The prognostic importance of cathepsin D and E-cadherin in early breast cancer: A single-institution experience

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Abstract. Molecular tools have increasingly been used for decision-making in patients with early breast cancer (EBC). Nevertheless, simple tools such as immunohistochemistry may still be required in particular cases to complement traditional and molecular prognosticators. In this study, the prognostic significance of three well-known immunohistochemical biomarkers, cathepsin D, E-cadherin and Ki67, was studied in 270 patients with EBC, followed by a median time of 126 months in a single institution. Histological examination was performed to confirm the histopathological diagnosis and select specimens. The specimens were evaluated using immunohistochemistry and survival curves were plotted. Results revealed the following patient characteristics: node-negative/1-3 lymph nodes in 228 (86%) patients, hormone receptor-positive in 217 (80%); triple-negative in 31 (11%), and Her2-overexpression in 23 (9%) patients. Breast cancer-related events occurred in 37 patients (14%). A total of 217 patients (80%) survived. Receiver operating characteristic analysis for breast cancer-specific survival showed an area under curve for the clinicopathological model of 0.75 (95% CI, 0.64-0.86), 0.79 (95% CI, 0.68-0.90) for the three-biomarker model, and 0.82 (95% CI, 0.72-0.92) for the E-cadherin and cathepsin D only model. We propose that a simple prognostic model based on combined scores of E-cadherin and cathepsin D may aid treatment decisions in patients with EBC.

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Abbreviations: AUC, area under the curve; BCREs, breast cancer-related events; BCSS, breast cancer-specific survival; CI, confidence interval; ER, estrogen receptor; FISH, fluorescence *in situ* hybridization; HR, hazard ratio; IHC, immunohistochemistry; PgR, progesterone receptor; Q, quartile; ROC, receiver operating characteristic; RFS, relapse-free survival; SUMC, Soroka University Medical Center

Key words: early breast cancer, cathepsin D, E-cadherin, Ki67, immunohistochemistry, biomarkers, prognostic model, treatment decisions

Introduction

Breast cancer is the most common type of cancer and a leading cause of cancer-related mortality in women in developed countries (1). Survival following diagnosis is dependent upon a range of biological factors, including tumor stage, lymph node involvement, pathological grade, hormone receptors and Her2 status. Nevertheless, breast cancer patients at the same stage of disease and sharing similar pathological diagnoses can experience markedly different clinical courses (2). Numerous beneficial prognostic indicators were constructed in patients with early breast cancer (EBC), including the Nottingham Prognostic Index (3), St. Gallen criteria (4), NIH consensus guidelines (5) and Adjuvant! Online (6). However, molecular classification of EBC with the use of minimal sets of genes expressed in the tumor appears to be a more powerful tool for prognostication or prediction of response than current prognostic indicators (7). The most widely clinically used test is the 21-gene recurrence score assay (*Oncotype* DX), which predicts risk of recurrence in patients with estrogen receptor (ER)-positive EBC by measuring the expression of 21 genes in paraffin-embedded tumor material (8). In Israel, *Oncotype* DX has been funded by one or more of the four nationwide health care organizations since 2006. Despite the obvious advantages of the molecular classification of EBC, these studies usually require sending specimens to a central laboratory, are costly and may delay treatment decisions. The present study reports an analysis of the use of three well-recognized immunohistochemical biomarkers, cathepsin D, E-cadherin and Ki67, in patients with EBC.

Patients and methods

Study population. Following approval by the Institutional Review Board, we searched our registry and computerized database to identify patients who were diagnosed at the Soroka University Medical Center (SUMC), Israel, with a first primary breast cancer between January 1st, 1993 and December 31st, 2000. Patient medical records were retrospectively reviewed, and demographical, clinical and pathological data were

recorded. Only patients with a pathological diagnosis of the infiltrating-ductal carcinoma type, for whom adequate pathological specimens and clinical data were available, were included in the study. Patients with a previous history of another primary tumor, or those who had previously received chemotherapy and/or radiotherapy were excluded from the study. Patients diagnosed with pure ductal carcinoma *in situ*, lobular invasive or *in situ* tumors, as well as patients with bilaterality were excluded from the study. The study population included 270 patients who comprised 35% of the total breast cancer patients screened for the study. SUMC is a regional referral hospital providing chemo- and radiotherapy for the population of southern Israel. Baseline clinicopathological data included tumor size, local invasion and lymph node metastasis according to American Joint Committee on Cancer classification for breast cancer, 2002 version (9). In all cases in which adequate surgery was performed, pathological staging was a determining factor. Patients received chemotherapy, radiotherapy and hormonal treatments according to standards of that time-period. No patients with Her2-overexpressing disease received trastuzumab in the adjuvant setting.

Histological examination. H&E-stained slides were reviewed for confirmation of histopathological diagnosis and for selection of adequate specimens for analysis. The histological identification of breast cancer was determined as recommended by the World Health Organization (WHO). Grade was determined according to the Allred score applying the modified Scarff-Bloom-Richardson scoring system (10) by a pathologist (N.S.V.) who was blinded to the clinical data. In each case, a representative paraffin block of the tumor was selected and sections were selected for immunohistochemical studies.

Immunohistochemistry (IHC). Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue sections with the use of standard techniques. The following immunohistochemical studies were performed: ER and progesterone receptors (PgR), Her2, Ki67, cathepsin D and E-cadherin. Expression for cases with a Her2 HercepTest score of 3 were scored as positive, and those of 0 or 1 were scored as negative, as described in the HercepTest (Dako) protocol (11). Cases with Her2 scores of 2 were re-evaluated by fluorescence *in situ* hybridization (FISH) assays. Table I shows data regarding the antibodies used in the present study.

Scoring of stained slides. The immunohistochemical localization of ER and PgR as well as that of E-cadherin, cathepsin D and Ki67, was scored by applying a semi-quantitative method, incorporating both the intensity and the distribution of specific staining as described by Detere *et al* (12). A minimum of 500 tumor cells were counted. If differences occurred between spot intensities, the most positive spot was considered. The evaluations were recorded as percentages of positively stained target cells in each of the four intensity categories, which were denoted as 0 (no staining), 1+ (weak but detectable above control), 2+ (distinct) or 3+ (strong). For each tissue, a value designating the H-Score was derived by adding the percentages of cells staining at each intensity (P_i) multiplied by the weighted intensity of staining, as in the formula: H-Score = $\Sigma P_i\ (i + 1)$, where $i = 1, 2, 3$ and P_i varies from 0 to 100%.

Statistical analysis. Descriptive statistics were calculated as frequencies to summarize clinicopathological characteristics. Outcome measures for this study were breast cancer-related events (BCREs), relapse-free and breast cancer-specific survival (RFS and BCSS). RFS was defined as the time from the date of pathological diagnosis to the first local, regional or distant recurrence or death from breast cancer prior to a recorded relapse. Locoregional recurrence was defined either as local recurrence in the original tumor bed with the same histological features of the primary tumor, or regional recurrence in the lymph nodes. Distant recurrence was defined as the presence of metastatic disease in all other locations.

For patients with multiple BCREs during follow-up, only the first episode was considered in the analysis. New ipsilateral breast cancer or other non-breast primary tumors were considered as censoring events. In the absence of any of these events, observation time was censored at the latest follow-up visit. Only breast cancer-related death was considered in the analysis for BCSS. Patients surviving to the end of the follow-up period or those who succumbed during follow-up of any cause other than breast cancer were censored from the BCSS analysis. The χ^2 and Mann-Whitney U tests were used as appropriate. Hazard ratios for RFS and BCSS were assessed using a Cox model that included characteristics such as age, menopausal status, tumor stage (T), nodal stage (N), ER and PgR status, Her2 status, grade and the scores of cathepsin D, E-cadherin and Ki67. Age was considered as a binary variable with a cut-off value defined at 50 years. The cut-off value for T was >20 vs. ≤20 mm. The cut-off value for N was >3 nodes involved vs. 0 or 1-3. The cut-off value for ER and PgR was defined as >10 vs. ≤10%. The cut-off value for grade was defined as high vs. intermediate or low. The cut-off value for the three biomarkers, cathepsin D, E-cadherin and Ki67, was dichotomized as the fourth quartile vs. the lower three quartiles.

Univariate Cox proportional hazards regression models were estimated by fitting a Cox regression model, and statistically significant variables were included in the multivariate Cox proportional hazards regression models using stepwise selection. Survival curves were plotted as Kaplan-Meier survivor functions. Follow-up was truncated at 130 months for the purposes of plotting. Receiver operating characteristic (ROC) analysis and corresponding area under the curve (AUC) statistics were used for the discriminatory accuracy of models. The following variables were included in the clinicopathological model: T, N, grade, percentage of ER- and PgR-positive cells, and Her2 status. Numerical H-Scores of each biomarker were analyzed as continuous variables and were included in the two- and three-biomarker models.

Statistical tests were two-sided and statistical significance was defined as $P<0.05$. Statistical analyses were conducted using SPSS version 17 for Windows (SPSS Inc., Chicago, IL, USA). This study was written in accordance with the Reporting Recommendations for Tumor Marker Prognostic Studies guidelines (13).

Results

Table II shows the frequencies of baseline characteristics, adjuvant treatments and immunohistochemical markers of all breast cancer patients, separated into two groups according to

Table I. Antibodies, suppliers, dilutions and techniques used for immunohistochemistry and the parameters evaluated.

Antibody	Clone/Ab	Source	Dilution	Technique	Parameters evaluated
Anti-human E-cadherin mouse, monoclonal	NCH-38	DakoCytomation, Denmark	1:100	Ventana Benchmark, Nexes	Percentage and intensity
Anti-human Cathepsin D mouse, monoclonal	DC2000	DakoCytomation, Denmark	1:100	Ventana Benchmark, Nexes	Percentage and intensity
Anti-human Ki-67 mouse, monoclonal	MIB-1	DakoCytomation, Denmark	1:300	Ventana Benchmark, Nexes	Percentage and intensity
Anti-human ER mouse, monoclonal	NCL-ERp	Novocastra, Newcastle upon Tyne, UK	1:100	Dako Autostained	Percentage and intensity
Anti-human PgR mouse, monoclonal	PgR636	Novocastra, Newcastle upon Tyne, UK	1:200	Dako Autostained	Percentage and intensity
Anti-human Her2/NEU mouse, monoclonal	TAB250	Zymed, South San Francisco, CA, USA	1:100	Dako Autostained	Percentage and intensity

Table II. Frequencies of conventional prognostic factors, adjuvant treatments and immunohistochemical markers in the early breast cancer patients.[a]

Characteristic	All patients (n=270)		Event (n=39)		No event (n=231)		P-value
	No.	%	No.	%	No.	%	
Age (year)							0.990
Mean	57.3±12.1		57.3±13.8		57.3±11.9		
Range	29-89		29-83		32-89		
≤40	16	6					
>40	254	94					
Menopause state							0.440
Pre- and peri-menopausal	69	26	12	31	57	25	
Post-menopausal	181	67	27	69	172	75	
Unknown	20	7					
Tumor stage, TNM class							<0.001
T1a	5	2	0	0	5	2	
T1b	31	12	1	2	30	14	
T1c	126	48	10	28	112	52	
T2	91	35	23	62	68	30	
T3	7	3	2	2	5	2	
T4	0	0	0	0	0	0	
Unknown	10	4					
Positive nodes							0.050
0-3	228	86	28	76	200	88	
0	163	60					
1-3	65	24					
>4	37	14	9	24	28	12	
4-10	24	9					
>10	13	5					
Unknown	5	2					

Table II. Continued.

Characteristic	Patients (n=270)		Event (n=39)		No event (n=231)		P-value
	No.	%	No.	%	No.	%	
Tumor grade							0.420
Low	43	16	4	12	39	20	
Intermediate	110	41	11	48	95	49	
High	71	26	13	39	58	30	
Undetermined/unknown	46	17					
ER status							0.040
ER positive status, >10% of tumor cells	199	74	29	64	170	78	
ER negative status, <10% of tumor cells	63	23	15	42	48	21	
Unknown	8	3					
PgR status							0.040
PgR-positive status, >10% of tumor cells	197	75	30	64	167	78	
PgR-negative status, <10% of tumor cells	64	24	16	42	48	21	
Unknown	9	3					
Her2/neu status							0.080
Her2/neu overexpression	15	6	4	11	11	5	
Her2/neu-negative status	245	94	33	88	212	95	
Unknown	10	4					
ER, PgR, Her2/neu (Triple) negative status	31	11	7	18	24	10	0.180
Event type							
Metastasis	34	13	34	90	0	0	
Locoregional relapse	1	0.4	1	3	0	0	
Contralateral breast cancer	2	0.7	2	5	0	0	
Adjuvant tamoxifen							
Yes	212	78					
No	54	20					
Unknown	4	2					
Adjuvant chemotherapy							
Yes	146	54					
No	121	45					
Unknown	3	1					
Adjuvant radiotherapy							
Yes	192	71					
No	72	27					
Unknown	8	2					
Ki67 score							
Yes	264	98					
No	6	2					
E-cadherin score							
Yes	261	97					
No	9	3					
Cathepsin D score							
Yes	252	93					
No	18	7					

[a]The sum of percentages may not be equal to 100 due to rounding. Baseline characteristics include demographics, clinical and pathological parameters according to the occurrence of breast cancer-related events.

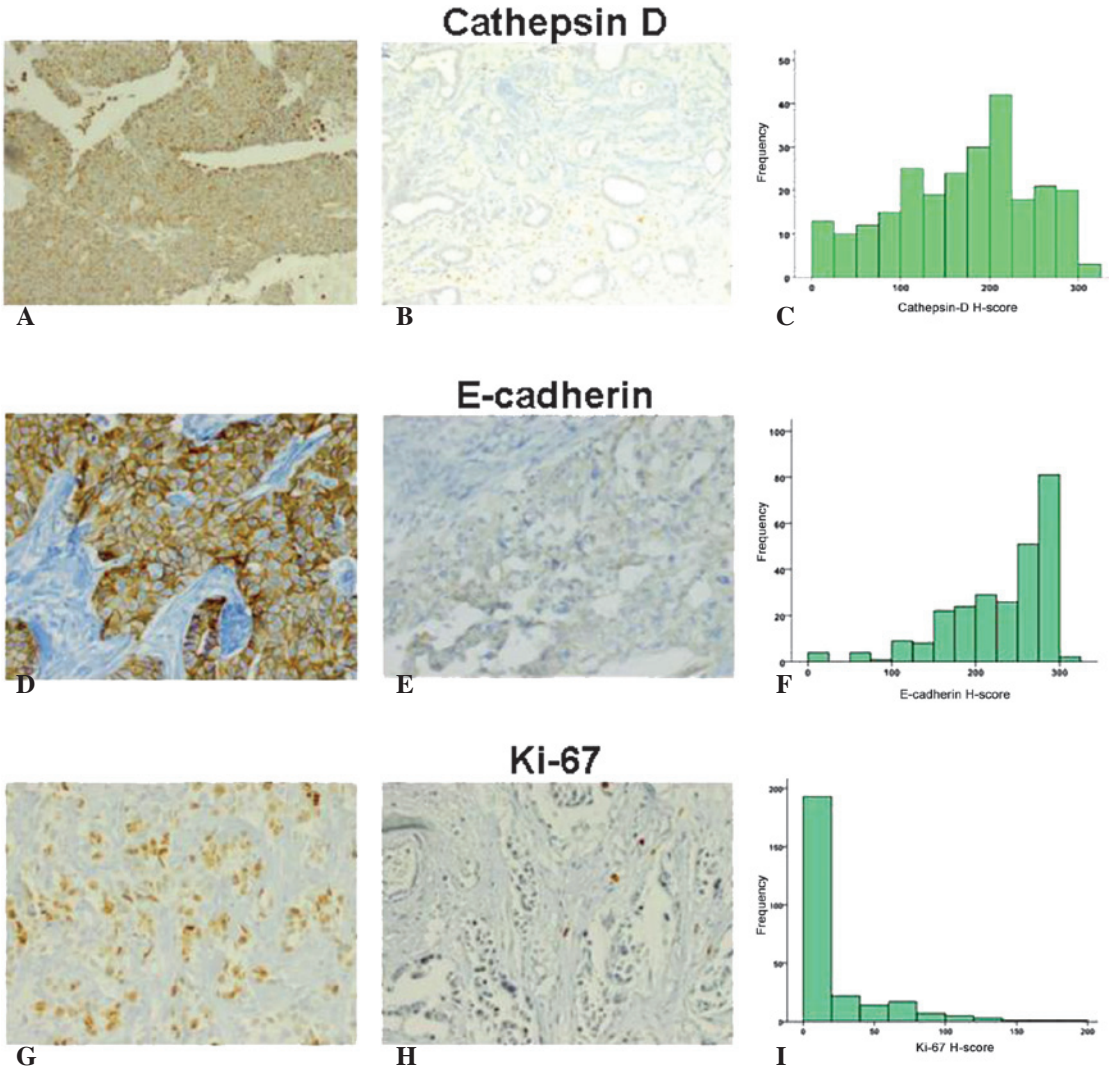

Figure 1. Immunohistochemical studies for cathepsin D, E-cadherin and Ki67 (IHC with diaminobenzidine; magnification, x400) in breast cancer (BC) patients. (A) BC case with a strongly-positive stain for cathepsin D (H-Score=300). (B) BC case with a mildly-positive stain for cathepsin D (H-Score=15). (C) A histogram of cathepsin D scores. (D) BC case with a strongly-positive stain for E-cadherin (H-Score=290). (E) BC case with a mildly-positive stain for E-cadherin (H-Score=20). (F) A histogram of E-cadherin scores. (G) BC case with a strongly-positive stain for Ki67 (H-Score=160). (H) BC case with a mildly-positive stain for Ki67 (H-Score=6). (I) A histogram of Ki67 scores.

the occurrence of BCREs. Fig. 1 shows the characteristics of the immunohistochemical stains for cathepsin D, E-cadherin and Ki67, including representative cases and histograms of each. In univariate analyses, the following parameters were associated with BCREs: i) tumor size (P<0.001), ii) ER-positive status (P=0.01), iii) PgR-positive status (P=0.01), iv) Her2-positive status (P=0.02) and v) lymph node involvement (P=0.05). No statistically significant correlation was found between tumor grade and BCRE. Table III shows the scores of immunohistochemical markers according to the occurrence of BCREs in all breast cancer patients. None of the three study biomarkers correlated with the occurrence of BCREs.

Analysis for RFS. Univariate Cox proportional hazard regression analyses for RFS were performed according to following variables: age <50 years, pre-/peri-menopausal status, tumor size >20 mm, involvement of lymph nodes of >3, positive ER, positive PgR, Her2 overexpression, high-grade tumors, and cathepsin D, E-cadherin and Ki67 in quartiles (highest quartile as the reference group). Among those models, the following parameters were statistically significant in the Cox regression analysis (Table IV): i) tumor size (HR=2.94; P=0.01, 95% CI, 1.41-6.10); ii) positive ER (HR=0.40; P=0.01, 95% CI, 0.21-0.78); iii) positive PgR (HR=0.42; P=0.01, 95% CI, 0.22-0.80); iv) Her2 overexpression (HR=3.11; P=0.01, 95% CI, 1.36-7.10). In the multivariate Cox proportional hazard regression analyses, the only variables that were statistically significant included tumor size (HR=2.63; P=0.01, 95% CI, 1.26-5.54) and PgR status (HR=0.41; P=0.01, 95% CI, 0.21-0.82). The Kaplan-Meier RFS curve for all breast cancer patients is shown in Fig. 2.

Analysis for BCSS. Univariate Cox proportional hazard regression analyses for BCSS were similarly performed according to the same variables. Among those models, the following parameters were statistically significant on the Cox regression analysis (Table IV): i) tumor size (HR=7.37; P=0.001, 95% CI, 2.18-24.93); ii) positive ER (HR=0.29; P=0.01, 95% CI, 0.13-0.68);

Table III. Scores of immunohistochemical markers according to the occurrence of breast cancer-related events in the early breast cancer patients.

Variable	All patients (n=270)	Event (n=39)	No event (n=231)	P-value
ER H-Score				
Mean	102±94	78±100	106±92	0.10
Median	90	15	90	
PgR H-Score				
Mean	86±84	64±80	90±85	0.09
Median	70	30	70	
Ki67 H-Score				
Mean	19±31	17±28	19±31	0.66
Median	5	2	5	
E-cadherin H-Score				
Mean	229±62	227±69	229±61	0.86
Median	250	250	250	
Cathepsin D H-Score				
Mean	168±77	172±84	168±76	0.75
Median	180	185	180	

iii) positive PgR (HR=0.36; P=0.01, 95% CI, 0.16-0.81); iv) Her2 +3 (HR=4.25; P=0.01, 95% CI, 1.67-10.8). Tumor grade showed a trend for statistical significance (HR=2.45; P=0.06, 95% CI, 0.97-6.2). In the multivariate Cox proportional hazard regression analyses, the only variables that were statistically significant included tumor size (HR=5.39; P=0.01, 95% CI, 1.56-18.64) and ER status (HR=0.33; P=0.02, 95% CI, 0.13-0.81). The Kaplan-Meier BCSS curve for all breast cancer patients is shown in Fig. 2.

ROC statistics. Results of the ROC analysis for RFS of the following models: i) combined clinicopathological data; ii) scores for E-cadherin, cathepsin D and Ki67; and iii) scores for E-cadherin and cathepsin D only are shown in Table V. Analysis for a combined clinicopathological model, including parameters such as tumor size, lymph node status, histological grade, ER and PgR scores and Her2 status, resulted in an AUC of 0.51 (95% CI, 0.39-0.64); a model using the scores of three immunohistochemical parameters resulted in an AUC of 0.73 (95% CI, 0.63-0.84), and a model using the scores of cathepsin D and E-cadherin only resulted in an AUC value of 0.75 (95% CI, 0.65-0.85). Results of the ROC analysis for BCSS related to similar models are shown in Table V. The AUC of a combined clinicopathological model was 0.75 (95% CI, 0.64-0.86). The AUC of the three biomarker models was 0.79 (95% CI, 0.68-0.90), and the AUC of a model of E-cadherin and cathepsin D was only 0.82 (95% CI, 0.72-0.92).

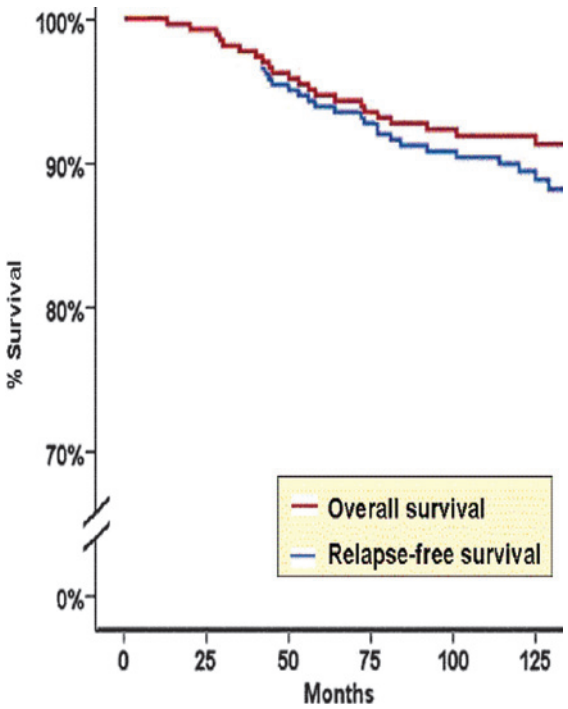

Figure 2. Kaplan-Meier relapse-free and disease-specific survival curves for the early breast cancer patients.

Discussion

In this single-institutional study, we showed that a model incorporating two immunohistochemical biomarkers, E-cadherin and cathepsin D, in tumors of patients with EBC was comparable to a model based on standard clinicopathological parameters in predicting BCSS. We used a simple scoring method that provided a quantitative measure for the degree of staining intensity of each biomarker. The combination of E-cadherin and cathepsin D proved valuable despite the fact that none of the individual biomarkers was capable of predicting prognosis. Overexpression of cathepsin D and low expression of E-cadherin may therefore be used to detect distinct sets of EBC in patients with a more aggressive form of the disease.

Each of the three biomarkers has been extensively studied in the past for its contribution to tumor aggressiveness. In

Table IV. Clinical, pathological and immunohistochemical parameters.

Variable	Relapse-free survival			Overall survival		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Age ≤50 vs. >50 years	1.09	0.57-2.08	0.79	1.09	0.48-2.49	0.840
Pre-/peri-menopause vs. post-menopause	1.27	0.64-2.50	0.50	1.18	0.49-2.85	0.710
Tumor size >20 vs. <20 mm	**2.94**	**1.41-6.10**	**0.01**	**7.37**	**2.18-24.93**	**0.001**
Positive lymph nodes >3 vs. 0-3	1.96	0.93-4.17	0.08	1.75	0.65-4.72	0.270
Positive ER status vs. negative	**0.40**	**0.21-0.78**	**0.01**	**0.29**	**0.13-0.68**	**0.010**
Positive PgR status vs. negative	**0.42**	**0.22-0.80**	**0.01**	**0.36**	**0.16-0.81**	**0.010**
Positive Her2/neu status vs. negative	**3.11**	**1.36-7.10**	**0.01**	**4.25**	**1.67-10.80**	**0.010**
High- vs. low- and intermediate-grade	1.77	0.87-3.57	0.11	2.45	0.97-6.20	0.060
Ki67 (quartiles) Q4 vs. Q1-3	0.91	0.60-1.19	0.47	0.86	0.61-1.22	0.400
E-cadherin Q4 vs. Q1-3	1.06	0.79-1.43	0.70	0.79	0.54-1.43	0.220
Cathepsin D Q4 vs. Q1-3	1.08	0.81-1.43	0.60	1.06	0.73-1.51	0.790

Univariate Cox proportional hazards regression analyses for relapse-free and overall survival. Bold, statistically significant. Q, quartiles. CI, confidence interval.

Table V. ROC analysis and corresponding area under the curve (AUC) statistics for prediction models of breast cancer-related events and breast cancer-specific death in patients with early breast cancer.

Prediction model	BC-related events		BC-specific death	
	95% CI	C-statistics (AUC)	95% CI	C-statistics (AUC)
Clinicopathological (combined)	0.51	0.39-0.64	0.75	0.64-0.86
Three markers (Ki67, cathepsin D and E-cadherin)	0.73	0.63-0.84	0.79	0.68-0.90
Two markers (cathepsin D and E-cadherin)	0.75	0.65-0.85	0.82	0.72-0.92

BC, breast cancer; CI, confidence interval; AUC, area under the curve.

clinical practice, however, there remains some controversy regarding the manner in which biomarkers support treatment decisions. A critical impediment to their wider use is the lack of standardization for the interpretation of staining results. Overexpression of cathepsin D in breast tumors was associated with increased metastatic potential and poor survival (14). Although it has been held that cathepsin D is involved in a non-specific protein degradation in a markedly acidic environment of lysosomes, an increasing number of studies have shown that cathepsin D interacts with other significant molecules and affects cell signaling. Procathepsin D, the proform of lysosomal aspartic peptidase cathepsin D secreted from cancer cells, acts as a mitogen on cancer and stromal cells and stimulates their pro-invasive and pro-metastatic properties (15). In a model of neuroblastoma, extracellular exogenous cathepsin D induced Akt-1 phosphorylation and doxorubicin resistance in sensitive cells (16). Unlike infiltrating lobular carcinomas, which consistently exhibit a loss of E-cadherin expression regardless of clinical staging or outcome, over 80% of infiltrating ductal carcinomas continue to express E-cadherin, albeit at a progres-

sively reduced level with an increasing stage or histological grade (17). E-cadherin is a transmembrane glycoprotein that mediates calcium-dependent intercellular adhesion and tissue architecture among epithelial cell layers (18,19). However, a reduced expression of E-cadherin may also underscore the 'stem-cell behavior' of breast cancer cells, since E-cadherin is regulated by Slug, Snail and Twist, which belong to the canonical Wnt/β-catenin signaling system (20,21). Notably, the addition of Ki67 to the model of cathepsin D and E-cadherin in our study did not improve prediction over and above the two-biomarker model. Multiple studies have previously shown that the immunohistochemical expression of nuclear Ki67 may be prognostic and predictive in patients with EBC (22), and the majority of gene expression-based predictors, including *Oncotype* DX, make use of proliferation phenotypes (23).

Our findings indicate that decision-making in patients with EBC is feasible. Our group has recently published its experience with the use of *Oncotype* DX in patients with EBC and ER-positive, node-negative tumors (24). Recommendations for chemotherapy were changed after obtaining assay results in 25% of patients, where the majority of changes (71%) were from chemotherapy to no chemotherapy. Most importantly, *Oncotype* DX correlated poorly with Adjuvant! Online predictions. Nevertheless, we experienced uncertainty in regard to the correct treatment for patients with intermediate risk and recurrence scores. In such cases, treatment decisions are based on numerous parameters, including histological grade, clinical judgment and patients' willingness to receive chemotherapy. Although histological grade is almost universally accepted by clinicians, included in consensus guidelines and used in treatment decision-making (25), it is considered by certain investigators to have poor reproducibility, particularly for nuclear pleomorphism and mitotic count. Clinical judgment remains highly subjective, and patients' willingness to receive chemotherapy may not be based on solid data. Moreover, treatment decisions in many cases are required while molecular studies are unavailable. IHC has shown good reproducibility when performed by skilled specialists and in experienced high-volume laboratories. Beyond its simplicity and relative cost efficacy, IHC may prove prudent in the setting of limited tissue availability through the use of needle or core biopsies (26). The present study included a heterogeneous population of patients with EBC who received various treatments, and none of the patients received certain treatment options that now are considered standard, such as trastuzumab or taxanes. Another limitation of the present study is the lack of validation of the proposed prognostic model.

In conclusion, our results show that a prognostic model based on the scores of cathepsin D and E-cadherin staining intensity on paraffin sections of breast tumors may be beneficial in treatment decisions for patients with EBC, and in particular cases complement traditional and molecular prognosticators.

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