Prognostic Utility of Breast Cancer Index to Stratify Distant Recurrence Risk in Invasive Lobular Carcinoma

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ABSTRACT

Purpose: The prognostic utility of Breast Cancer Index (BCI) for risk assessment of overall (0–10 years), early (0–5 years), and late (5–10 years) distant recurrence (DR) in hormone receptor–positive (HR+) invasive lobular carcinoma (ILC) was evaluated.

Experimental Design: BCI gene expression analysis was performed blinded to clinical outcome utilizing tumor specimens from patients with HR+ ILC from a multi-institutional cohort. The primary endpoint was time to DR. Kaplan–Meier analyses of overall, early, and late DR risk were performed, and statistical significance was evaluated by log-rank test and Cox proportional hazards regression. The prognostic contribution of BCI in addition to clinicopathologic factors was evaluated by likelihood ratio analysis.

Results: Analysis of 307 patients (99% ER+, 53% T1, 42% N+, 70% grade II) showed significant differences in DR over 10 years based on BCI risk categories. BCI low- and intermediate-risk patients demonstrated similar DR rates of 7.6% and 8.0%, respectively, compared with 27.0% for BCI high-risk patients. BCI was a significant independent prognostic factor for overall 10-year DR [HR = 4.09; 95% confidence interval (CI), 2.00–8.34; $P = 0.0001$] as well as for both early (HR = 8.19; 95% CI, 1.85–36.30; $P = 0.0042$) and late (HR = 3.04; 95% CI, 1.32–7.00; $P = 0.0224$) DR. In multivariate analysis, BCI remained the only statistically significant prognostic factor for DR (HR = 3.49; 95% CI, 1.28–9.54; $P = 0.0150$).

Conclusions: BCI is an independent prognostic factor for ILC and significantly stratified patients for cumulative risk of 10-year, early, and late DR. BCI added prognostic value beyond clinicopathologic characteristics in this distinct subtype of breast cancer.

Introduction

Invasive lobular carcinoma (ILC) is the second most common histologic subtype of invasive breast cancer and accounts for approximately 10% to 15% of all breast cancers (1). ILC displays distinct pathologic, molecular, and clinical characteristics compared with those of the more commonly diagnosed invasive ductal carcinoma (IDC; refs. 1–5). Loss of E-cadherin expression is a defining characteristic of ILC and results in reduced cell–cell adhesion and tumor morphology in which cells invade tissues in a chain-like single-file manner (1). ILC tumors are predominantly estrogen receptor positive (ER+), HER2 negative (HER2−), and of low grade and low proliferative index (1–5). Although these tumor characteristics are generally associated with favorable prognoses, ILC tumors have an increased risk of late distant recurrence (DR) and can display aggressive metastatic behavior associated with poorer long-term outcomes when compared with stage-matched IDC (2, 5–9). Despite the unique clinical challenges of ILC versus IDC, current clinical practice guidelines recommend similar treatment paradigms for both histologic subtypes (5, 10). Thus, there is an unmet medical need for enhanced approaches that interrogate underlying ILC tumor biology to better individualize treatment and long-term disease management (2, 11).

The Breast Cancer Index (BCI) is a gene expression–based signature that incorporates two functional biomarker panels: (i) the 2-gene ratio, *HOXB13/IL17BR* (H/I), and (ii) the 5-gene Molecular Grade Index (MGI). The BCI test is indicated for patients with early-stage, hormone receptor–positive (HR+) breast cancer and reports both a predictive and a prognostic result. The predictive component, BCI (H/I), reports a categoric prediction of high versus low likelihood of benefit from extended endocrine therapy (12–14), whereas the prognostic component, the BCI score, is based on the algorithmic combination of H/I and MGI and stratifies risk for overall (0–10 years) and late (5–10 years post-diagnosis) DR (12, 13, 15).

BCI prognostic models have been developed for both node-negative (N0) and node-positive (N+) disease (13, 16). The N0 prognostic model is based on gene expression alone and categorizes patients into low-, intermediate-, and high-risk groups (13), whereas the N+ prognostic model incorporates tumor size and grade with gene expression and dichotomizes patients with N+ tumors into low- and high-risk categories (16). BCI prognostic ability has been validated in multiple studies of breast cancer patients, which also included approximately 12% patients with ILC (13, 15–17). This study examines prognostic risk stratification of BCI specifically in ILC in a blinded multi-institutional analysis.

Materials and Methods

Study design and patient samples

In this retrospective study, formalin-fixed paraffin-embedded (FFPE) tumor specimens from 376 patients diagnosed with ILC

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Translational Relevance

Invasive lobular breast cancer (ILC) is a heterogenous disease with diverse clinical outcomes and considerable risk of late metastasis. Enhanced molecular approaches that provide information on the tumor biology of this distinct subtype of invasive breast cancer are needed to inform prognosis and individualized treatment. In this study, Breast Cancer Index (BCI) significantly stratified patients with ILC into risk groups based on risk of overall 10-year, early, and late distant recurrence. BCI provided distinct and additive prognostic information beyond clinicopathologic factors and reclassified a meaningful number of clinically low-risk tumors as high genomic risk and clinically high-risk tumors as low genomic risk. These findings demonstrate that BCI is an independent prognostic factor for ILC and suggest its potential role to enhance individualization of ILC treatment.

between 1992 and 2011 were collected from The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University (JHU, $N = 111$), Dana-Farber Cancer Institute (DFCI, $N = 95$), Massachusetts General Hospital (MGH, $N = 76$), and the Pitt Biospecimen Core (PBC) at the University of Pittsburgh and University of Pittsburgh Medical Center (UPMC) Magee-Womens Hospital (UPMC, $N = 94$). Inclusion criteria included HR+ (≥1% positive stained cells for ER or progesterone receptor (PR) based on ASCO/CAP guidelines; ref. 18), stage I to III based on AJCC 7th Ed. (19), any nodal status, and pure lobular or mixed lobular/ductal histology. HER2 status was determined according to standard procedures at the time of patient diagnosis by IHC and FISH analysis. Patients were excluded if treated with neoadjuvant therapy, missing clinical information (i.e., tumor size or nodal status), or inadequate survival follow-up.

Breast cancer index assay

BCI gene expression analysis of FFPE primary tumor specimens was performed blinded to clinical outcome as described previously (13). Briefly, macro-dissection was performed on FFPE sections to enrich tumor content followed by RNA extraction. Patients were excluded if their specimen had less than 40% tumor content according to assay requirements. Total RNA was reverse transcribed, and the resulting cDNA was pre-amplified by PCR using the PreAmp Master Mix Kit (Thermo Fisher Scientific) prior to TaqMan RT-PCR analysis. Calculation of risk scores and categorical risk stratification were performed using prespecified cut points for the N0 and N+ prognostic models (12, 13). The BCI prognostic model for N0 has three reporting categories (low-, intermediate-, and high-risk) whereas the N+ model (BCIN+) has two (low- and high-risk). To examine risk stratification in the overall cohort, patients were divided into three risk groups using both BCI models. The low-risk group for the overall cohort combined the low-risk patients from the N0 (using BCI gene expression alone) and N+ subsets (using BCIN+). The intermediate-risk group consisted of intermediate-risk patients from the N0 subset. The high-risk group combined the high-risk patients from the N0 and N+ subsets. For simplification, "BCI" throughout the manuscript refers to the combined risk groups in the overall cohort, which integrates prognostic risk categories from both the N0 (BCI gene expression alone) and N+ (BCIN+, gene expression with tumor size and grade) models. Calculation of a BCI predictive score [BCI (H/I)] for response to extended endocrine therapy was conducted using a pre-specified and validated assay cut-point to categorize patients as either BCI (H/I)-high (likely to respond) or BCI (H/I)-low (unlikely to respond; ref. 20).

Pilot study comparing laser capture microdissection with standard macro-dissection

To determine the analytical impact of tissue stroma and further define sample requirements based on cellularity, 23 tumor samples were analyzed in a pilot study comparing tissue processing by either the standard manual macro-dissection or laser capture microdissection (LCM). It was hypothesized that LCM would enrich for invasive tumors cells, thus impacting BCI results. Pre-specified criteria to determine equivalence between dissection methods were based on an empirically defined ≥80% concordance using the BCI assay variability threshold of two SDs of BCI score difference (1.2 BCI units).

Study endpoints

The primary endpoint was time to DR, defined as the time from diagnosis to first DR. Contralateral disease, local and regional recurrence, and other second primary cancers were not considered as events nor censored. The survival analysis was censored at 10 years. The primary objective was to evaluate the prognostic performance of BCI for risk of overall 10-year, early (0–5 years), and late (5–10 years) DR. The secondary objective was to evaluate the prognostic performance of BCI in clinically relevant subsets.

Statistical considerations and analyses

Kaplan–Meier analysis was used to estimate the overall 10-year, early (0–5 years), and late (5–10 years) DR risk for BCI risk groups. The log-rank test was used to test the equality of survival curves and a univariate Cox proportional hazards regression model was used to estimate HRs and the associated 95% confidence intervals (CI). A multivariate Cox proportional hazards regression model was used to evaluate whether BCI provided independent prognostic information in addition to standard clinicopathologic factors that were significant in the univariate analysis (age, adjuvant endocrine therapy, adjuvant chemotherapy, tumor size, tumor grade, nodal status) using Wald tests. Likelihood ratio statistics (ΔLR-χ^2) were calculated on the basis of Cox proportional hazards regression models to measure the relative contributions of BCI gene expression alone or together with tumor size and grade. A two-sided P value of less than 0.05 was considered statistically significant. All analyses were performed using R statistical package (version 3.5.2, http://www.r-project.org).

Results

BCI testing and clinicopathologic characteristics

Prior to standard BCI testing, a pilot study was conducted to evaluate the impact of tumor cellularity on BCI results comparing LCM to standard manual macro-dissection in 23 ILC tumor specimens. Tumor content in selected areas ranged between 15% to 85% (IQR: 30%) with two cases being excluded due to insufficient tumor quantity and three cases due to insufficient RNA yield in the LCM sample (Supplementary Fig. S1). In the remaining 18 paired samples, a high concordance (86%) in BCI scores was observed across both tissue dissection methods, with three samples being above the predetermined concordance threshold of >1.2 BCI units' difference (Supplementary Fig. S2). The two methods of LCM and manual micro-dissection were considered equivalent based on the prespecified concordance threshold. On the basis of this data, subsequent analysis for the study was performed following the standard manual macro-dissection. BCI results were generated in 307 patients (JHU, $N = 80$; DFCI, $N =$

Table 1. Clinicopathologic characteristics of ILC cohort ($N = 307$).

	Patients, *n* (%)
Age	
<50	88 (29%)
≥50	219 (71%)
Primary surgery	
Lumpectomy	130 (46%)
Mastectomy	148 (52%)
Other	7 (2%)
Unknown	22
ER status	
Positive	306 (100%)
Unknown	1
PR status	
Positive	273 (92%)
Negative	25 (8%)
Unknown	9
HER2 status	
Positive	15 (6%)
Negative	224 (94%)
Unknown	68
Histological type	
Lobular	292 (95%)
Mixed	15 (5%)
T stage	
T1	163 (53%)
T2	116 (38%)
T3	27 (9%)
Unknown	1
Tumor grade	
Well	59 (20%)
Moderate	202 (70%)
Poor	29 (10%)
Unknown	17
Stage	
Stage I	107 (35%)
Stage II	167 (55%)
Stage III	32 (10%)
Unknown	1
Nodal status	
N0	179 (58%)
N1	103 (34%)
N2	15 (5%)
N3	10 (3%)
Adjuvant chemotherapy	
No	142 (50%)
Yes	140 (50%)
Unknown	25
Adjuvant endocrine therapy	
No	21 (7%)
Yes	263 (93%)
Unknown	23

76; MGH, $N = 76$; UPMC, $N = 75$) of 376 HR+ evaluated cases. Samples were excluded ($N = 69$) due to previous neoadjuvant chemotherapy, a history of remote breast cancer, insufficient tumor content, or missing clinical information for an attrition rate of 18% (Supplementary Fig. S3). BCI assay failure rate was 0%.

Patient and tumor characteristics are summarized in **Table 1**. Of the 307 patients, 71% were ≥50 years old and 42% had N+ tumors, with 95% of tumors exhibiting pure ILC histology and 5% with mixed ILC and IDC histology. Among patients with available tumor information, 100% were ER+ (306/306), 94% were HER2− (224/239), 47% were classified as T2 or higher (143/306), 70% had moderate-grade tumors (202/290), 55% were stage II (167/306), 50% were treated with adjuvant chemotherapy (140/282), and 93% received adjuvant endocrine therapy (263/284). HER2 status was not available for 68 patients either because the biomarker was not routinely assessed in older cases, accounting for the majority with unknown HER2 status, or that the data were lost when clinical information was migrated from medical record databases. Among the 233 patients with specific information on the type of endocrine therapy, 35% received tamoxifen monotherapy, 35% received aromatase inhibitor (AI) monotherapy, and 30% received tamoxifen-AI sequential treatment.

The median follow-up for the overall population was 10 years (7.4, 14.4, 10.0, and 11.0 years for the JHU, DFCI, MGH, and UPMC cohorts, respectively). There were 41 DR events in the entire cohort (13% of patients), 63% of which occurred more than 5 years after diagnosis. For each BCI risk group (BCI low/intermediate or BCI high), differences in clinicopathologic variables were evaluated (Supplementary Table S1). As expected, the group of patients classified as BCI low/intermediate were more likely to be T stage T1 (60% vs. 46%; $P = 0.0496$), well-differentiated (37% vs. 1%; $P < 0.0001$), stage I (53% vs. 14%; $P < 0.0001$), and N0 (87% vs. 24%; $P < 0.0001$; Supplementary Table S1).

BCI is a significant prognostic factor in ILC

Significant differences in outcome based on BCI risk stratification were observed. In the overall cohort ($N = 307$), BCI classified 40% of patients ($N = 123$) as low-risk with a 10-year DR rate of 7.6% (95% CI, 2.0%–12.9%), 14% of patients ($N = 44$) as intermediate-risk with a 10-year DR rate of 8.0% (95% CI, 0.0%–16.4%), and 46% of patients ($N = 140$) as high-risk with a 10-year DR rate of 27.0% (95% CI, 18.3%–34.9%; **Fig. 1A**). The low- and intermediate-risk patients displayed similar rates of DR, and therefore were combined into a single low/intermediate-risk group with a 10-year DR rate of 7.8% (95% CI, 3.0%–12.4%; **Fig. 1B**). BCI significantly stratified patients with ILC into high- and low/intermediate-risk groups based on overall 10-year (HR = 4.09; 95% CI, 2.00–8.34; $P = 0.0001$), early (0–5 years) (HR = 8.19; 95% CI, 1.85–36.30; $P = 0.0042$), and late (5–10 years) DR (HR = 3.04; 95% CI, 1.32–7.00; $P = 0.0224$; **Fig. 1B–D**). Of the 248 patients that remained free of DR for at least 5 years, 57% were classified as low/intermediate-risk with a late DR rate of 6.5% (95% CI, 2.0%–10.9%) compared with a DR rate of 18.7% (95% CI, 10.4%–26.3%) in the high-risk group (**Fig. 1D**). Overall, the low/intermediate-risk group had a favorable prognosis with DRs predominantly occurring late (post-5 years; 6.5%; 95% CI, 1.3%–7.0%) rather than early (1.4%; 95% CI, 0.0%–3.2%). In contrast, the high-risk group demonstrated a persistent and increasing risk of DR over the entire 10-year period (**Fig. 1B–D**). Results in patients that received adjuvant endocrine therapy ($N = 263$) were similar to the overall cohort (Supplementary Fig. S4). The overall cohort was then stratified by BCI (H/I) to evaluate the likelihood of benefit from extended endocrine therapy. Of the BCI low/intermediate-risk patients, 34% were classified as BCI (H/I)-high and predicted to benefit from extended endocrine therapy (Supplementary Fig. S5). In addition, 49% of the BCI high-risk patients were classified as BCI (H/I)-low and not predicted to benefit from extended endocrine therapy (Supplementary Fig. S5).

In the N0 subset ($N = 179$), BCI gene expression classified 81% of patients as low/intermediate-risk and 19% as high-risk, and the 10-year DR rates were 8.2% (95% CI, 2.8%–13.3%) and 24.7% (95% CI, 6.5%–39.5%), respectively (HR = 3.85; 95% CI, 1.43–10.35; $P = 0.0158$; Supplementary Fig. S6A). In contrast, BCIN+ classified

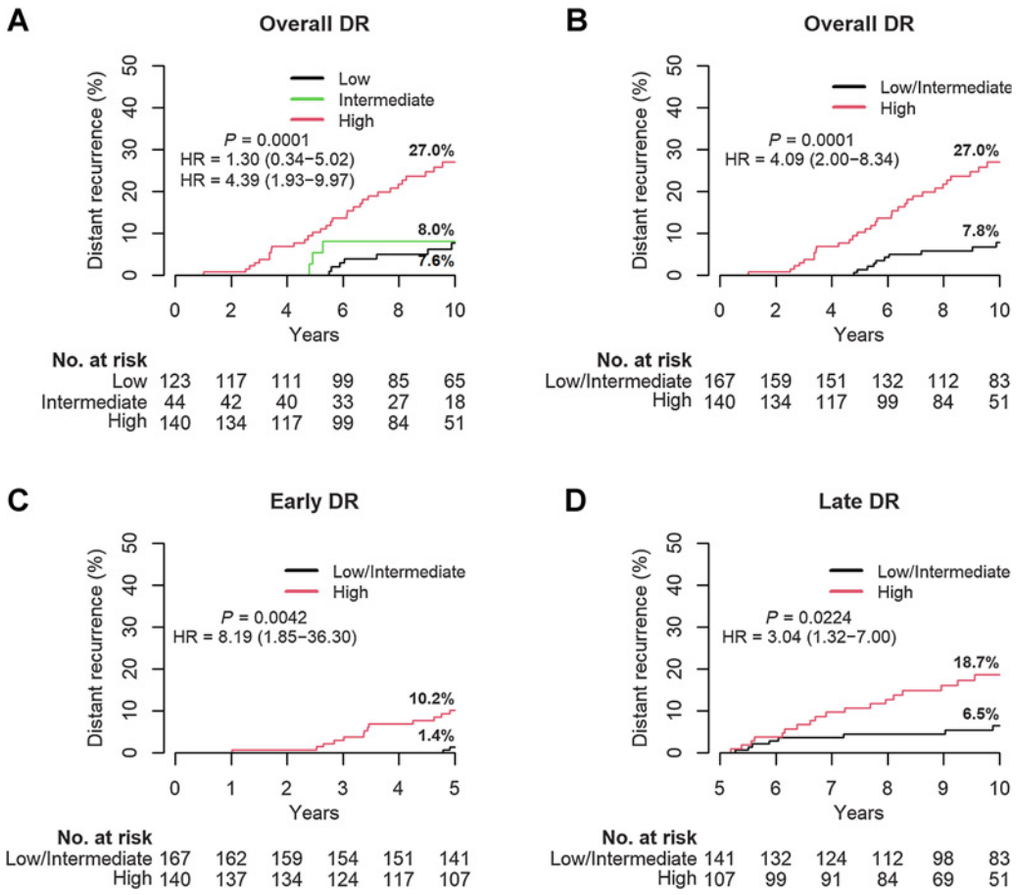

Figure 1.

Prognostic performance of BCI for overall 10-year, early (0–5 years), and late (5–10 years) DR rate for all patients in the lobular cohort utilizing (**A**) BCI risk stratification by three prognostic risk groups (low, intermediate, high) and (**B–D**) BCI risk stratification by two prognostic groups (low/intermediate, high).

17% of N+ patients ($N = 128$) into the low-risk group compared with 83% in the high-risk group (Supplementary Table S2), and the 10-year DR rates were 5.6% (95% CI, 0.0%–15.6%) and 27.9% (95% CI, 17.6%–37.0%), respectively (HR = 5.90; 95% CI, 0.80–43.64; $P = 0.1419$; Supplementary Fig. S6B).

Patients with well- and moderately-differentiated tumors accounted for 90% of the overall cohort, and within this group 53% were N0 and 47% were N+. Among these patients with well- and moderately-differentiated tumors, the 10-year rate of DR was 16.3% (95% CI, 11.0%–21.2%), and BCI stratified 45% of patients into a high-risk group with a 10-year rate of DR of 26.0% (95% CI, 16.6%–34.4%), and stratified 55% of patients into a low/intermediate-risk group with a 10-year rate of DR of 8.2% (95% CI, 2.8%–13.4%; HR = 3.78; 95% CI, 1.76–8.09; $P = 0.0012$). In addition, BCI significantly stratified patients for both early (HR = 11.64; 95% CI, 1.47–91.90; $P = 0.0126$) and late (HR = 2.78; 95% CI, 1.19–6.49; $P = 0.0484$) DR (**Fig. 2A**).

In the 65% of patients with stage II and III breast cancer (36% N0, 64% N+), the overall 10-year DR rate was 29.5% (95% CI, 19.7%–38.1%) for the high-risk group and 10.5% (95% CI, 1.9%–18.3%) for the low/intermediate-risk group (HR = 3.37; 95% CI, 1.40–8.12; $P = 0.0158$). These patients with stage II and III disease could also be further stratified for both early (HR = 4.09; 95% CI, 0.91–18.27; $P = 0.1352$) and late (HR = 3.01; 95% CI, 1.01–8.95; $P = 0.1139$) DR (**Fig. 2B**) with 39% classified as low/intermediate-risk. Patients treated with chemotherapy were similarly stratified for overall 10-year (HR = 4.62; 95% CI, 1.60–13.36; $P = 0.0081$), early ($P = 0.0246$), and late (HR = 2.73; 95% CI, 0.89–8.38; $P = 0.1876$) DR risk (**Fig. 2C**).

BCI was prognostic in patients ≥50 years of age for overall (HR = 4.16; 95% CI, 1.88–9.23; $P = 0.0007$), early (HR = 4.97; 95% CI, 1.06–23.41; $P = 0.0789$), and late (HR = 3.89; 95% CI, 1.53–9.86; $P = 0.0087$) DR. Since patients under 50 years of age primarily had early DRs, BCI was significantly prognostic for early DR ($P = 0.0414$) but not late DR ($P = 0.9235$) within this age group (**Fig. 3**).

BCI is an independent prognostic factor beyond clinicopathologic parameters

Univariate analysis in the overall cohort showed that adjuvant chemotherapy, tumor size, tumor grade, nodal status, and BCI each provided significant prognostic information for overall 10-year DR rate, whereas age and adjuvant endocrine therapy did not. However, in the multivariate analysis, after adjusting for other significant prognostic factors identified in the univariate analysis, BCI remained the only significant and independent prognostic factor for overall 10-year DR rate (HR = 3.49; 95% CI, 1.28–9.54; $P = 0.015$; **Table 2**).

To further examine the additive prognostic value of BCI gene expression versus tumor size and grade, likelihood ratio statistics ($\Delta LR\text{-}\chi^2$) were calculated for the N0 and N+ subsets. As shown

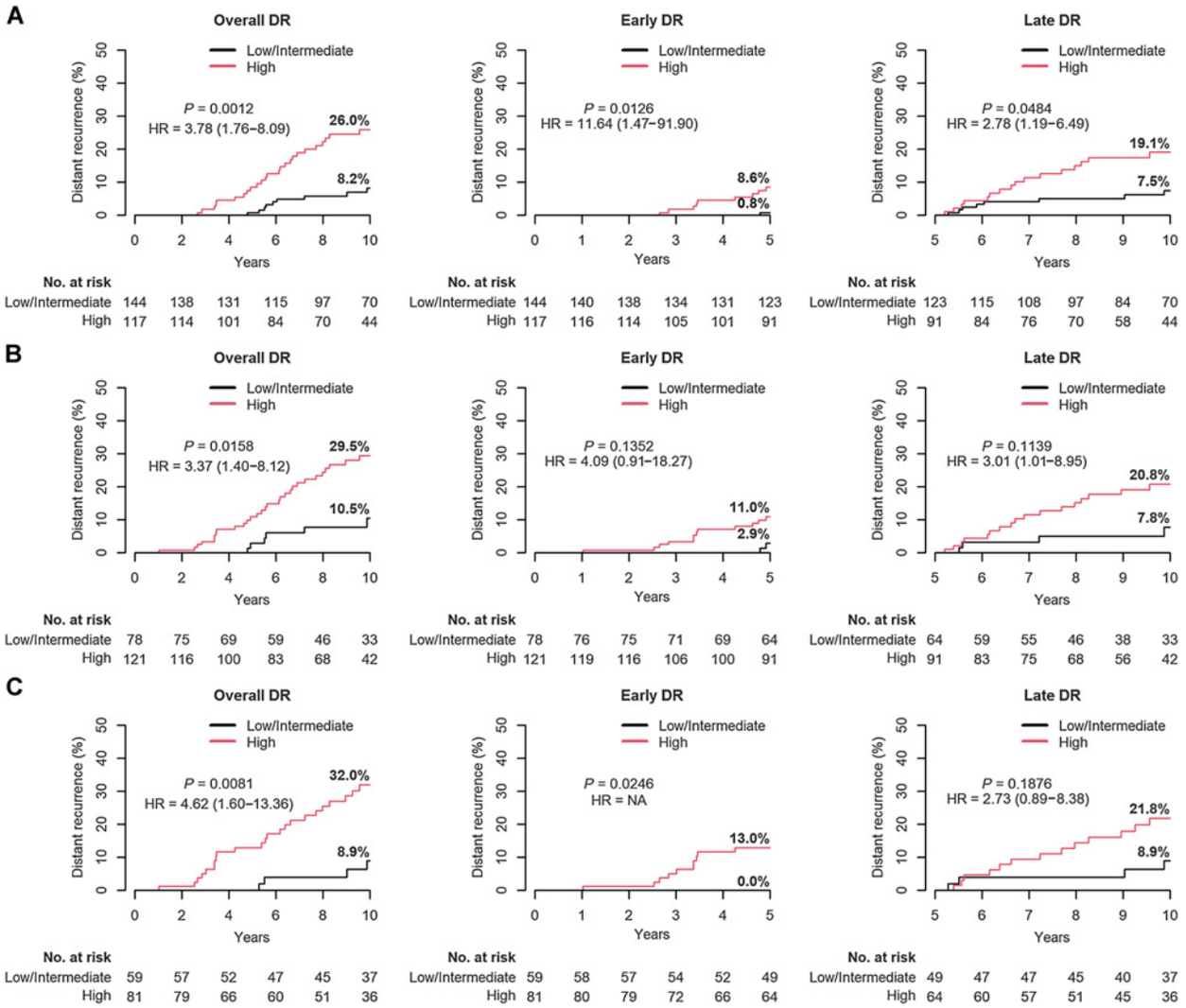

Figure 2.

Prognostic performance of BCI for overall 10-year, early (0–5 years), and late (5–10 years) DR rate for patients with (**A**) well- and moderately-differentiated tumors, (**B**) stage II and III tumors, and (**C**) chemotherapy treatment.

in **Fig. 4**, BCI gene expression was highly prognostic in both N0 (ΔLR-$\chi^2 = 6.28$) and N+ (ΔLR-$\chi^2 = 6.82$) patients. In the N0 subset, BCI was more prognostic than tumor size (ΔLR-$\chi^2 = 2.52$), but not more than tumor grade (ΔLR-$\chi^2 = 7.74$) or tumor size plus grade (ΔLR-$\chi^2 = 10.05$). BCI added independent prognostic information to tumor grade, tumor size, and tumor size plus grade, and conversely, tumor grade and tumor size plus grade added independent prognostic information to BCI gene expression. In the N+ subset, BCI provided greater prognostic information versus tumor size (ΔLR-$\chi^2 = 0.02$), grade (ΔLR-$\chi^2 = 2.10$), or tumor size plus grade (ΔLR-$\chi^2 = 2.14$), and added more prognostic information to tumor size and grade (ΔLR-$\chi^2 = 5.35$) than these variables added to BCI (ΔLR-$\chi^2 = 0.67$).

Discussion

Findings from this study, which investigated the prognostic ability of BCI in a multi-institutional cohort of patients with ILC treated with endocrine or chemo-endocrine therapy, demonstrate that BCI is a significant and independent prognostic factor in ILC and provides risk stratification for cumulative 10-year DR, as well as both early (0–5 years) and late (5–10 years) DR. Patients with ILC were stratified into a low/intermediate-risk group comprising 54% of the patients, with a DR rate of approximately 7.8%, and a high-risk group including 46% of patients with a DR rate of approximately 27.0%. Importantly, BCI also significantly stratified patients with ILC for late DR, with prognostic performance consistent across all clinical subsets examined. It has been previously reported that lymph node status, tumor size, age, S-phase, PR status, and ER status are significant clinical factors independently associated with recurrence and survival (21). In addition, increased tumor size and nodal status have been shown to be associated with risk of late DR (22). However, results from the current study underscore the utility of genomic risk assessment in ILC, as BCI classified 19% of patients with N0 tumors and 45% of patients with well/moderately-differentiated tumors as high-risk. Conversely, 17% of patients with

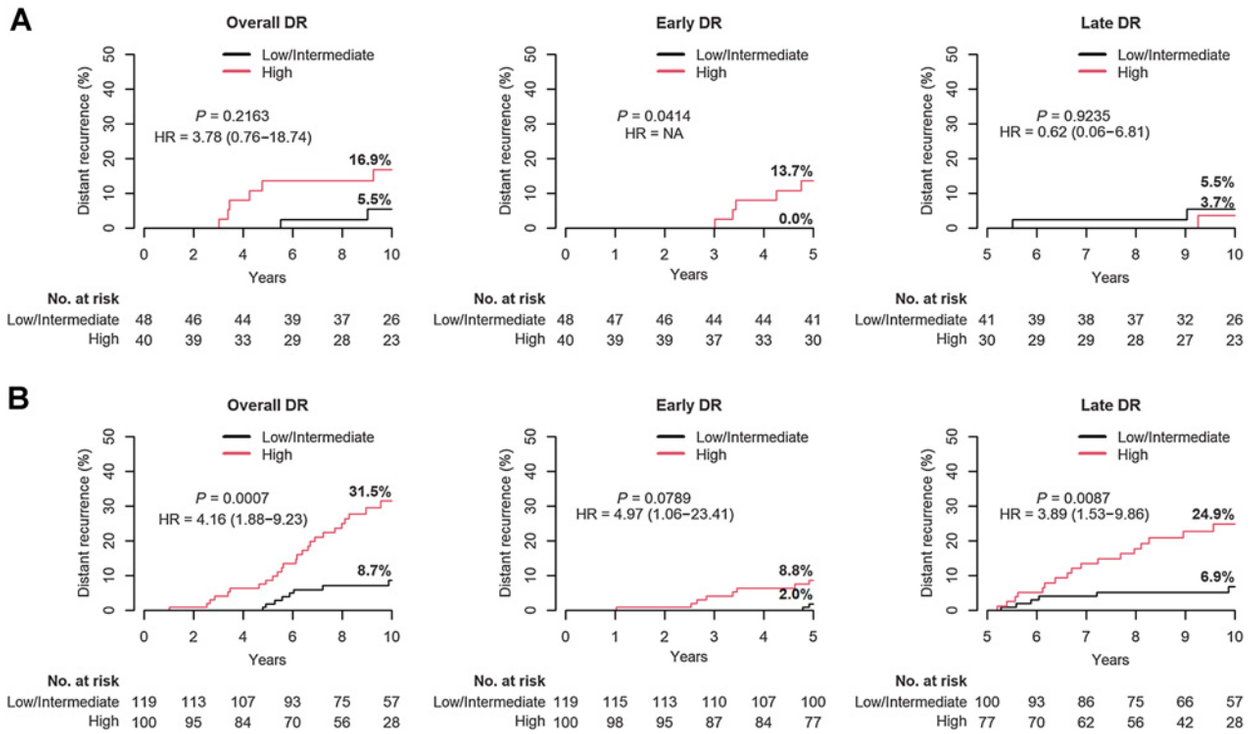

Figure 3.

Prognostic performance of BCI for overall 10-year, early (0–5 years), and late (5–10 years) DR rate for patients (**A**) <50 years old and (**B**) ≥50 years old, respectively.

N+ tumors and 39% of patients with stage II/III tumors were classified as low/intermediate-risk by BCI, indicating these patients have a favorable long-term prognosis despite their high-risk clinicopathologic features.

As ILC has a propensity for late DR, an apparent feature of BCI prognostic stratification is that DRs in low/intermediate-risk patients occurred almost entirely in the late follow-up period (post-5 years from diagnosis), whereas recurrences in high-risk patients showed a steady increase in cumulative risk over 10 years (**Fig. 1**). Similar recurrence patterns to those of the overall cohort were also observed in high- and low/intermediate-risk patients with well- and moderately-differentiated tumors (**Fig. 2A**), stage II/III tumors (**Fig. 2B**), and N+ tumors (Supplementary Fig. S6B). In addition, patients with ILC did not demonstrate any reduction in risk or shift in prognostic profile with chemotherapy treatment, although this should be interpreted with caution given the retrospective nature of this study (**Fig. 2C**).

Table 2. Univariate and multivariate Cox regression analysis of prognostic performance of BCI for overall 10-year DR rate in the ILC cohort.

	Univariate analysis		Multivariate analysis[a]	
Variable	**HR**	***P* value**	**HR**	***P* value**
Age (years)				
≥50 vs. <50	1.82 (0.84–3.95)	0.129	—	—
Adjuvant endocrine therapy				
Yes vs. no	0.53 (0.19–1.49)	0.229	—	—
Adjuvant chemotherapy				
Chemo vs. no chemo	1.96 (1.03–3.74)	0.041	1.21 (0.58–2.51)	0.611
Tumor size (cm)				
T2–3 vs. T1	2.22 (1.17–4.22)	0.015	1.65 (0.84–3.24)	0.149
Tumor grade (differentiation)				
Moderate vs. well	5.06 (1.21–21.13)	0.026	2.09 (0.44–9.93)	0.352
Poor vs. well	9.33 (1.94–44.93)	0.005	2.76 (0.48–15.77)	0.254
Nodal status				
N+ vs. N0	2.24 (1.19–4.19)	0.012	0.77 (0.33–1.81)	0.545
BCI				
High vs. low/Intermediate	4.09 (2.00–8.34)	0.0001	3.49 (1.28–9.54)	0.015

[a]Only significant prognostic factors in the univariate analysis were included in the multivariate model.

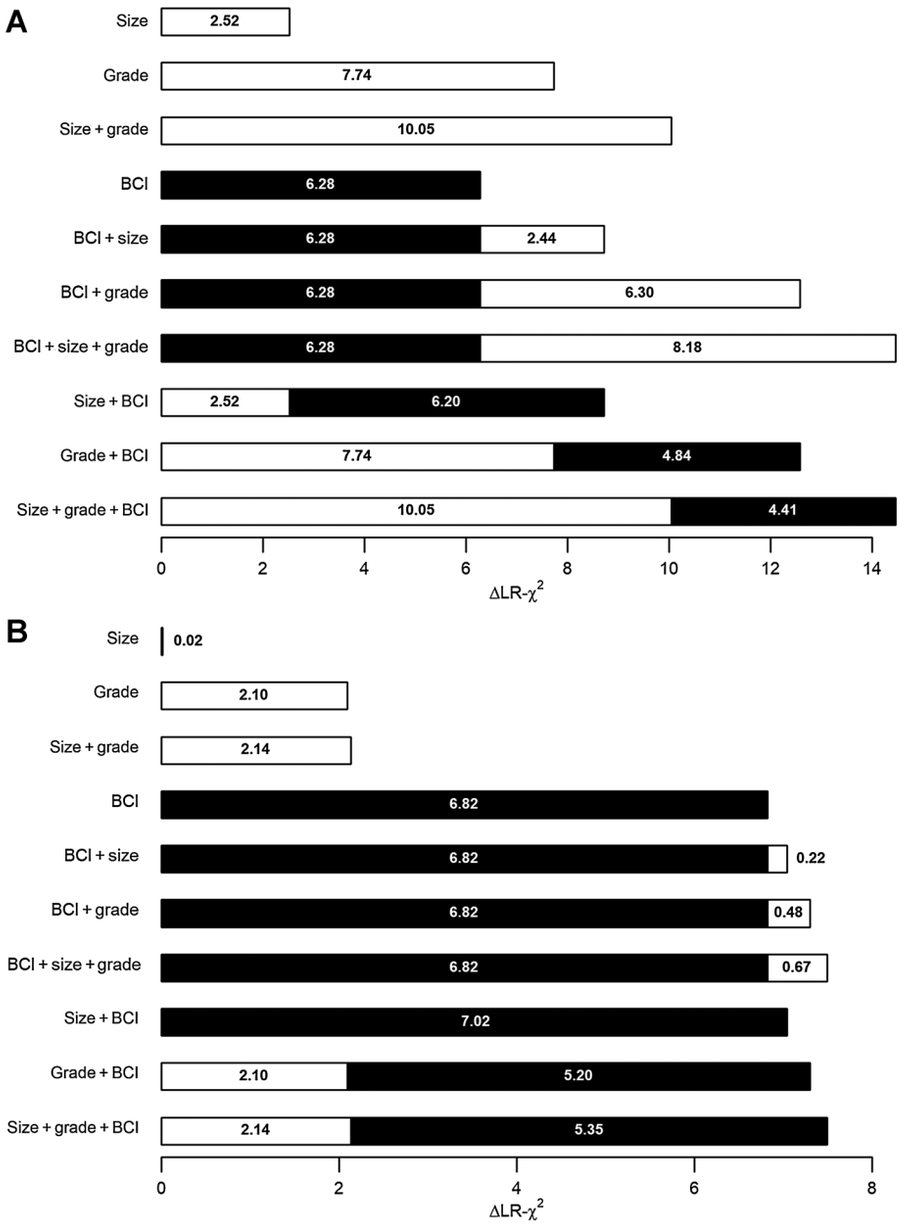

Figure 4.

Prognostic value of BCI (gene expression only) and improvement by incorporation of tumor size and grade as measured by the change in likelihood ratio statistic, χ^2 (ΔLR-χ^2) for patients with N0 (**A**) and N+ (**B**) tumors in the lobular cohort. Bars represent relative contributions of BCI (black) and clinicopathologic factors, including tumor size and/or grade (white).

Consistent with the distinct biological characteristics of ILC, there are notable differences between BCI risk stratification of patients with ILC versus IDC. Previous studies have shown that BCI stratified N0 IDC tumors into low-, intermediate-, and high-risk groups comprising approximately 55–60%, 25–30%, and 15–20% of patients, respectively (13, 15). For patients with N+ IDC, BCI stratified ∼20% as low-risk and ∼80% as high-risk (16). In contrast, only two risk groups were classified by BCI in ILC in patients with both N0 and N+ tumors. Thus, BCI identifies two clinical entities in ILC: a low/intermediate-risk category consisting of 54% of patients and a high-risk category comprising 46% of patients. Importantly, findings from this study indicate that tumor biology and genomic classification provide increased resolution to prognostication of ILC, which is highly heterogenous and therefore likely requires individualized assessment of gene expression to accurately assess DR risk. Although nodal status showed correlation with BCI risk groups, in multivariate analysis, BCI remained the only significant independent prognostic factor for risk of DR.

In addition to BCI, several other prognostic classifiers have been investigated in patients with ILC (22–28). Similar prognostic classifications to those reported here have been reported for the 70-gene MammaPrint assay (24) and EndoPredict (27), each of which classified patients with ILC into a high-risk and a low-risk category. The 97-gene Genomic Grade Index (GGI) outperformed histologic grade in patients with ILC patients, classifying 64% of tumors as GG low (GG1), 17% as GG high (GG3), and 19% as equivocal (not classified as GG1 or GG3; ref. 23). Studies evaluating the 21-gene OncotypeDX score in ILC tumors have also been reported wherein a majority of tumors (71%) were classified as intermediate-risk with very limited differences observed in breast cancer–specific survival between low/

intermediate-risk (99%) and high-risk (96%) groups (25). In the prospective PlanB study, the prevalence of high recurrence score (RS) was 3-fold lower in patients who had lobular breast cancer compared with those who had nonlobular breast cancer, but 5-year disease-free survival estimates for lobular and nonlobular breast cancer were similar, suggesting that RS alone may not add the same prognostic information in ILC (29). The prognostic performance of Prosigna was compared in patients with ILC and patients with IDC, in a cohort of postmenopausal women receiving 5 years of endocrine therapy, with significant prognostic value for Prosigna demonstrated in N0 and N+ subsets; however, 28% of ILC tumors classified as intermediate-risk (26). Finally, Conforti and colleagues reported the prognostic performance of Clinical Treatment Score post-5 years (CTS5), which incorporates age, tumor size, nodal status, and tumor grade (22). In this study, 95% of patients were classified as low-risk, with only 3 out of 1,361 patients (0.2%) classified as high-risk; therefore the clinical utility of CTS5 in patients with ILC remains to be clearly established (22). Overall, it is notable that the classifiers described above, including BCI, were not developed specifically for prognostication of lobular cancer, which may provide a basis for the variability in performance.

Knowledge of both prognostic risk of recurrence as well as predicted response to extended endocrine therapy may be useful for treatment decisions. Aside from BCI prognostic results, the BCI assay also provides a predictive result that reports whether the patient has a high or low likelihood to benefit from extended endocrine therapy. It is notable that 34% of the BCI low/intermediate-risk patients were predicted to benefit from extended endocrine therapy and, conversely, that 49% of the BCI high-risk patients were not predicted to benefit from extended endocrine therapy (Supplementary Fig. S5). For patients with a high risk of recurrence but low likelihood of benefit from extended endocrine therapy, alternative therapies may warrant consideration. For example, the monarchE study demonstrated that addition of abemaciclib, a cyclin-dependent kinase 4/6 inhibitor, to adjuvant endocrine therapy in patients with HR+, HER2−, high-risk, early-stage breast cancer resulted in improved invasive disease-free survival (30). This study enrolled patients with poor prognosis, including those with one to three positive nodes and grade 3 tumors or a Ki-67 ≥20%. Although outcomes for patients with ILC were not disclosed in the monarchE trial, there is a growing importance for biologic markers to select patients who remain at high risk for recurrence despite optimal standard therapy and for whom addition of novel agents may be particularly beneficial. This study highlights the value of BCI in selecting this higher risk population among patients with ILC, a subtype of breast cancer whose biology remains understudied.

This study has several key strengths and limitations. This was a multi-institutional study, conducted in a well-annotated cohort in which 95% of patients had pure lobular histology. The study was prospectively defined and BCI testing was performed blinded to clinical outcome. Results from the comparative tissue dissection study showed a high concordance of BCI-risk group classification between methods, adding meaningful confidence in the study findings. Nevertheless, due to the unique growth pattern of lobular breast cancers, approximately 10% of samples were excluded since they had a tumor cellularity below 40%. Although useful information was gained from subset analyses overall, further examination of BCI prognostic activity in patients with N0 ILC is warranted given the low event rate in this study. Approximately 22% of patients had unknown HER2 status, although patients with HER2+ tumors represented ∼5% of the overall cohort. Finally, details regarding chemotherapy and endocrine therapy were unavailable for 11% and 7% of patients, respectively, and specific information on duration of endocrine therapy was not available for a majority (70%) of patients in the study.

In summary, the findings presented here indicate that BCI is a significant and independent prognostic factor for risk stratification in ILC. Findings from this study may help individualize prognosis and inform treatment approaches. Furthermore, although the addition of novel agents may result in improved outcome, it can also result in increased side effects and adverse events. BCI may be a useful tool in selecting a population of patients with ILC and a high risk of recurrence for whom escalation of therapy may be particularly beneficial; conversely, BCI may be useful in identifying patients with a low risk of recurrence who may be appropriate for de-escalation of treatment. Overall, these results support a role for BCI to define prognostic risk groups based on individual tumor biology to better inform treatment strategies for patients with ILC.

Authors' Disclosures

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