among breast cancer patients using ER-positivity thresholds of 1% and 10%.

Methods: The study population consisted of patients with primary breast carcinoma treated at our center from January 1990 to December 2011 and whose records included complete data on ER status. Patients were separated into three groups: ≥10% positive staining for ER (ER-positive ≥10%), 1%–9% positive staining for ER (ER-positive 1%–9%) and <1% positive staining (ER-negative).

Results: Of 9639 patients included, 80.5% had tumors that were ER-positive ≥10%, 2.6% had tumors that were ER-positive 1%–9% and 16.9% had tumors that were ER-negative. Patients with ER-positive 1%–9% tumors were younger with more advanced disease compared with patients with ER-positive ≥10% tumors. At a median follow-up of 5.1 years, patients with ER-positive 1%–9% tumors had worse survival rates than did patients with ER-positive ≥10% tumors, with and without adjustment for clinical stage and grade. Survival rates did not differ significantly between patients with ER-positive 1%–9% and ER-negative tumors.

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Conclusions: Patients with tumors that are ER-positive 1%–9% have clinical and pathologic characteristics different from those with tumors that are ER-positive ≥10%. Similar to patients with ER-negative tumors, those with ER-positive 1%–9% disease do not appear to benefit from endocrine therapy; further study of its clinical benefit in this group is warranted. Also, there is a need to better define which patients of this group belong to basal or luminal subtypes.
Key words: estrogen receptor, breast cancer, survival, low positive

introduction

Estrogen receptor (ER) is a prognostic factor in breast cancer and a predictor of response to endocrine therapy [1]. Recently, guidelines from the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) recommended that ER status be considered positive if 1% or more of tumor cells demonstrate positive nuclear staining on immunohistochemistry [2]. However, in routine practice, a wide range of arbitrary cutoffs in percentage of stained cells are being used (i.e. >0% [3, 4], 1% [5], 5%–10% and 20% [3]). Many clinicians consider patient eligibility for endocrine therapy with 10% or greater nuclear staining [6–10]. Results of a meta-analysis from the Early Breast Cancer Trialists' Collaborative Group revealed tamoxifen was ineffective with low ER expression (<10 fmol/mg by ligand-binding assay, LBA) [11]. Prospective data addressing the optimal cutoff for defining positivity based on the efficacy of endocrine therapy are lacking.

A recent study demonstrated that half of tumors staining ER-positive 1%–9% on immunohistochemistry have molecular characteristics more similar to the ER-negative, basal-like phenotype [12]. Unfortunately, limited clinical information is available for these subtypes regarding prediction of treatment effect. In principle, those retrospective results need to be validated, particularly with regard to clinical endocrine responsiveness. In this study, we examined patient, tumor and treatment differences among patients with different ER status: at least 10% of cells staining positive (ER-positive ≥10%), between 1% and 9% of cells positive (ER-positive 1%–9%) and <1% positive (ER-negative). We compared survival outcomes among patients with different ER-positivity thresholds, for the whole cohort and for subgroups based on treatment with endocrine therapy.

patients and methods

We used the Surgical Breast Oncology Database at The University of Texas MD Anderson Cancer Center to identify patients with primary invasive breast carcinoma treated at our center from January 1990 to December 2011 with known ER status. Patients presenting with recurrent or metastatic disease were excluded. This study was approved by our center's Institutional Review Board.

Hormone receptor evaluation was carried out on core biopsy or surgical specimens. When ER and PR immunohistochemistry were carried out at a referring institution, the slides were evaluated at our institution. Less than 40% of cases were referred from other centers. Largely due to a lack of availability of tumor blocks, routine re-staining for ER/PR was not done on these outside cases. When immunohistochemistry slides were not available for review, ER and PR were repeated at our institution. Multiple pathologists were involved in signing the markers. The slides from 6% of tumors were available for re-review by a single pathologist (LH) for confirmation. From 2007 to present, the polymeric biotin-free horseradish peroxidase method was used for ER staining on a Leica Bond-Max stainer (Leica Microsystems, Buffalo Grove, IL). One whole-slide 4-μm-thick unstained tissue section from a representative paraffin block of the invasive carcinoma was incubated at 60°C for 20 min. Following heat-induced epitope retrieval with citrate buffer for 30 min at 100°C, slides were incubated with mouse monoclonal antibody to ER (clone 6F11, 1:35, Novocastra Laboratories, Leica Microsystems). The Refine Polymer Detection kit was used to detect bound antibody, with 3,3-diaminobenzidine as the chromogen (Leica Microsystems). Slides were counterstained with Mayer's hematoxylin and results evaluated with positive and negative tissue controls. ER staining was carried out using antibody clone 6F11 on a DAKO autostainer (Dako North America, Inc., Carpinteria, CA) from 2002 to 2007, and using antibody clone 1D5 (Dako North America, Inc.) before 2002. Any invasive tumor cell with strong, moderate or weak nuclear staining is considered positive (supplementary Figure S1, available at *Annals of Oncology* online). Assessment of percentage of stained tumor cells is an estimate of the entire invasive tumor on a given slide, regardless of whether there are heterogeneously stained tumor areas.

For analysis, patients were separated into three groups: ER-positive ≥10%, ER-positive 1%–9% and ER-negative. Patient, tumor and treatment characteristics were evaluated and compared between groups. Kaplan–Meier survival curves were calculated, and log-rank test used to compare overall survival (OS) (time from surgery to death from any cause), recurrence-free survival (RFS) (time from surgery to first recurrence), and distant recurrence-free survival (DRFS) (time from surgery to death due to breast cancer or first distant recurrence) between groups. A multivariate stratified Cox proportional hazards model was used to identify significant predictors of DFS stratifying by clinical TNM stage and tumor grade. STATA statistical software (SE 9, StataCorp LP, College Station, TX) was used for statistical analyses. All P values were two tailed, and $P \leq 0.05$ was considered significant.

results

patient and tumor characteristics

Of 9639 patients included in this study, 7764 (80.5%) had tumors ER-positive ≥10%, 1625 (16.9) were ER-negative and 250 (2.6%) were ER-positive 1%–9%. Median percentage of ER positivity in the 1%–9% group was 4 (mean: 3.5, range: 1–9). Of the 250 ER-positive 1%–9%, 230 (92%) were ER-positive ≤5%. For the entire cohort, median age at diagnosis was 55 years (mean 56, range: 21–99). The majority had stage I (50.5%) or II (36.5%) disease and tumors were grade II in 48.2% and grade III in 38.4%.

Patient and tumor characteristics of the three groups are summarized in Table 1. Compared with patients whose tumors were ER-positive ≥10%, those with ER-positive 1%–9% were younger (median age 53 versus 56 years, $P < 0.0001$), less likely to be white (74.2% versus 66.4%, $P = 0.008$), more likely to have ductal histology (83.6% versus 73.0%, $P < 0.0001$) with more advanced disease (clinical stage II/III 61.6% versus 43.7%, $P < 0.0001$) and more likely to receive neoadjuvant

Table 1. Comparison of patient, tumor and pathologic factors by level of ER staining in the primary tumor

Factors	ER staining				
	≥10% ($n = 7764$)	$n = 250$	P value[a]	Negative ($n = 1625$)	P value[b]
Age at diagnosis, years					
Mean	56.6	51.9	<0.0001	52.3	0.7
Median (range)	56 (21–93)	53 (22–84)		52 (23–99)	
Race					
White	5762 (74.2)	166 (66.4)	0.007	1058 (65.1)	0.2
Black	1061 (13.7)	47 (18.8)		331 (20.4)	
Hispanic	660 (8.5)	22 (8.8)		173 (10.6)	
Asian	232 (3.0)	15 (6.0)		54 (3.3)	
Others	49 (0.6)	0 (0)		9 (0.6)	
Clinical TNM stage					
I	4292 (55.3)	93 (37.2)	<0.0001[c]	486 (29.9)	0.04[c]
II	2654 (34.2)	114 (45.6)		749 (46.1)	
III	741 (9.5)	40 (16.0)		363 (22.3)	
IV	77 (1.0)	3 (1.2)		27 (1.7)	
Clinical tumor size, cm					
Mean	2.3	2.9	<0.0001[d]	3.1	0.2[d]
Median (range)	1.8 (0.03–20)	2.5 (0.05–18)		2.5 (0.01–38)	
Histology					
IDC/DCIS	5671 (73.0)	209 (83.6)	<0.0001[c]	1432 (88.1)	0.001[c]
ILC/DCIS	768 (9.9)	11 (4.4)		20 (1.2)	
Mixed	718 (9.3)	12 (4.8)		36 (2.2)	
Others	607 (7.8)	18 (7.2)		137 (8.4)	
Tumor grade					
I	1148 (15.0)	7 (2.9)	<0.0001	16 (1.0)	0.049[c]
II	4380 (57.1)	38 (15.5)		228 (14.2)	
III	2141 (27.9)	200 (81.6)		1362 (84.8)	
Unknown	95	5		19	
Pathologic nodal stage					
N0	4828 (62.2)	182 (72.8)	0.003[c]	1080 (66.5)	0.3[c]
N1	1979 (25.5)	40 (16.0)		332 (20.4)	
N2	471 (6.1)	14 (5.6)		96 (5.9)	
N3	486 (6.2)	14 (5.6)		117 (7.2)	
HER-2 status					
Positive	890 (13.1)	64 (27.6)	<0.0001	436 (28.6)	0.8[c]
Negative	5903 (86.9)	168 (72.4)		1088 (71.4)	
Unknown	971	18		101	
PR status					
Positive ≥10%	5956 (77.0)	38 (15.2)	<0.0001	149 (9.2)	<0.0001
Positive 1%–9%	609 (7.9)	66 (26.4)		110 (6.8)	
Negative	1169 (15.1)	146 (58.4)		1360 (84.0)	
Unknown	30	0		6	
Preoperative chemotherapy					
No	5496 (70.8)	131 (52.4)	<0.0001	732 (45.1)	0.03
Yes	2268 (29.2)	119 (47.6)		893 (54.9)	

IDC, invasive ductal carcinoma; DCIS, ductal carcinoma *in situ*; ILC, invasive lobular carcinoma; PR, progesterone receptor.
[a]Comparisons between ≥10% and 1%–9%.
[b]Comparisons between 1%–9% and negative.
[c]Fisher's exact test.
[d]Wilcoxon scores rank sum test.

chemotherapy (47.6% versus 29.2%, $P < 0.0001$). They were also more likely to have HER-2-positive (27.6% versus 13.1%, $P < 0.0001$) and grade III (81.6% versus 27.9%, $P < 0.0001$) disease. Of 250 patients with ER-positive 1%–9% status, 66 (26.4%) were progesterone (PR) positive 1%–9%, while only 609 (7.9%) patients with ER-positive ≥10% status and 110 (6.8%) of patients with ER-negative tumors were PR-positive 1%–9%. Overall, 63.7% of patients had tumors that were PR-positive

≥10% and 8.1% of patients had tumors that were PR-positive 1%–9%. Compared with patients with ER-negative tumors, patients with ER-positive 1%–9% tumors had earlier stage disease and were less likely to have ductal histology.

Adjuvant treatments, follow-up and recurrence in the three different groups based on ER status are shown in Table 2. Patients with ER-positive 1%–9% disease were more likely to receive adjuvant chemotherapy (49.2% versus 35.5%, $P < 0.0001$) and less likely to receive adjuvant endocrine therapy (20.4% versus 83.6%, $P < 0.0001$) than patients with ER-positive ≥10% tumors. Compared with patients with ER-negative tumors, patients with ER-positive 1%–9% were more likely to receive adjuvant endocrine therapy (20.4% versus 12.9%, $P = 0.002$). Follow-up time was longer in patients with ER-positive tumors at ≥10%. Patients with ER-positive 1%–9% tumors were more likely to experience recurrence (17.2%) than patients

Table 2. Adjuvant therapy, follow-up and recurrence status among patients with three different levels of ER staining

Factors	ER staining				
	≥10% ($n = 7764$)	$n = 250$	P value[a]	Negative ($n = 1625$)	P value[b]
Adjuvant chemotherapy					
Yes	2742 (35.5)	123 (49.2)	<0.0001	805 (49.7)	0.9[c]
No	4981 (64.5)	127 (50.8)		815 (50.3)	
Unknown	41	0		5	
Adjuvant endocrine therapy					
Yes	6454 (83.6)	51 (20.4)	<0.0001	208 (12.9)	0.002[c]
No	1265 (16.4)	199 (79.6)		1409 (87.1)	
Unknown	45	0		8	
Adjuvant radiation therapy					
Yes	5174 (67.1)	160 (64.3)	0.4[c]	1134 (70.0)	0.08[c]
No	2536 (32.9)	89 (35.7)		485 (30.0)	
Unknown	54	1		6	
Follow-up time, years					
Mean	6.2	4.6	<0.0001[d]	5.8	<0.0001[d]
Median (range)	5.4 (1–19.8)	3.8 (1–19.3)		5.3 (1.1–19.5)	
Recurrence					
Yes	685 (9.1)	42 (17.2)	<0.0001	307 (19.4)	0.5[c]
No	6841 (90.9)	202 (82.8)		1276 (80.6)	
Unknown	238	6		42	
Local recurrence					
Yes	181 (2.4)	12 (5.0)	0.01[c]	91 (5.8)	0.8[c]
No	7313 (97.6)	230 (95.0)		1478 (94.2)	
Unknown	270	8		56	
Regional recurrence					
Yes	124 (1.7)	9 (3.7)	0.02[c]	99 (6.3)	0.1[c]
No	7372 (98.3)	232 (96.3)		1469 (93.7)	
Unknown	268	9		57	
Distant recurrence					
Yes	561 (7.5)	35 (14.4)	<0.0001	264 (16.6)	0.4[c]
No	6963 (92.5)	208 (85.6)		1323 (83.4)	
Unknown	240	7		38	
Patients who received endocrine therapy					
Recurrence					
Yes	500 (7.7)	9 (17.7)	0.02[c]	48 (23.1)	0.5[c]
No	5954 (92.3)	42 (82.3)		160 (76.9)	
Patients who did not receive endocrine therapy					
Recurrence					
Yes	183 (14.5)	33 (16.6)	0.5[c]	258 (18.3)	0.6[c]
No	1082 (85.5)	166 (83.4)		1151 (81.7)	

[a]Comparisons between ≥10% and 1%–10%.
[b]Comparisons between 1%–10% and negative.
[c]Fisher's exact test.
[d]Wilcoxon scores rank sum test.

with ER-positive ≥10% tumors (9.1%) ($P < 0.0001$), including more local, regional and distant recurrences. There was no significant difference in recurrences between patients with ER-positive 1%–9% and ER-negative tumors (19.4%) ($P = 0.5$). For patients receiving endocrine therapy, recurrence rates were higher in patients whose tumors were ER-positive1%–9% compared with those that were ER-positive ≥10% (17.7% versus 7.7%, $P = 0.02$). There was no significant difference in total recurrences between these groups for patients who did not receive endocrine therapy.

survival outcomes

At a median follow-up of 5.1 years, patients with ER-positive tumors at 1%–9% or ER-negative tumors had worse DRFS ($P < 0.0001$), RFS ($P < 0.0001$) and OS ($P < 0.0001$) rates compared with ER-positive tumors at ≥10%. Patients with ER-positive tumors at 1%–9% had similar DRFS ($P = 0.8$), RFS ($P = 0.96$) and OS ($P = 0.1$) rates as ER-negative tumors (Figure 1).

Figure 2 shows survival outcomes between patients with ER-positive tumors at 1%–9% and ER-positive tumors at ≥10% with/without endocrine therapy. Patients with ER-positive tumors at 1%–9% had worse DRFS ($P = 0.0003$), RFS ($P = 0.0005$) and OS ($P < 0.0001$) rates than did patients with ER-positive ≥10% tumors even in patients who received endocrine therapy (Figure 2, upper). Among patients who did not receive endocrine therapy (Figure 2, lower), those with ER-positive 1%–9% tumors had worse DRFS ($P = 0.02$), RFS ($P = 0.0003$) and OS ($P = 0.0002$) rates than those with ER-positive ≥10% tumors. There were no DRFS and RFS survival differences between patients with ER-positive 1%–9% tumors who received endocrine therapy and patients with ER-negative tumors who did not receive endocrine therapy (supplementary Figure S2, available at *Annals of Oncology* online).

Table 3 shows the stratified Cox proportion regression models for different groups by ER positivity associated with survival outcomes. Patients with ER-positive ≥10% tumors had better DRFS, RFS and OS rates than patients with ER-positive 1%–9% tumors even when stratified by clinical stage and tumor grade. Patients with ER-negative tumors had similar DRFS and RFS rates as patients with ER-positive 1%–9% tumors when stratified by clinical stage and tumor grade.

discussion

Recent ASCO/CAP guidelines have decreased the threshold for ER positivity by immunohistochemistry to 1%. A finding of 1%–9% ER positivity is rare; our study indicates that only about

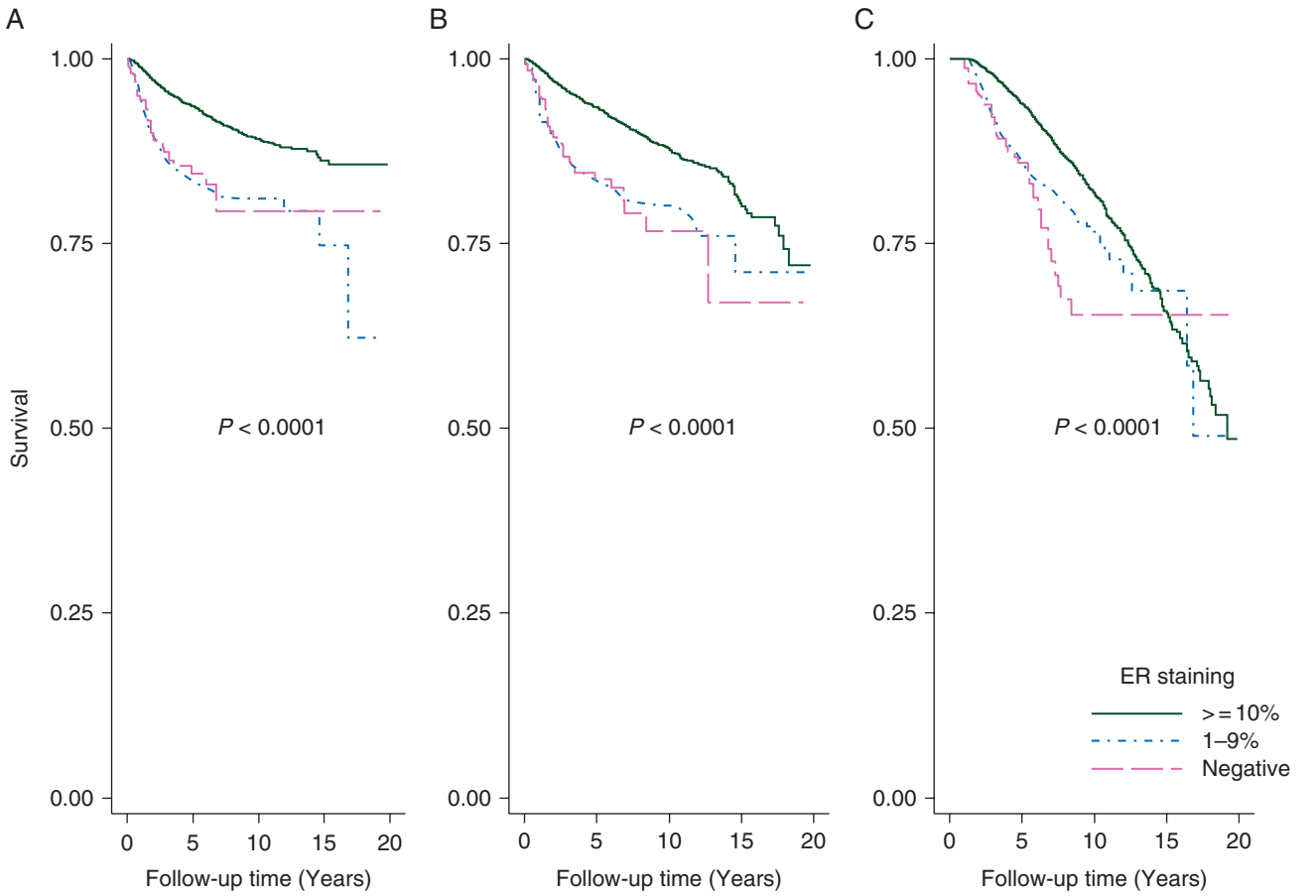

Figure 1. Comparison of survival outcomes among patients with three different levels of ER expression in the primary tumor: (A) distant recurrence-free survival, (B) recurrence-free survival, (C) overall survival.

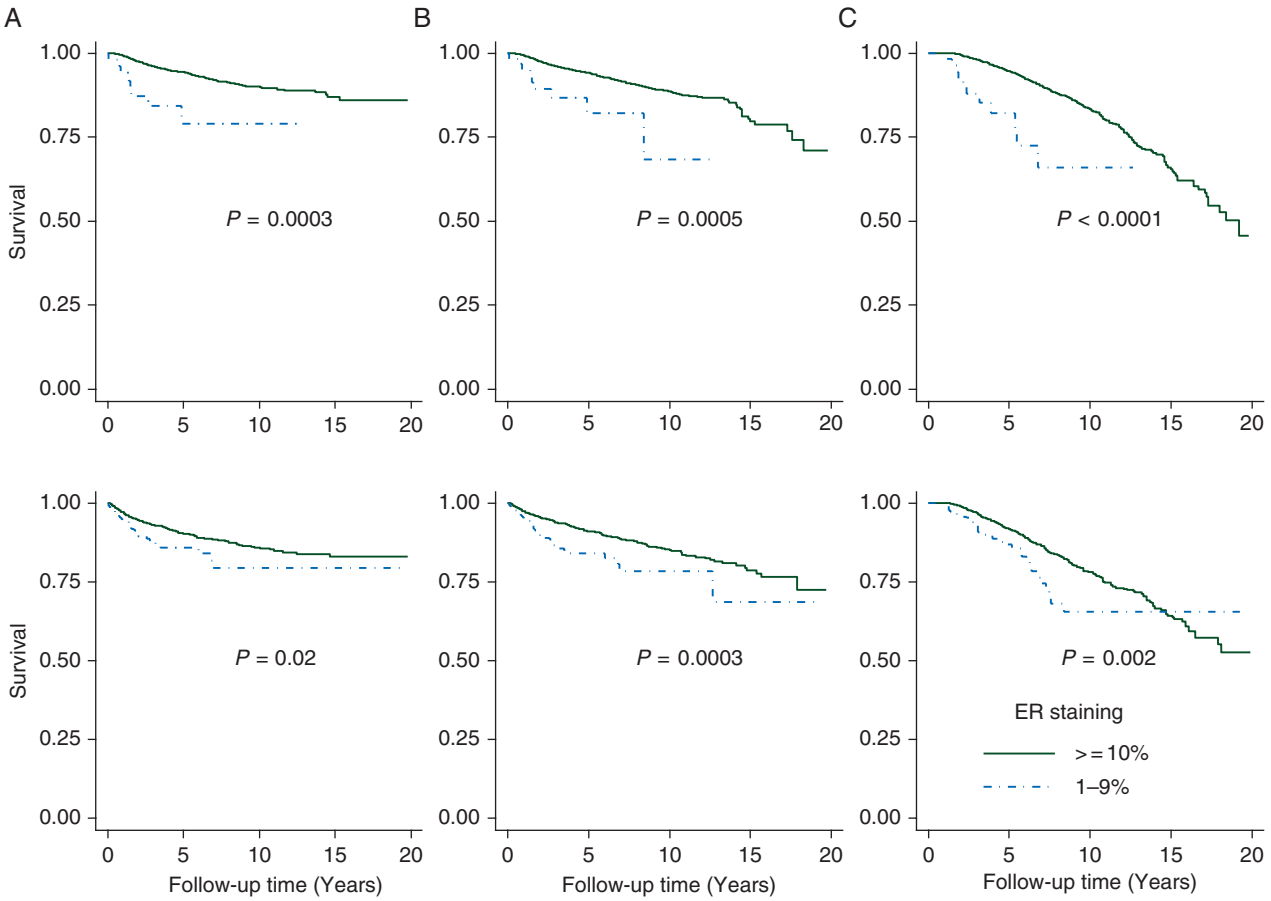

Figure 2. Comparison of survival outcomes between patients with ER-positive tumors at 1%–9% and patients with ER-positive tumors ≥10% among patients: (A) distant recurrence-free survival, (B) recurrence-free survival, (C) overall survival; upper received endocrine therapy; lower: not received endocrine therapy.

Table 3. Cox regression stratified model for survival outcomes among patients with different levels of ER staining in the primary tumor

	HR[a]	SE	*P* value	95% CI	
Distant recurrence-free survival					
ER staining					
1%–9%	Reference				
≥10%	0.7	0.06	<0.001	0.6	0.8
Negative	1.2	0.2	0.3	0.8	1.7
Recurrence-free survival					
ER staining					
1%–9%	Reference				
≥10%	0.7	0.06	<0.001	0.6	0.8
Negative	1.2	0.2	0.2	0.9	1.7
Overall survival					
ER staining					
1%–9%	Reference				
≥10%	0.8	0.06	0.002	0.7	0.9
Negative	1.5	0.2	0.02	1.1	2.0

[a]Stratified by tumor grade and clinical stage.
HR, hazard ratio; SE, standard error; CI, confidence interval.

3% of breast cancers fit this category, a lower rate than other studies have reported [12, 13]. Our study shows that patients with ER-positive 1%–9% tumors have clinical and pathologic characteristics different from those with ER-positive ≥10% tumors. Similar to patients with ER-negative tumors, those with ER-positive 1%–9% disease do not appear to benefit from endocrine therapy. Our findings are consistent with reports from the Oxford Overview [11]; all 20 trials included in that meta-analysis defined values of 10 fmol/mg or greater on biochemical assays as ER-positive. The study showed little apparent benefit from adjuvant tamoxifen if ER levels were just below 10 fmol/mg, but a significant and increasing benefit with higher levels, beginning at the cutoff [11].

A number of methods have been developed to determine ER status; however, retrospective studies showed that semiquantitative immunohistochemistry analysis of ER expression was superior for prognostic and predictive purposes compared with standardized LBAs [7, 14].The heterogeneity of hormone receptor expression in breast cancer can be visualized with immunohistochemistry [15]. Tumors have variable expression ER and PR, with some cells staining positively, whereas the others do not [16]. St Gallen 2005 guidelines suggested three categories for scoring ER status: endocrine responsive, with strong ER expression; endocrine response uncertain, with low ER expression

and endocrine nonresponsive, with no ER expression [17]. The boundary between 'endocrine responsive' and 'endocrine response uncertain' was not provided, although the authors suggest that tumors with 1%–10% positive cells are 'usually considered' to have low ER expression. This endocrine-response-uncertain group may have potential resistance to particular endocrine therapies due to lack of PR [17]. The panel suggested this group should receive endocrine therapy and adjuvant chemotherapy. In an effort to improve accuracy of hormone receptor testing by immunohistochemistry, ASCO/CAP guidelines were revised in 2010 to recommend a cutoff of 1% positive cells be used to define ER-positive status [2]. The panel recommended considering endocrine therapy when tumors show at least 1% ER-positive cells and withholding endocrine therapy if tumors had <1% ER-positive cells [2]. This would increase the proportion of patients receiving endocrine therapy; based on our study, 3% more patients could receive endocrine therapy than if a 10% threshold is used. Although updated guidelines recommend that patients whose tumors show at least 1% ER-positive cells be considered for endocrine therapy, clinicians must consider benefits of endocrine therapy versus cost and side-effects. Tamoxifen is the least expensive of all the endocrine therapies; a generic version costs about $100/month in the USA, according to Susan G. Komen for the Cure (http://ww5.komen.org/uploadedfiles/content_binaries/806–326a.pdf 3 January 2013, date last accessed). Aromatase inhibitors usually cost significantly more than tamoxifen [18]. The side-effects of tamoxifen include vasomotor symptoms, gynecologic symptoms, sexual dysfunction, and increased rates of endometrial cancer, stroke, pulmonary embolism and deep vein thrombosis [19, 20]. Aromatase inhibitors are better tolerated with fewer side-effects but are associated with increased risk of osteopenia, osteoporosis and fractures [21].

Studies have shown that the benefit of tamoxifen increases with increasing ER expression, and that there is a potential benefit for therapy in patients with as little as 1% ER expression [11, 22]. Other clinically relevant genes aside from ER are likely to affect therapeutic benefit. Onco*type* DX® (Genomic Health, Redwood City, CA) is a commercial assay designed to assess recurrence probability in node-negative ER-positive breast cancers. Some have recommended Onco*type* DX® replace immunohistochemistry for ER and PR. However, studies that investigated Onco*type* DX and the predictive validity of the recurrence score in ER-positive breast cancer found no correlation between expression of the hormonal receptor-related genes to clinical outcome [23–25]. A recent study that compared immunohistochemistry with Onco*type* DX® qRT-PCR assay for ER and PR found that immunohistochemistry is preferable to qRT-PCR for determining ER and PR expression [26].

The current study has limitations. First, we retrospectively collected data, and treatment was not assigned in a randomized fashion. Second, because of the limited sample size of patients with ER-positive 1%–9% tumors, we cannot perform subset analyses based on adjuvant chemotherapy and endocrine therapy. Also, we cannot assess predictive ability of ER at different cutoffs by examining the interaction at various cutoff points between patients who received endocrine therapy versus those who did not. Third, some patients had ER determined outside our center, and those ER slides were only reviewed and not restained. We cannot account for differences in ER evaluation method and heterogeneity in methodology may affect results.

In conclusion, patients with ER positive 1%–9% tumors have clinical and pathologic characteristics more similar to tumors that are ER-negative than those with ER positive ≥10% tumors. Similar to patients with ER-negative tumors, those with ER-positive 1%–9% tumors do not appear to benefit from endocrine therapy. Although the substantial benefit of endocrine therapy in ER-positive cases is indisputable, its application to patients with a low level of ER expression, specifically those with ER at 1%–9%, requires further study.

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disclosure

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Phase III placebo-controlled double-blind randomized trial of radiotherapy for stage IIB–IVA cervical cancer with or without immunomodulator Z-100: a JGOG study

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Background: Based on the result of our previous study showing better overall survival (OS) at the lower dose (0.2 μg) of immunomodulator Z-100 than higher dose (40 μg) in patients with locally advanced cervical cancer who received radiotherapy, we conducted a placebo-controlled double-blind randomized trial.

Patients and methods: Patients of stages IIB–IVA squamous cell carcinoma of the uterine cervix were randomly assigned to receive Z-100 at 0.2 μg (Z) or placebo (P). The study agent was given subcutaneously twice a week during the radiotherapy, followed by maintenance therapy by administering once every 2 weeks until disease progression. Primary end point was OS, and secondary end points were recurrence-free survival, and toxicity.

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