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ORIGINAL RESEARCH

Novel Signatures Based on the Lymphocyte-to-C-Reactive Protein Ratio Predict the Prognosis of Patients with Early Breast Cancer: A Retrospective Study

Li Wang[1]*, Yu-Ling Zhang[2]*, Chang Jiang[1]*, Fang-Fang Duan[1], Zhong-Yu Yuan[1], Jia-Jia Huang[1], Xi-Wen Bi[1]

[1]Department of Medical Oncology, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-Sen University Cancer Center, Guangzhou, People's Republic of China; [2]Department of Endocrinology, Jiangxi Provincial People's Hospital, Nanchang, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xi-Wen Bi; Jia-Jia Huang, Department of Medical Oncology, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, 651 Dongfeng Road East, Guangzhou, 510060, People's Republic of China, Email bixw@sysucc.org.cn; huangjiaj@sysucc.org.cn

Background: The value of the lymphocyte-to-C-reactive protein (CRP) ratio (LCR) in early breast cancer (BC) is unclear. We explored the correlation between the LCR and survival of patients with early BC and established effective LCR-based prognostic signatures for predicting prognosis.

Methods: In this retrospective study, we randomized 623 patients with early-stage BC diagnosed in December 2010 to October 2012 at the Sun Yat-sen University Cancer Center to training and verification datasets. The median follow-up of all patients was 109 months. The survival differences were calculated by Kaplan–Meier method using the Log rank test. For overall survival (OS) and disease-free survival (DFS), the independent factors in the training dataset were identified using univariate and multivariate Cox analyses, in which two-tailed P-values < 0.05 were considered statistically significant. Based on this, we respectively constructed novel signatures for survival prediction and validated the efficiency of signatures through the concordance index (C-index), calibration and receiver operating characteristic (ROC) curves in both datasets.

Results: The LCR, lymphatic vessel invasion (LVI), progesterone receptor (PR) status, and Ki67 index were independent prognostic factors of OS. And the LCR and LVI are associated to DFS too. High LCR was associated with better OS and DFS. We constructed the prediction signatures based on those independent prognostic factors and calculated the risk scores. Patients in the training dataset with higher risk scores had significantly worse prognosis ($P < 0.001$). The signature had excellent discrimination capacity, with an OS C-index of 0.785 [95% confidence interval (CI): 0.713–0.857] and 0.750 (95% CI: 0.669–0.832) in the training and verification datasets, respectively. The time–ROC curves also suggest accurate prediction by the signature.

Conclusion: The LCR was a significant prognostic predictor of OS and DFS in early BC. The LCR-based prognostic signatures could be a useful tool for individualized therapeutic guidance.

Keywords: survival, nomogram, early breast cancer, LCR

Introduction

In women, breast cancer (BC) is the most common type of cancer.[1] Although screening and diagnostic methods have been increasingly improved in the clinic, BC remains a threat to women's health.[2,3] In the clinic, clinicians typically choose treatments in terms of the molecular type of BC (estrogen receptor [ER]; progesterone receptor [PR]; human epidermal growth factor receptor 2 [Her2]; Ki67). Beside the molecular subtypes, various factors such as age, stage, and

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OncoType Dx test may affect the decision. There are several therapeutic regimens for each subtype.[4–6] Despite this, BC recurrence and metastasis remain common clinical problems, resulting in poor curative effects and high mortality rates. Therefore, it is greatly necessary and important to search preferable prognostic factors for planning appropriate treatment. Currently, except for the tumor-node-metastasis (TNM) staging system,[7] multifarious prognostic models have been proposed and trialed on a small scale.[8–10] Nevertheless, owing to individual heterogeneity, accurate prediction of prognosis remains challenging worldwide. In addition, in many medium and small cities, people do not have access to Oncotype DX test, moreover, it's costly too. Accordingly, identifying a reliable and robust indicator for patients with early BC is important and justified.

Inflammation is linked with cancer; specifically it plays decisive roles in almost all phases of tumor development, from initiation to metastasis.[11,12] In recent years, inflammatory factor-related prognostic models have been reported in several cancers, for example, lung cancer and colorectal tumors.[13–16] With regard to BC, the neutrophil-to-lymphocyte ratio (NLR),[17,18] lymphocyte-to-monocyte ratio (LMR),[19,20] and C-reactive protein (CRP)-to-albumin ratio (CAR) can be used as prognostic biomarkers.[21] In infection, the lymphocyte count increases;[22] meanwhile, the liver will generate more CRP.[23] Therefore, the LCR, which combines the lymphocyte and CRP inflammatory indexes, might be a better reflection of the inflammatory condition than either index individually.

Nomograms integrating multifarious prognostic and decisive factors for forecasting event probability are now widely used in medical statistics.[24,25] As far as we know, there remains a lack of studies on LCR as a precise prognostic factor of early BC. In the present article, we probed and tested the prognostic ability of LCR in patients with early BC. Based on the LCR, we established a graphic nomogram for predicting the survival outcomes of such patients, which might facilitate individualized survival predictions in the clinic.

Patients and Methods

Patient Selection

A total of 623 patients initially diagnosed as BC in December 2010 to October 2012 at the Sun Yat-sen University Cancer Center (SYSUCC; Guangzhou, China) were randomly allocated to a training dataset and verification dataset in a 6:4 ratio. The inclusive criteria were: 1) age ≥ 18 years old; 2) pathological diagnosis as invasive BC; 3) without distant metastasis (lung, bone, liver, and brain); 4) available complete baseline laboratory data and specific follow-up data. We excluded patients who: 1) were pregnant or breastfeeding: 2) had a pathological diagnosis of ductal carcinoma in situ; 3) had synchronal malignancies; 4) had taken any medicine inducing an immune or inflammatory response in the most recent 3 months; 5) had any inflammatory disease (autoimmune diseases included).

In this study, all processes concerning human participants were conducted following the ethical principles of SYSUCC, the Helsinki declaration (1604) and its later revisions, and its analogical ethics. As this was a retrospective study, the requirement for signed informed consent by the patients was waived. In addition, all patients' information was confidential.

Data Collection and Variable Categorization

All patient clinicopathological characteristics were manually extracted from the SYSUCC electronic medical records system. We extracted the patient age at diagnosis; postoperative pathological classification (PPC); lymphatic vessel invasion (LVI); T and N stage; ER, PR, and Her2 status; Ki67 index [stained with MIB1 monoclonal antibody (ZSGB-BIO, Beijing, China) and assessed by two professional pathologists]; and pathological grade (PG). Every participant underwent pretreatment assessments, including illness history inquiry, physical examination, and hematological and biochemical tests. The blood parameters CRP and lymphocyte before any anti-tumor treatment were also collected from the electronic medical records system.

BC specimens with >1% tumor nuclei positive for ER or PR via immunohistochemistry (IHC) testing were defined as ER- or PR-positive, respectively.[26] For Her2 status, only tumor cells that scored 3+ on IHC or 2+ with *ERBB2* gene amplification in Fluorescence in situ hybridization (FISH) were identified as Her2-positive.[27] The continuous laboratory variable LCR was classified as a categorical variable based on its optimal cut-off value (0.33), calculated by maximally selected rank statistics analysis.

Outcome and Follow-Up

Overall survival (OS) time was specified as the interval from the time of diagnosis to death from any cause or to the last follow-up. Disease-free survival (DFS) time was referred to the time from randomization to disease recurrence or patient death from any cause. Patients were followed using outpatient examination and telephone interviews.

Statistical Analysis

The continuous variables and categorical variables were described by median values with interquartile ranges (IQR) and frequencies with percentages, respectively. Both variable comparisons and analysis of the relationship of LCR grade with other clinicopathological characteristics were conducted by chi-square test or Fisher's exact test.

The LCR cut-off value was derived using the R package maxstat.[28] Next, the LCR was classified as a categorical variable scored as 0 or 1. Cox proportional hazards matrixes were applied to conduct univariate and multivariate analyses. Only factors with two-tailed *P*-values < 0.2 in the univariate Cox regression analysis would be recruited to conduct the Proportional Hazards (PH) test. In multivariate analyses, two-tailed *P*-values < 0.05 were considered statistically significant. Based on the univariate and multivariate analyses results, nomograms with the terminal points of 5-year and 8-year OS and 5- and 8-year DFS were developed through the rms package in R software. Furthermore, the concordance index (C-index), calibration curves, and receiver operating characteristic (ROC) curves for OS and DFS were generated to evaluate the predictive performance and accuracy of the nomogram in both the training and validation datasets.

Furthermore, we counted the risk scores of all patients in the training and validation datasets using the corresponding regression coefficient of every recruited prognostic factor. The following formula was used: scores = $e^{\text{sum (every prognostic factor} \times \text{corresponding coefficient)}}$. Then, based on the median risk scores, the patients in both datasets were divided into high- (total scores ≥ median scores) and low-risk (total scores < median scores) groups. Next, Kaplan-Meier survival analyses between the low- and high-risk groups in both datasets were performed using the survminer package and their significance was determined by the Log rank test.

All analyses were carried out in R software (version 4.1–0, Vanderbilt University, Nashville, TN, USA). $P < 0.05$ was considered significant except where specified otherwise.

Results

Patients' Characteristics

We recruited 623 patients with early BC to the study. Table 1 shows their clinicopathological features. The whole cohort was randomized to a training and validation dataset in a 6:4 ratio (375 vs 248, respectively). Including LCR, all 12 clinicopathological variables and 4 treatment regimens were well balanced between the two datasets. The median age of all patients was 46 years (IQR: 39.0–55.0). Nearly half of the patients were T2 (53.5%) and N0 (49.6%). More than half of the patients (56.5%) had LVI. Most patients (91.8%) were diagnosed with invasive ductal carcinoma. The positive: negative ratio for hormone receptors (PR, ER) was approximately 2:1. Patients who were Her2-negative accounted for 70.8% (n = 441) of the whole cohort. Ki67 status was positive (>15%) in 418 patients (67.1%) and negative (≤15%) in 205 patients (32.9%). The majority of total patients received chemotherapy (85.1%) and more than half of patients received endocrine therapy (56.8%, 57.3% in training and validation datasets, respectively). There was no statistical difference between the LCR level and other clinicopathologic characteristics ($P > 0.05$) in the datasets (Table 2).

Survival Analysis of Training Dataset Based on the LCR

In the training dataset, the LCR was correlated with OS and DFS. And the median follow-up of all patients in this group is 109 months. Compared to patients in the LCR-low (LCR ≤ 0.33) group, patients in the LCR-high (LCR > 0.33) group had better overall and disease-free survival (Figures 1 and 2, $P < 0.001$).

Univariate and multivariate analyses for OS revealed that the LCR was a significant individual prognostic variable for OS (Table 3) and DFS (Table 4).

Table 1 Comparison of Baseline Clinicopathological Characteristics Between the Training and Validation Dataset

Variables	All (n,%) N=623	Training (n,%) N=375	Validation (n,%) N=248
Age (years), median (IQR)	46(39,55)	45(39,55)	47(40,55)
Age at diagnosis			
≤45 years old	301(48.3)	188(50.1)	113(45.6)
>45 years old	322(51.7)	187(49.9)	135(54.4)
Menstrual status			
Premenopausal	410 (65.8)	248(66.1)	162(65.3)
T stage[#]			
T1	219(35.2)	135(36.0)	84(33.9)
T2	333(53.5)	196(52.3)	137(55.2)
T3	27(4.3)	18(4.8)	9(3.6)
T4	44(7.1)	26(6.9)	18(7.3)
N stage[#]			
N0	309(49.6)	190(50.7)	119(48.0)
N1	174(27.9)	108(28.8)	66(26.6)
N2	87(14.0)	53(14.1)	34(13.7)
N3	53(8.5)	24(6.4)	29(11.7)
LVI			
No	271(43.5)	165(44.0)	106(42.7)
Yes	352(56.5)	210(56.0)	142(57.3)
PPC			
IDC	572(91.8)	344(91.7)	228(91.9)
Others	51(8.2)	31(8.3)	20(8.1)
PR status			
Negative	235(37.7)	144(38.4)	91(36.7)
Positive	388(62.3)	231(61.6)	157(63.3)
ER status			
Negative	193(31.0)	121(32.3)	72(29.0)
Positive	430(69.0)	254(67.7)	176(71.0)
HER-2 status			
Negative	441(70.8)	267(71.2)	174(70.2)
Positive	182(29.2)	108(28.8)	74(29.8)
KI67 index[##]			
≤15%	205(32.9)	118(31.5)	87(35.1)
>15%	418(67.1)	257(68.5)	161(64.9)
Pathological Grade			
≤1	27(4.3)	19(5.1)	8(3.2)
2	458(73.5)	266(70.9)	192(77.4)
3	138(22.2)	90(24.0)	48(19.4)
Endocrine therapy			
Yes	355(57.0)	213(56.8)	142(57.3)
Only AI	79(12.7)	47(12.5)	32(12.9)
Only ER-anti	234(37.6)	155(41.3)	79(31.9)
Others	42(6.7)	11(2.9)	31(12.5)
No	268(43.0)	162(43.2)	106(42.7)
Chemotherapy			
Yes	530(85.1)	325(86.7)	205(82.7)
Taxane based	48(7.7)	32(8.5)	16(6.5)
Anthracycline based	118(18.9)	76(20.3)	42(16.9)
Anthracyclines and taxane	364(58.4)	217(57.9)	147(59.3)
No	93(14.9)	50(13.3)	43(17.3)

(*Continued*)

Table 1 (Continued).

Variables	All (n,%) N=623	Training (n,%) N=375	Validation (n,%) N=248
Radiotherapy			
Yes	212(34.0)	132(35.2)	80(32.3)
No	411(66.0)	243(64.8)	168(67.7)
Targeted therapy			
Yes	53(8.5)	32(8.5)	21(8.5)
No	570(91.5)	343(91.5)	227(91.5)
LCR			
≤0.33	79(12.7)	43(11.5)	36(14.5)
>0.33	544(87.3)	332(88.5)	212(85.5)

Notes: #According to the 7th edition of the UICC/AJCC staging system. ##Indicating DNA synthetic activity as measured using immunocytochemistry.

Abbreviations: IQR, interquartile ranges; LVI, lymphatic vessel invaded; PPC, postoperative pathological classification; IDC, invasive ductal carcinoma; PR, Progesterone receptor; ER-anti, estrogen receptor antagonist; ER, estrogen receptor; HER-2, human epidermal growth factor receptor-2; AI, aromatase inhibitor; LCR, lymphocyte/C-reactive protein ratio.

Table 2 The Relationship Between LCR Grade and Other Clinicopathological Characteristics in Training and Validation Sets

Baseline Characteristics	Training Set (n,%)			Validation Set (n,%)		
	LCR-Low	LCR-High	P-value	LCR-Low	LCR-High	P-value
Age at diagnosis			0.407			0.829
≤45 years old	19(44.2)	169(50.9)		17(47.2)	96(45.3)	
>45 years old	24(55.8)	163(49.1)		19(52.8)	116(54.7)	
T stage#			0.210			0.175
T1	13(30.2)	122(36.7)		7(19.4)	77(36.3)	
T2	21(48.8)	175(52.7)		24(66.7)	113(53.3)	
T3	4(9.4)	14(4.2)		2(5.6)	7(3.3)	
T4	5(11.6)	21(6.3)		3(8.3)	15(7.1)	
N stage#			0.052			0.280
N0	15(34.9)	175(52.7)		13(36.1)	106(50.0)	
N1	15(34.9)	93(28.0)		10(27.8)	56(26.4)	
N2	11(25.6)	42(12.7)		8(22.2)	26(12.3)	
N3	2(4.6)	22(6.6)		5(13.9)	24(11.3)	
LVI			0.340			0.217
No	16(37.2)	149(44.9)		12(33.3)	94(44.3)	
Yes	27(62.8)	183(55.1)		24(66.7)	118(55.7)	
PPC			0.999			0.324
IDC	40(93.0)	304(91.6)		35(97.2)	193(91.0)	
Others	3(7.0)	28(8.4)		1(2.8)	19(9.0)	
PR status			0.461			0.159
Negative	16(37.2)	105(31.6)		14(38.9)	58(27.4)	
Positive	27(62.8)	227(68.4)		22(61.1)	154(72.6)	
ER status			0.407			0.503
Negative	19(44.2)	125(37.7)		15(41.7)	76(35.8)	
Positive	24(55.8)	207(62.3)		21(58.3)	136(64.2)	
HER-2 status			0.099			0.374
Negative	26(60.5)	241(72.6)		23(63.9)	151(71.2)	
Positive	17(39.5)	91(27.4)		13(36.1)	61(28.8)	

(*Continued*)

Table 2 (Continued).

Baseline Characteristics	Training Set (n,%)			Validation Set (n,%)		
	LCR-Low	LCR-High	P-value	LCR-Low	LCR-High	P-value
KI67 index[##]			0.389			0.538
≤15%	16(37.2)	102(30.7)		11(30.6)	76(35.8)	
>15%	27(62.8)	230(69.3)		25(69.4)	136(64.2)	
Endocrine therapy			0.148			0.341
Yes	20(46.5)	193(58.1)		18(50.0)	124(58.5)	
No	23(53.5)	139(41.9)		18(50.0)	88(41.5)	
Chemotherapy			0.280			0.718
Yes	35(81.4)	290(87.3)		29(80.6)	176(83.0)	
No	8(18.6)	42(12.7)		7(19.4)	36(17.0)	
Radiotherapy			0.331			0.001
Yes	18(41.9)	114(34.3)		20(55.6)	60(28.3)	
No	25(58.1)	218(65.7)		16(44.4)	152(71.7)	
Targeted therapy			0.848			0.051
Yes	4(9.3)	28(8.4)		0(0.0)	21(9.9)	
No	39(90.7)	304(91.6)		36(100.0)	191(90.1)	

Notes: [#]According to the 7th edition of the UICC/AJCC staging system. [##]Indicating DNA synthetic activity as measured using immunocytochemistry.
Abbreviations: LCR, lymphocyte/C-reactive protein ratio; LVI, lymphatic vessel invaded; PPC, postoperative pathological classification; IDC, invasive ductal carcinoma; PR, progesterone receptor; ER, estrogen receptor; HER-2, human epidermal growth factor receptor-2.

Nomogram Establishment

We used the Cox proportional hazards regression model to respectively build prognostic signatures based on the stepwise regression method for OS (Figure 3A) and DFS (Figure 4A). Subsequently, combining the statistical results and clinical significance, we predicted 5- and 8-year of OS rates using the LVI, PR, Ki67, and LCR (Figure 3B). And the T stage, LVI and LCR were applied for predicting 5- and 8-year of DFS (Figure 4B).

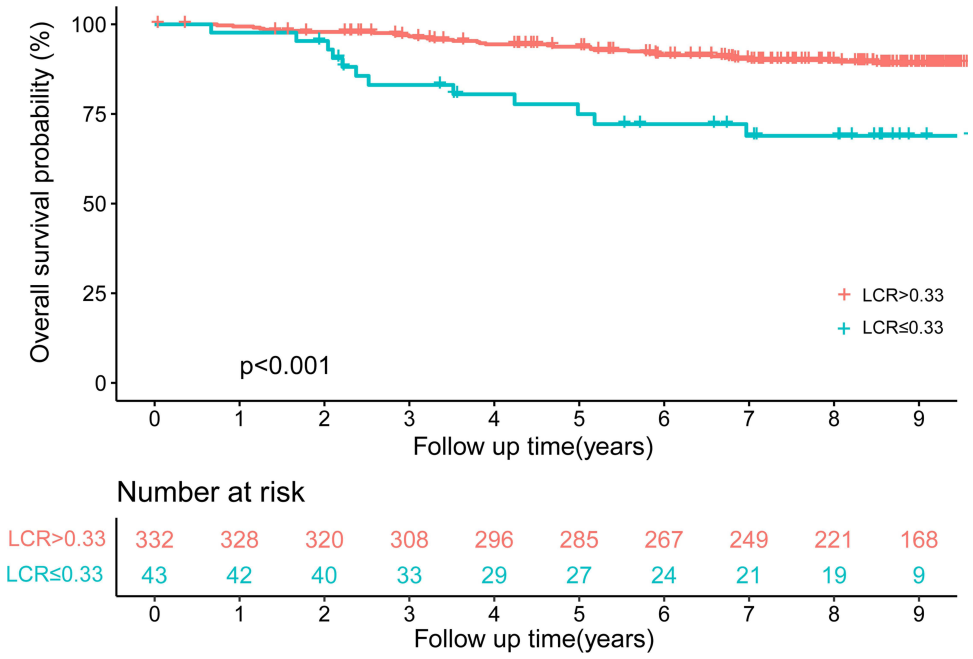

Figure 1 Kaplan-Meier curves for the overall survival (OS) of patients based on lymphocyte/c-reactive protein ratio (LCR).

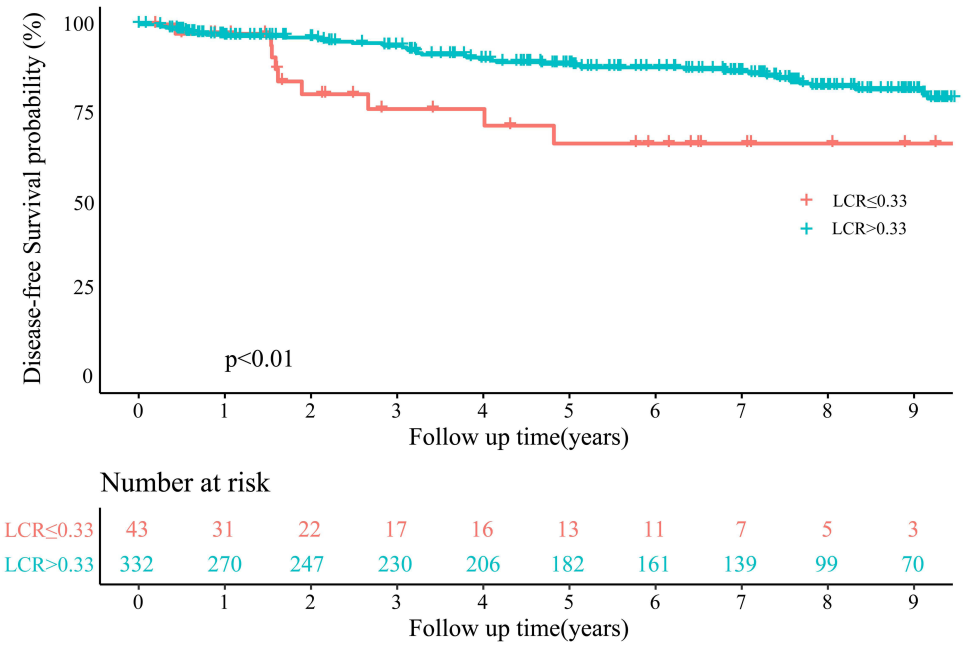

Figure 2 Kaplan-Meier curves for the overall survival (DFS) of patients based on lymphocyte/c-reactive protein ratio (LCR).

Nomogram Verification

To validate the robustness of the OS prognostic model, which included four prognostic factors from the training set, we performed further verification in both datasets. The predictive nomogram exhibited a good predictive performance with a C-index of 0.785 (95% CI: 0.713–0.857) and 0.750 (95% CI: 0.669–0.832) in the training and validation dataset, respectively. The results also revealed an excellent area under the ROC curve (AUC) for OS of the nomogram in both the training dataset (5-year AUC: 0.809, 8-year AUC: 0.768) (Figure 5A) and validation dataset (5-year AUC: 0.770, 8-year AUC: 0.707) (Figure 5B). Furthermore, the calibration curves showed good consistency for the 5-and 8-year of OS between the actual observed and predicted survival rate in the training (Figure 5C and D) and validation datasets (Figure 5E and F). Similarly, we also verified the prediction model of DFS on the training and validation sets. The C-index of 0.815 (95% CI: 0.749–0.881) and 0.678 (95% CI: 0.582–0.774) in the training and validation dataset, respectively. The ROC curves for the DFS in training set and validation set are shown in the Figure 6A and B, respectively. In addition, the results of 5-and 8-year of DFS between the actual observed and predicted survival rate in the training (Figure 6C and D) and validation datasets (Figure 6E and F) also showed good consistency.

We counted the risk scores of every patient in both datasets (score = $e^{\text{sum (every prognostic factor} \times \text{corresponding coefficient)}}$) and then divided them into low- and high-risk groups according to the median risk score (Figures 7A, B and 8A, B). Survival analysis showed that patients in the high-risk group had poorer OS (Figure 7C and D) and DFS (Figure 8C and D) than those in the low-risk group. And in the high-risk group, the 5- and 8-year OS probability are respectively 88.6% and 84.6%. Correspondingly, the 5- and 8-year OS probability of patients in low-risk group achieve at 94.3% and 89.6%, respectively. Consistently, the high-risk group had higher mortality (Figure 7E and F) and high risk of recurrence or metastasis (Figure 8E and F).

Discussion

In this study, 623 BC patients were enlisted and randomized into training and validation datasets in a 6:4 ratio. According to the optimal LCR cut-off value of 0.33, the participants were grouped into LCR-low and LCR-high groups. The multivariate analysis results for the training dataset revealed that LVI, PR status, and the LCR were independent predictors of OS for patients with early-stage BC. And T stage, LVI, and the LCR were independently to predict DFS. The multivariate Cox regression analysis revealed no obvious statistical significance for the Ki67 index. Nevertheless,

Table 3 Univariate and Multivariate Cox Regression Analysis of OS in Training Set

Baseline Characteristics	Univariate Analysis		Multivariate Cox Regression Analysis	
	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Age at diagnosis				
≤45 years old				
>45 years old	1.46(0.815,2.616)	0.203		
T stage[#]				
T1				
T2	1.94(0.939,4.008)	0.074		
T3	3.21(1.007,10.242)	0.049		
T4	3.53(1.283,9.716)	0.015		
N stage[#]				
N0				
N1	3.17(1.436,6.980)	0.004		
N2	5.63(2.500,12.680)	<0.001		
N3	6.94(2.639,18.250)	<0.001		
LVI				
No				
Yes	4.24(1.980,9.074)	<0.001	4.07(1.898,8.739)	<0.001
PPC				
IDC				
Others	1.01(0.363,2.825)	0.980		
PR status				
Negative				
Positive	0.43(0.240,0.763)	0.004	0.52(0.285,0.940)	0.030
ER status				
Negative				
Positive	0.80(0.440,1.445)	0.455		
HER-2 status				
Negative				
Positive	1.72(0.960,3.079)	0.068		
KI67 index[##]				
≤15%				
>15%	2.00(0.968,4.140)	0.061	1.81(0.863,3.801)	0.110
Endocrine therapy				
No				
Yes	1.09(0.607,1.947)	0.779		
Chemotherapy				
No				
Yes	0.77(0.363,1.660)	0.513		
Radiotherapy				
No				
Yes	1.55(0.873,2.764)	0.134		
Targeted therapy				
No				
Yes	1.29(0.508,3.250)	0.596		
LCR				
≤0.33				
>0.33	0.30(0.156,0.579)	<0.001	0.35(0.181,0.689)	<0.001

Notes: [#]According to the 7th edition of the UICC/AJCC staging system. [##]Indicating DNA synthetic activity as measured using immunocytochemistry.

Abbreviations: CI, confidence interval; LVI, lymphatic vessel invaded; PPC, postoperative pathological classification; IDC, invasive ductal carcinoma; PR, Progesterone receptor; ER, estrogen receptor; HER-2, human epidermal growth factor receptor-2; LCR, lymphocyte/C-reactive protein ratio.

Table 4 Univariate and Multivariate Cox Regression Analysis of DFS in Training Set

Baseline Characteristics	Univariate Analysis		Multivariate Cox Regression Analysis	
	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Age at diagnosis				
≤45 years old				
>45 years old	1.09(0.636,1.869)	0.755		
T stage[#]				
T1				
T2	2.20(1.106,4.381)	0.027	1.96(0.981,3.921)	0.06
T3	2.94(0.817,10.590)	0.099	1.96(0.542,7.110)	0.31
T4	7.68(3.071,19.185)	<0.001	5.22(2.080,13.120)	<0.001
N stage[#]				
N0				
N1	4.12(1.887,9.009)	<0.001		
N2	6.43(2.812,14.695)	<0.001		
N3	9.17(3.532,23.817)	<0.001		
LVI				
No				
Yes	7.88(3.138,19.810)	<0.001	6.77(2.681,17.110)	<0.001
PPC				
IDC				
Others	0.56(0.175,1.802)	0.332		
PR status				
Negative				
Positive	0.94(0.538,1.635)	0.821		
ER status				
Negative				
Positive	1.12(0.617,2.04)	0.705		
HER-2 status				
Negative				
Positive	1.24(0.689,2.235)	0.473		
KI67 index[##]				
≤15%				
>15%	1.07(0.598,1.931)	0.811		
Endocrine therapy				
No				
Yes	1.06(0.603,1.855)	0.846		
Chemotherapy				
No				
Yes	2.31(0.721,7.428)	0.158		
Radiotherapy				
No				
Yes	1.44(0.836,2.465)	0.191		
Targeted therapy				
No				
Yes	1.20(0.512,2.804)	0.677		
LCR				
≤0.33				
>0.33	0.39(0.192,0.814)	0.012	0.45(0.211,0.930)	0.03

Notes: [#]According to the 7th edition of the UICC/AJCC staging system. [##] Indicating DNA synthetic activity as measured using immunocytochemistry.

Abbreviations: CI, confidence interval; LVI, lymphatic vessel invaded; PPC, postoperative pathological classification; IDC, invasive ductal carcinoma; PR, progesterone receptor; ER, estrogen receptor; HER-2, human epidermal growth factor receptor-2; LCR, lymphocyte/C-reactive protein ratio.

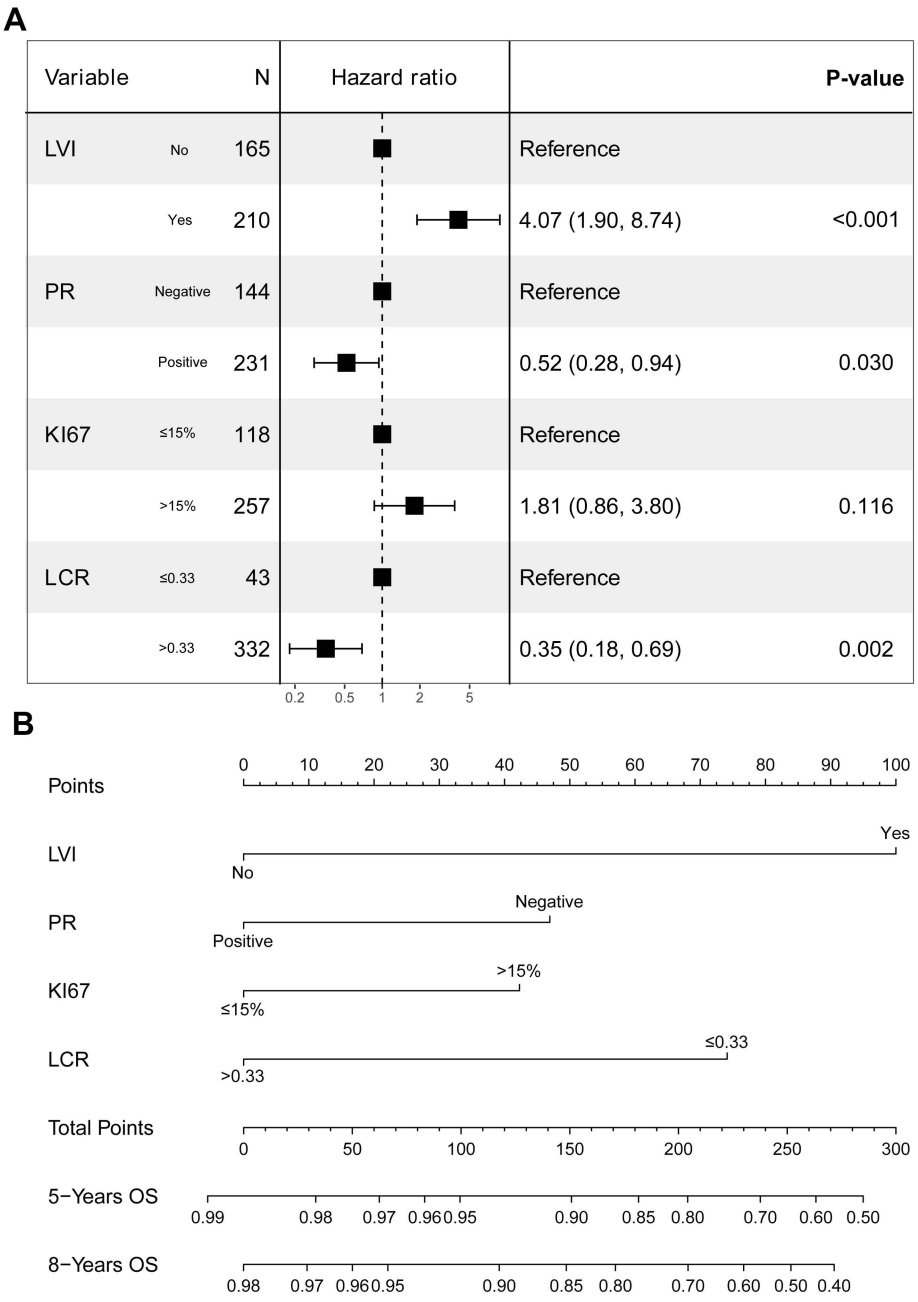

Figure 3 Development of the prognostic signature of OS. (**A**) Results of the stepwise multivariate Cox regression analysis in the training dataset. (**B**) A nomogram of the current prognostic model for individualized OS time predictions.

considering its clinical significance, we incorporated it into the nomogram construction for individualized prognostic prediction, which showed satisfactory discrimination and calibration. In addition, the model underwent verification in the validation dataset. The C-index, calibration curves, and ROC curve AUC also revealed good discriminative ability and satisfactory prognostic accuracy.

The relevance of inflammation to cancer was first proposed by Virchow in 1863.[29] Subsequently, numerous studies have explored and reported on this relationship.[30–32] At present, there is consensus that inflammation contributes to tumor development. Inflammation is involved in the construction of the tumor microenvironment and systemic changes for promoting tumor expansion. Additionally, inflammation can cause changes in the peripheral blood, such as achroacytosis and neutrophilia.[33] Lymphocytes can inhibit tumor development by boosting immunosurveillance.[34] Consequently, the

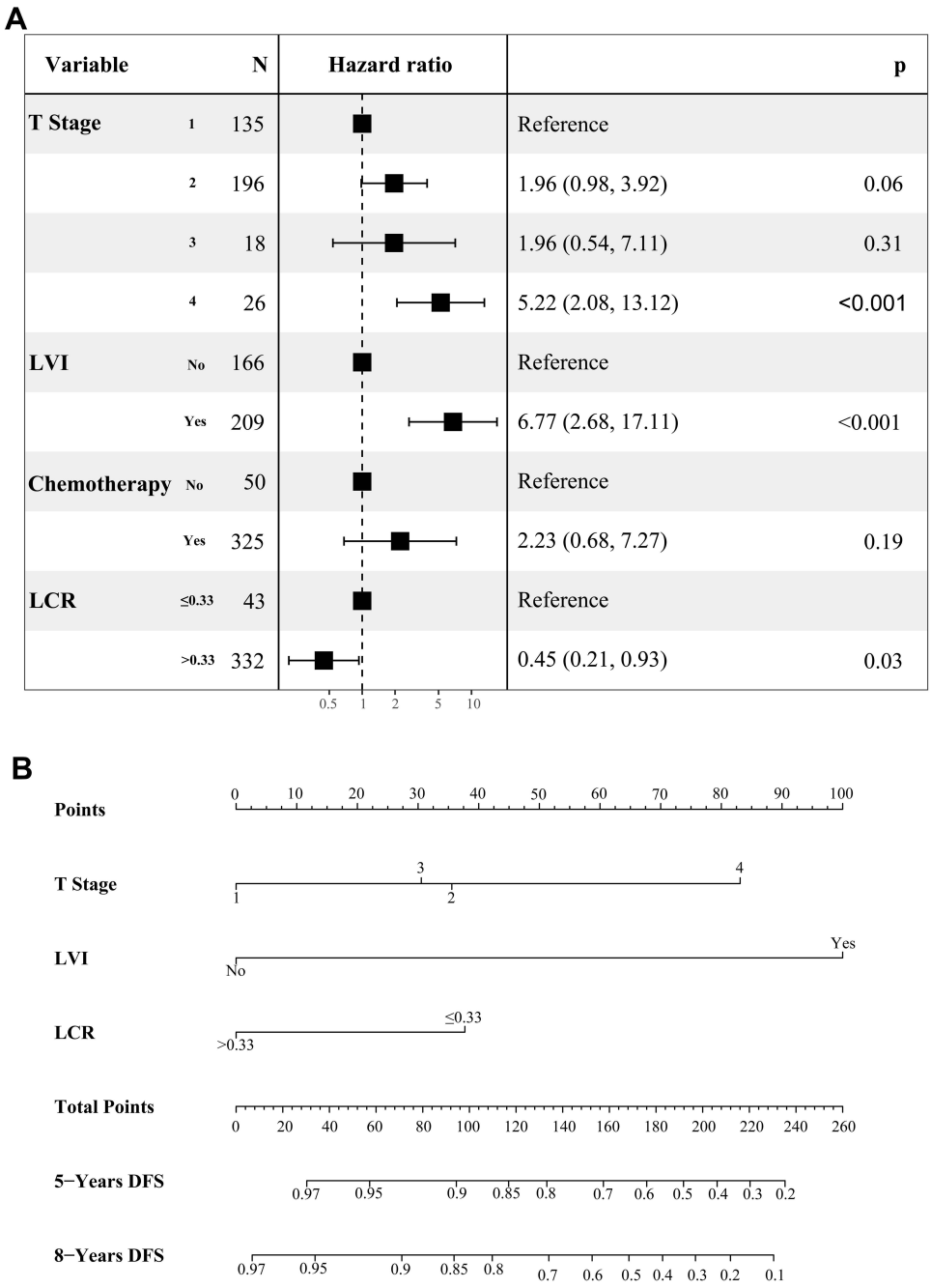

Figure 4 Development of the prognostic signature of DFS. (**A**) Results of the stepwise multivariate Cox regression analysis of DFS in the training dataset. (**B**) A nomogram of the current prognostic model for individualized DFS time predictions.

increased lymphocyte number can indicate enhanced regulatory inhibition of tumor. Moreover, CRP accumulates massively in plasma when the body or tissue experiences infection or injury. It can activate complement and strengthen phagocytosis of phagocytes to regulate and eliminate pathogenic microorganisms.[35] To summarize, as an inflammatory marker, lymphocytes have high specificity but low sensitivity, while CRP has high sensitivity but low specificity. Combining the two to complement each other is a good option. Moreover, the high LCR value linked to longer survival has been validated in other cancers.[36–38]

Second, it had been confirmed that the first step of metastatic dissemination involves local invasion of the adjacent organizations and entry to the microvasculature consisting of lymph and blood systems. Then, the tumor cells translocate

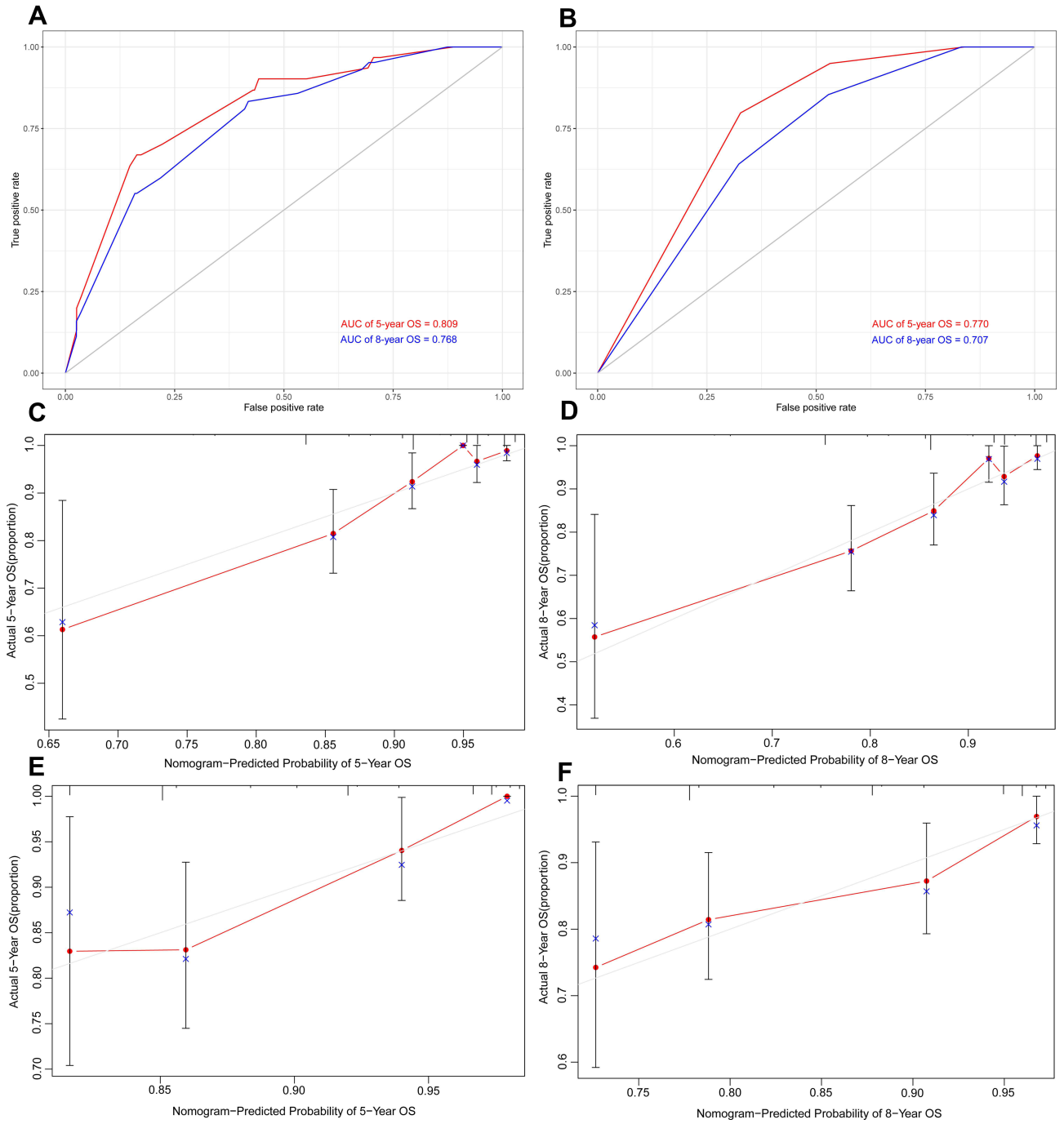

Figure 5 The validation of the prognostic model of OS. (**A**) Receiver operating characteristics (ROC) curves in training dataset. (**B**) Receiver operating characteristics (ROC) curves in validation dataset. (**C**) Calibration plot of the nomogram model at 5-year in the training dataset. (**D**) Calibration plot of the nomogram model at 8-year in the training dataset. (**E**) Calibration plot of the nomogram model at 5-year in the validation dataset. (**F**) Calibration plot of the nomogram model at 8-year in the validation dataset.

to the micro-vessels of distant tissues and organs through the bloodstream. Therefore, LVI is an independent prognostic factor of poor OS and DFS.[39,40]

Third, *PR* is not only a gene target of ERα but also can modulate its behavior.[41] In Erα-positive BC cells with PR expression, estrogen-mediated proliferation and ERα transcriptional activity is blocked.[42] Blows explore the prognostic value of PR for patients with Erα-positive BC in a meta-analysis based on 10,159 patients, in which the mortality hazard

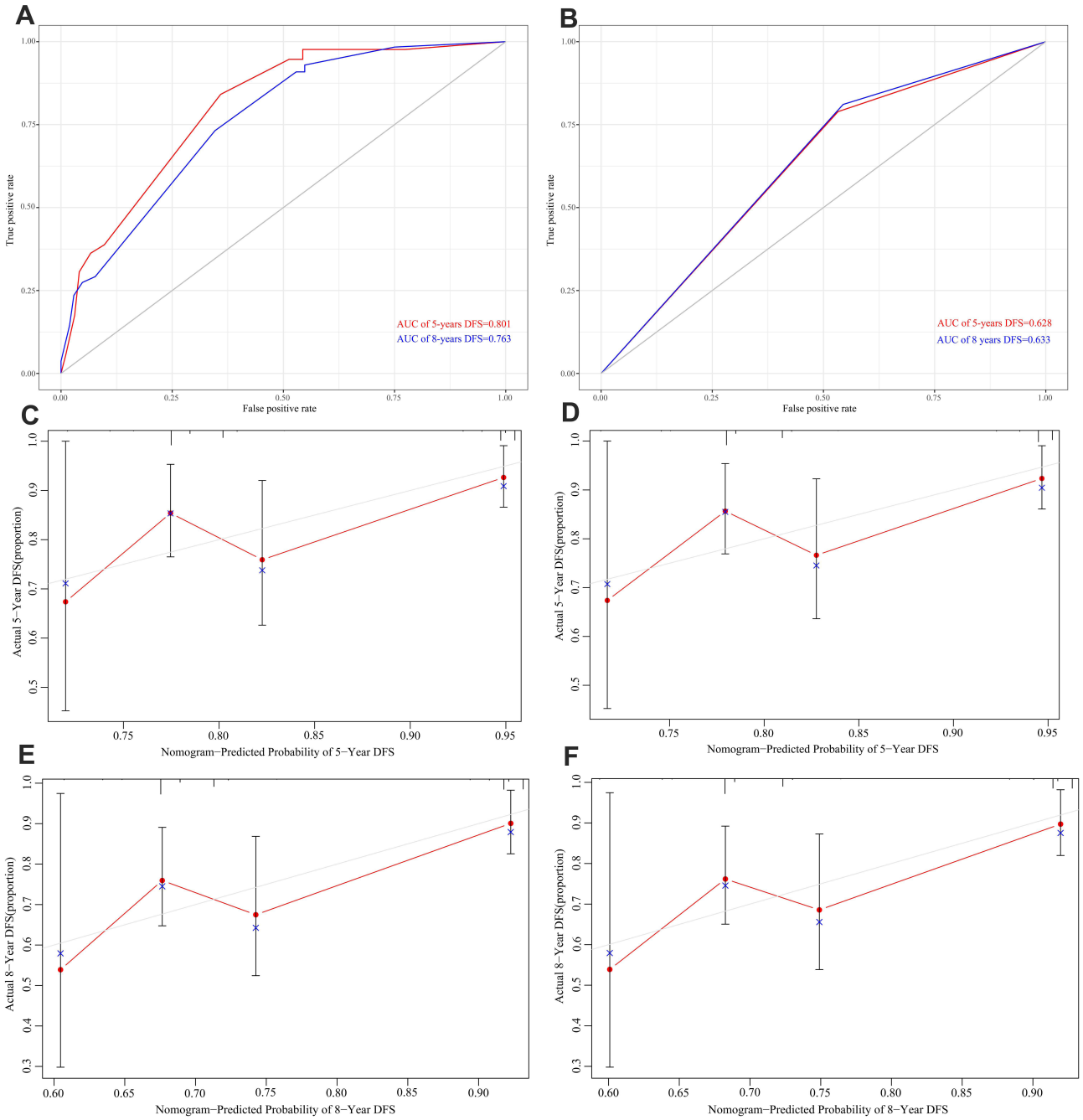

Figure 6 The validation of the prognostic model of DFS. (**A**) Receiver operating characteristics (ROC) curves in training dataset. (**B**) Receiver operating characteristics (ROC) curves in validation dataset. (**C**) Calibration plot of the nomogram model at 5-year in the training dataset. (**D**) Calibration plot of the nomogram model at 8-year in the training dataset. (**E**) Calibration plot of the nomogram model at 5-year in the validation dataset. (**F**) Calibration plot of the nomogram model at 8-year in the validation dataset.

ratio for PR status is showed to be time-dependent.[43] In addition, high PR levels are associated with fewer metastatic events in early BC.[44]

Fourth, the Ki67 index, named for the expression level of the cell cycle antigen Ki67 in IHC staining, is calculated as follows: Ki67 index = positive-staining cells/total malignant cells in the tumor tissue. The Ki67 index is widely acknowledged as a proliferative marker and is used as an independent prognostic signature in early BC. High Ki67 levels are associated with higher mortality and shorter OS.[45,46]

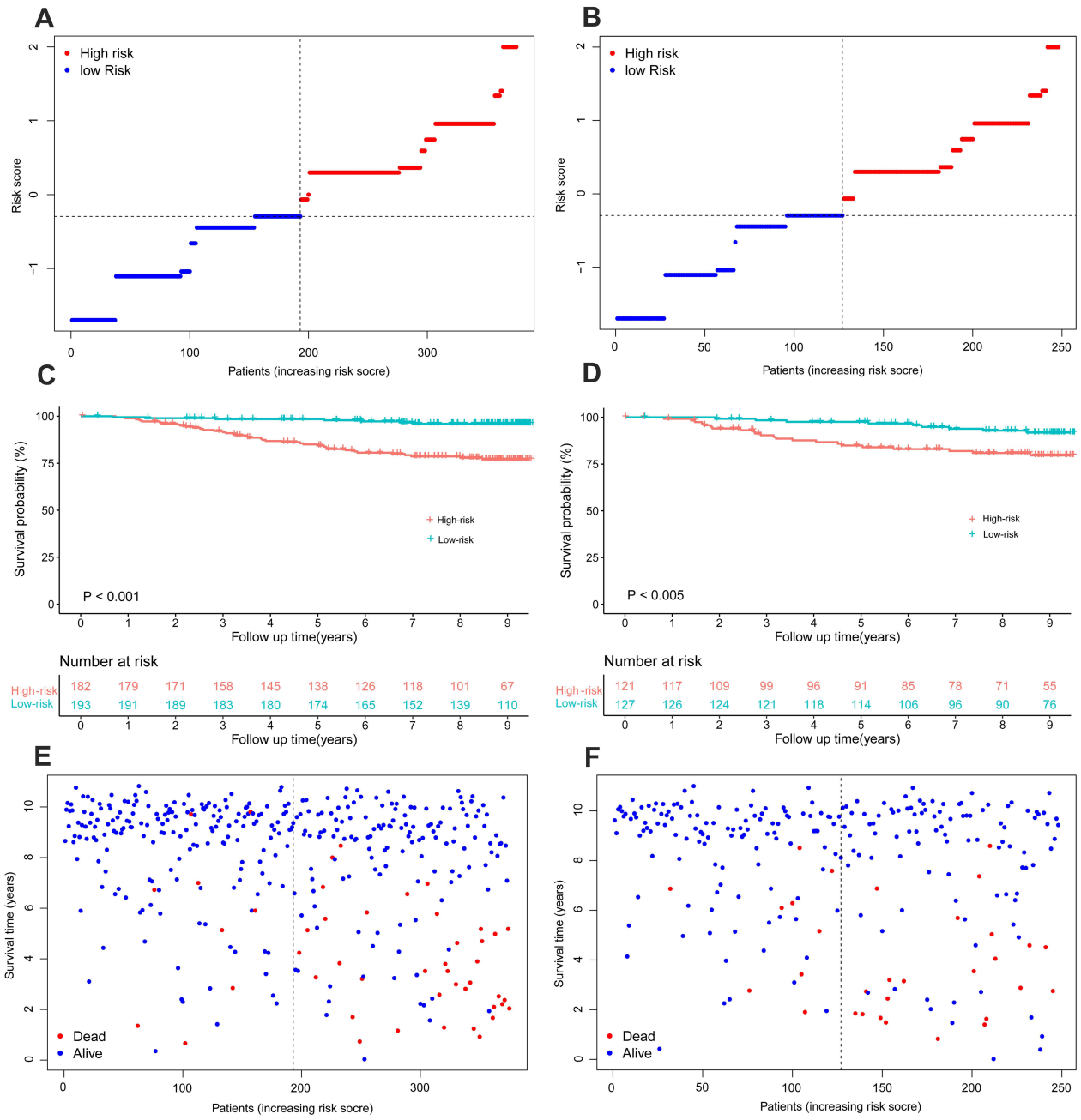

Figure 7 Overall survival analysis based on risk scores. (**A**) The distribution and the median value of the risk scores in the training dataset. (**B**) The distribution and the median value of the risk scores in the validation dataset. (**C**) Kaplan-Meier curves for the OS of patients in the high- and low-risk group in the training dataset. (**D**) Kaplan-Meier curves for the OS of patients in the high- and low-risk group in the validation dataset. (**E**) The distributions of OS status, OS and risk scores in the training dataset. (**F**) The distributions of OS status, OS and risk scores in the validation dataset.

Taking the individual patient's condition into consideration, we believe that this prognostic signature is optimal for patients with early BC. Although several models based on NLR, LMR, CAR, or genes may predict patient prognosis,[18,21,47] our model has its own characteristics. First, we used the C-index, calibration curves, and ROC curve survival analysis together to validate the robustness of our signature, and these methods are rarely used together. Moreover, our nomogram demonstrates a better AUC. Second, with regard to real-world feasibility, all information required in our model was obtained through basic characteristics inquiry, hematological and biochemical tests, and

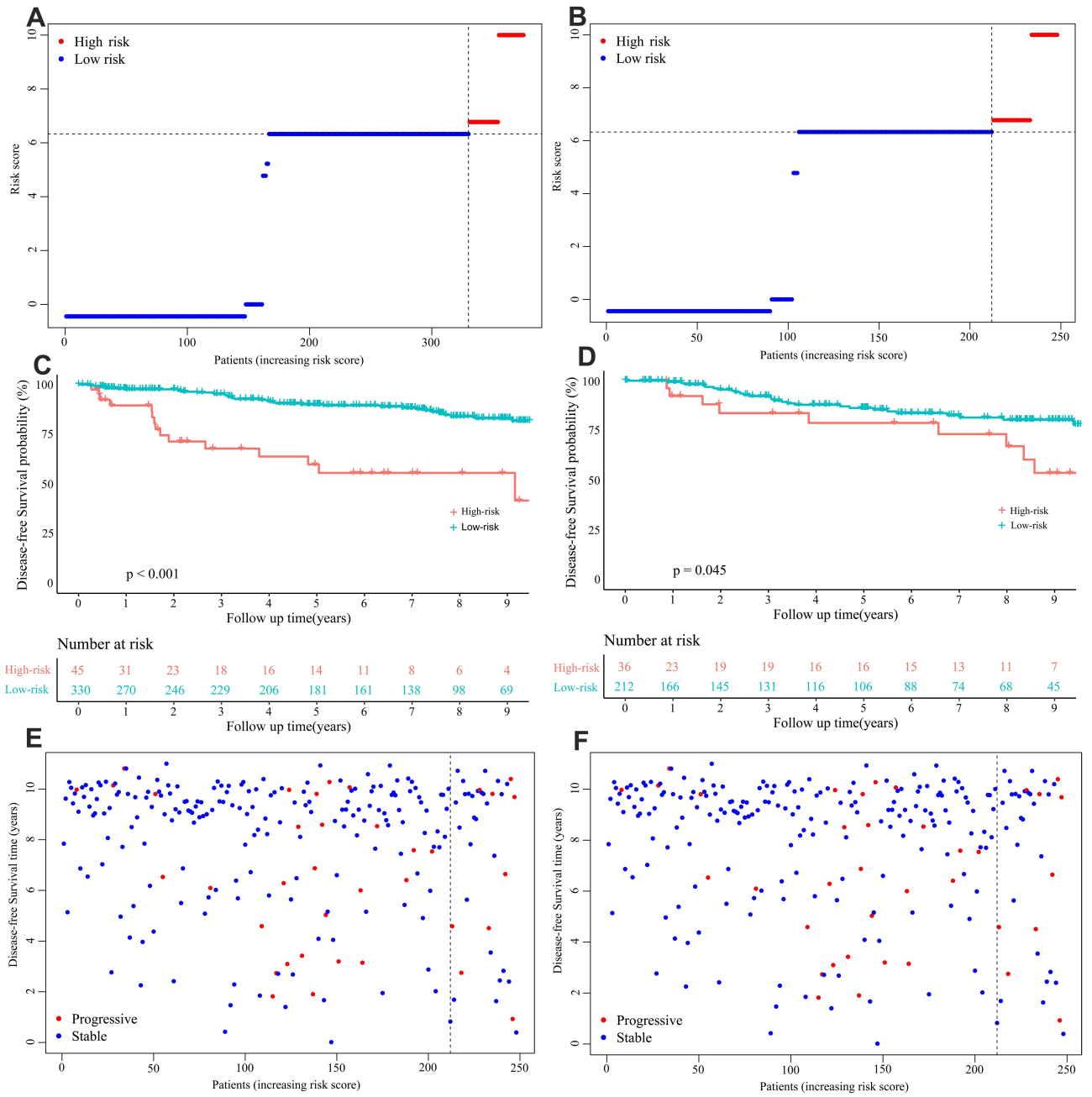

Figure 8 Disease-free survival analysis based on risk scores. (**A**) The distribution and the median value of the risk scores in the training dataset. (**B**) The distribution and the median value of the risk scores in the validation dataset. (**C**) Kaplan-Meier curves for the DFS of patients in the high- and low-risk group in the training dataset. (**D**) Kaplan-Meier curves for the DFS of patients in the high- and low-risk group in the validation dataset. (**E**) The distributions of DFS status, DFS and risk scores in the training dataset. (**F**) The distributions of DFS status, DFS and risk scores in the validation dataset.

pathological examination, which is more convenient and economical than that required for gene-based prognosis models.[48]

Our study has some limitations. First, selection bias is common in retrospective studies, and our study is no exception. Second, this was a single-center study, which might have affected the stability of the results somewhat. Third, we focused only on the pre-treatment LCR and did not explore its dynamic change during therapy. Finally, our nomogram was not validated externally and might require further validation in a prospective, multicenter, and larger-sample cohort in the future.

Conclusion

We innovatively explored the clinical value of a novel inflammatory marker in patients with early-stage BC, LCR, and revealed that it is a significant prognostic predictor of OS and DFS. The LCR-based prognostic signature demonstrated good predictive probability and accuracy. Therefore, it can be used as a tool for promoting individualized survival prediction.

Abbreviations

CRP, C-reactive protein; LCR, lymphocyte-to-C-reactive protein ratio; BC, breast cancer; OS, overall survival; C-index, concordance index; ROC, receiver operating characteristic; LVI, lymphatic vessel invasion; PR, progesterone receptor; AUC, area under the curve; ER, estrogen receptor; Her2, human epidermal growth factor receptor 2; TNM, tumor-node-metastasis; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; CAR, C-reactive protein-to-albumin ratio; SYSUCC, Sun Yat-sen University Cancer Center; PPC, postoperative pathological classification; PG, pathological grade; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; IQR, interquartile ranges; PH, proportional hazards.

Data Sharing Statement

The data analyzed in this study are available from the corresponding author (Xi-Wen Bi, E-mail: bixw@sysucc.org.cn) on reasonable request.

Ethics Approval and Consent to Participate

This study was approved by ethics committee of Sun Yat-sen University Cancer Center (registration number: B2022-277-01). Owing to we just retrospectively reviewed their medical data and did not impair their health, patient's informed consent was waived.

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Disclosure

The authors declare that they have no conflicts of interest.

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