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21-Gene recurrence score and locoregional recurrence in lymph node-negative, estrogen receptor-positive breast cancer

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Abstract

Background/purpose—The 21-gene recurrence score (RS) assay evaluates the likelihood of distant recurrence and benefit of chemotherapy in lymph node-negative, estrogen receptor (ER)-positive, HER2-negative breast cancer patients. The RS categories are associated with the risk of locoregional recurrence (LRR) in some, but not all studies.

Methods—We reviewed the institutional database to identify consecutive female patients with node-negative, ER+/HER2– breast carcinoma tested for the 21-gene RS assay and treated at our center from 2008 to 2013. We collected data on clinicopathologic features, treatment, and outcome. Statistical analysis was performed using SAS version 9.4 or R version 3.3.2.

Results—Of 2326 patients, 60% (1394) were in the low RS group, 33.4% (777) in the intermediate RS group, and 6.6% (155) in the high RS group. Median follow-up was 53 months. A total of 44 LRRs were observed, with a cumulative incidence of 0.17% at 12 months and 1.6% at 48 months. The cumulative incidence of LRR at 48 months was 0.84%, 2.72% and 2.80% for low, intermediate, and high RS groups, respectively ($p < 0.01$). Univariate analysis showed that the risk of LRR was associated with the RS categories ($p < 0.01$), T stage ($p < 0.01$) and lymphovascular invasion (LVI) ($p = 0.009$). There was no difference in LRR rates by initial local treatment (total

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Compliance with ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

The study was approved by the Institutional Review Board. For this type (retrospective) of study formal consent is not required.

Ethical Standards

The experiments (data analyses) comply with the current laws of the country in which they were performed (United States of America).

mastectomy vs. breast-conserving surgery plus radiation therapy). The RS remained significantly associated with LRR after adjusting for LVI and T stage. Compared to patients with low RS, the risk of LRR was increased more than 4-fold (hazard ratio: 4.61, 95% CI 1.90–11.19, $p < 0.01$), and 3-fold (hazard ratio: 2.81, 95% CI 1.41–5.56, $p < 0.01$) for high and intermediate risk categories, respectively.

Conclusions—Our study confirms that RS is significantly associated with the risk of LRR in node-negative, ER+/HER2– breast cancer patients. Our findings suggest that in addition to its value for prognostic stage grouping and decision-making regarding adjuvant systemic therapy, the role of the RS in identifying patients not requiring radiotherapy should be studied.

Keywords

breast cancer; 21-gene recurrence score assay; recurrence score; locoregional recurrence

Introduction

Estrogen receptor (ER)-positive breast cancer accounts for approximately 80% of all invasive breast tumors[1]. Following endocrine therapy, this subtype generally has a favorable prognosis with 5-year locoregional recurrence (LRR) and distant recurrence rates of approximately 10%[2–6] and low rates of breast cancer specific mortality. Many clinicopathologic factors have been reported to be associated with an increased risk of LRR, including patient age, tumor size, lymphovascular invasion (LVI), nodal status, ER and progesterone receptor (PR) status, molecular subtypes (luminal A vs. luminal B) and the duration of endocrine therapy [7,2,5,6,4,8,9].

Multigene prognostic classifiers developed in the last two decades have been clinically used to identify patients at a higher risk of distant recurrence. The 21-gene recurrence score (RS) assay (Oncotype Dx™, Genomic Health, Redwood City, CA)[10] is recommended by the American Society of Clinical Oncology (ASCO) and National Comprehensive Cancer Network (NCCN) for patients with early stage, ER+/HER2– invasive breast cancer[11,12], and has recently been included in the prognostic stage group in the 8th edition of American Joint Commission of Cancer (AJCC) staging manual[13]. Many prospective and retrospective studies have reported a strong, consistent association between the RS and the likelihood of distant recurrence, determining the magnitude of chemotherapy benefit[14–27]. Studies have investigated the relationship between the RS and the risk of LRR with conflicting results. The association between the RS and LRR was first suggested in large patient cohorts from National Surgical Adjuvant Breast and Bowel Project (NSABP) trials B-14 and B-20[28]. However, subsequent smaller studies, including retrospective analysis of a subset of patients in the E2197 prospective randomized trial by the Eastern Cooperative Oncology Group (ECOG), did not confirm this finding[29–31].

This study aimed to evaluate the value of the 21-gene RS assay for predicting the risk of LRR in a cohort of lymph node negative, ER+/HER2– breast cancer patients treated at a single institution.

Materials and methods

Patient cohort

Our retrospective cohort included unselected, consecutive female patients with lymph node negative (pN0 and pN0[i+])[32], ER+/HER2– invasive breast carcinoma with known 21-gene RS assay results treated at our center between September 2008 and August 2013. The exclusion criteria were male sex and failure of the assay for any technical reasons. Patients receiving neoadjuvant therapy were also excluded. At our institution, standard management of patients with early stage, lymph node negative, ER+/HER2– invasive breast cancer who are candidates for chemotherapy includes evaluation of tumor sample with the 21-gene RS assay. Carefully selected representative paraffin blocks from tumors measuring 0.5 cm are submitted to Genomic Health for testing. Occasional smaller tumors are also analyzed upon treating physician's request.

The 21-gene RS assay is based on reverse transcriptase polymerase chain reaction (RT-PCR) using the RNA isolated from formalin-fixed paraffin-embedded tissue. It evaluates the expression of 16 cancer related genes normalized to the expression of five reference genes and yields a numeric variable of RS on a scale of 0 to 100[14,15,33]. Based on the RS values, patients were stratified into three risk categories: low risk (RS < 17), intermediate risk (RS 18–30), and high risk (RS ≥ 31), as previously reported[14]. The results of the 21-gene RS assay were prospectively incorporated in the treatment plan as recommended[16–25]. Most low risk patients were treated with adjuvant endocrine therapy, whereas most high risk patients received a combination of endocrine therapy and chemotherapy. Treatment of patients with intermediate RS was variable depending on various clinicopathologic features and individual choices. RS was not routinely considered in the selection of locoregional therapy.

Clinicopathologic variables included patient age at breast cancer diagnosis, tumor size, histologic type of tumor, LVI, 21-gene RS result, local and systemic treatment, and clinical outcome. For multifocal/multicentric carcinomas, the size of the largest tumor and the highest RS result were recorded. For one patient with metachronous bilateral ER+/HER2– breast carcinomas with low RS, only the data pertaining to the first tumor were included. The institutional database and electronic medical records were reviewed to record date of last follow-up, date of death, date and type of LRR and distant recurrence. The Institutional Review Board approved the study.

Statistical analysis

Clinical and pathologic characteristics stratified by RS were summarized using frequency and percentage for categorical covariates, and the median and range for continuous factors. Categorical and continuous variables were compared using Fisher's exact test and Wilcoxon rank-sum test, respectively. Overall survival was calculated from date of pathologic diagnosis of breast cancer to date of last follow-up, estimated using Kaplan-Meier methods and compared using the log-rank test. Cumulative incidence of LRR and distant recurrence were estimated using competing risk methods and compared between RS or between other clinical characteristics using Gray's test[34]. LRR was defined as invasive breast cancer

involving the ipsilateral breast parenchyma, axilla, regional lymph nodes, chest wall, or skin identified more than six months from the initial diagnosis of breast cancer[35]. The cut-off date for clinical follow-up was 9/1/2016.

Multivariable competing risk regression was used to examine the independent effect of RS on LRR, adjusting for other factors that were significantly associated with LRR from the univariate analysis[36]. Univariate association of RS score on LRR was also examined among the subset of women treated with endocrine therapy and chemotherapy using the methods described above. Statistical analyses were performed using SAS version 9.4 (SAS Institute, INC., Cary, NC, USA) or R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria). P-values were two-sided and <0.05 were considered statistically significant.

Results

Patient cohort

We identified 2326 consecutive female patients with lymph node negative, ER+/HER2– breast carcinoma tested for RS and treated at our center during the study period (Table 1). The median age at the initial diagnosis of breast cancer was 57 years (range 22–90), and 6% (128/2326) of women were <40 years old. Most patients (60%; 1394/2326) were in the low RS group, 33.4% (777/2326) were in the intermediate RS group, and 6.6% (155/2326) were in the high RS group. The median tumor size was 1.2 cm (range 0.3–5.9), and 18% (420/2326) were multifocal/multicentric. Most tumors (79%; 1831/2326) were invasive ductal carcinoma not otherwise specified, 13% (305/2326) were invasive lobular carcinoma, 5% (113/2326) were mixed ductal and lobular, and 3% (77/2326) were special histologic subtypes. LVI was identified in 21% (486/2326) of patients.

Overall, 66.2% (1539/2326) of patients were treated with breast-conserving surgery (BCS) and radiation therapy, 3.8% (89/2326) with BCS alone, and 29.3% (682/2326) with total mastectomy. Most patients (64.7%; 1505/2326) received endocrine therapy alone, and 30.1% (699/2326) were treated with chemotherapy plus endocrine therapy. Only 0.9% (22/2326) of women received chemotherapy alone, and 3.4% (78/2326) received no systemic therapy.

Association of RS with LRR

Median follow-up was 53 months (range 0.9–108). We observed a total of 44 LRRs (Table 2). Of these, 37 LRRs occurred as first recurrences, six occurred as simultaneous events within three months of distant metastases and one LRR developed seven months after the diagnosis of distant recurrence. LRR was seen in 1.8% (27/1539) of patients treated with BCS and radiation therapy, 3.4% (3/89) of those having BCS alone and 2.1% (14/682) of those undergoing total mastectomy. All patients with LRR had negative surgical resection margins at the initial surgery. The ipsilateral breast was the most common site of LRR (45%; 20/44), followed by the ipsilateral axilla (16%; 7/44), and the ipsilateral chest wall (16%; 7/44). Two patients recurred in the ipsilateral supraclavicular lymph nodes and one in the ipsilateral internal mammary nodes, while 16% (7/44) developed LRR in multiple sites. The

cumulative incidence of LRR was 0.17% at 12 months and 1.6% at 48 months (Fig. 1). The cumulative incidence of LRR at 48 months was 0.84%, 2.7% and 2.8% for low, intermediate and high RS groups, respectively (Gray's test $p < 0.01$) (Fig. 2).

Univariate analysis showed that the risk of LRR was associated with RS categories (intermediate vs. low and high vs. low, both $p < 0.01$), LVI ($p = 0.009$), and T stage ($p < 0.01$) (Table 3). The risk of LRR in the intermediate RS group was approximately half that in the high RS group (hazard ratio: 0.53, 95% CI: 0.25–1.16, $p = 0.11$) but did not reach statistical significance, likely due to the small number of patients and events in the high risk category. Multifocality/multicentricity and age were not significantly associated with LRR in this population. The rates of LRR did not differ between patients treated with BCS plus radiation therapy vs. total mastectomy ($p = 0.781$).

Of the 44 women with LRR, 16 were treated with endocrine therapy alone, 25 were treated with endocrine therapy plus chemotherapy, two were treated with chemotherapy alone, and one received no systemic therapy. Of the 25 LRRs observed in the 699 endocrine therapy plus chemotherapy treated patients, RS was low in four patients, intermediate in 15 patients, and high in six patients. There was no significant association between RS and the rates of LRR ($p = 0.63$) among patients treated with endocrine therapy and chemotherapy. Subgroup analysis among patients treated with endocrine therapy alone was not possible due to low number of events (eight in the low RS group, six in the intermediate RS group and two in the high RS group).

Multivariable competing risk analysis

Multivariate competing risk analysis demonstrated that the RS categories were associated with the risk of LRR after adjusting for LVI and T stage (Table 4). There was a statistically significant association between LRR and high vs. low RS risk categories (hazard ratio: 4.61, 95% CI: 1.90–11.19, $p < 0.01$), and intermediate vs. low RS risk categories (hazard ratio: 2.81, 95% CI: 1.41–5.56, $p < 0.01$). However, the risk of LRR in intermediate vs. high RS risk categories did not vary significantly (hazard ratio: 0.61, 95% CI: 0.27–1.35, $p = 0.23$).

Association of RS with overall survival and distant metastases

During the follow-up period, 37 patients developed distant metastases and there were 36 deaths, eight due to breast cancer. Overall survival was significantly associated with the RS risk categories (log-rank $p = 0.01$) (Fig. 3A). The cumulative incidence of distant recurrence at 48 months was 0.41%, 1.77% and 3.6% for low, intermediate and high RS risk categories, respectively (Gray's test $p < 0.01$) (Fig. 3B).

Discussion

Our study confirms that the 21-gene RS risk categories are significantly associated with the risk of LRR in a cohort of 2326 node negative, ER+/HER2– breast cancer patients by univariate and multivariate analyses. Mamounas *et al.* first reported this association in 895 tamoxifen treated patients from the NSABP B-14 and B-20 trials ($p < 0.001$), 424 chemotherapy and tamoxifen treated patients from the B-20 trial ($p = 0.028$), and 355 placebo patients from the B-14 trial ($p = 0.022$) [28]. LRR was associated with RS > 24 in patients

treated with total mastectomy in another smaller study[29]. In contrast, a retrospective analysis of 388 patients treated with breast conservation, radiation and adjuvant systemic chemotherapy in the ECOG E2197 trial showed no association between LRR and RS. However, in the subset of ER+ and/or PR+/HER2– tumors for which the RS was designed to be used, the RS as a continuous variable was significantly associated with LRR (hazard ratio: 2.66; p=0.03)[30].

The overall LRR rates were very low in our study, with only 44 LRR events and a 1.6% cumulative incidence of LRR at 48 months, including 0.84%, 2.72% and 2.8% LRR rates for low, intermediate and high RS risk categories, respectively (p<0.01). Other studies examining RS as a predictor of LRR have reported higher rates of LRR. The 10-year Kaplan-Meier estimate of LRR was 4.3%, 7.2% and 15.8% in low, intermediate and high RS groups in tamoxifen treated patients in the NSABP B-14 and B-20 trials in the report by Mamounas *et al* [28]. However, in the B-14 study high RS patients did not receive chemotherapy as was done in our study, likely accounting for the higher rate of LRR. Data from the B-20 study shows that the use of chemotherapy plus endocrine therapy in high RS patients reduces, but does not eliminate the difference in LRR seen on the basis of RS[28]. The ECOG E2197 trial included both node negative and 1–3 node positive patients and reported the 10-year LRR rates of 3.8%, 5.1%, and 12 % for the low, intermediate, and high RS risk categories, respectively[30].

We found no significant difference in the LRR rates by patient age or the type of initial local treatment (total mastectomy vs. BCS with radiation therapy). Given the very low absolute risk of LRR in the high RS group and similar LRR rates irrespective of the surgical procedure, our study provides no evidence that total mastectomy is superior to BCS with radiation therapy with regard to reducing the risk of future LRR.

The distribution of the RS risk categories in our cohort was 60%, 33.4% and 6.6% in the low, intermediate and high RS groups, respectively. This is comparable with other studies of ER+/HER2– breast cancers reporting RS distributions of 47–54%, 21–37% and 15–27% for the three risk categories[37,14,15,30]. The differences in the distribution of the RS risk categories among institutions most likely reflect patient population and selection bias with variable testing thresholds exercised by clinicians[37]. Nevertheless, the 21-gene RS assay has become an integral part of standard management of node negative, ER+/HER2– breast cancer patients. With the recent implementation of the RS results in prognostic stage groupings of early stage (T1-2 N0M0), ER+/HER2– invasive breast carcinomas by the AJCC[13], the 21-gene RS assay will likely continue to guide treatment decisions for the foreseeable future.

Strengths of this study include a large, unselected, consecutive population of breast cancer patients with clinical outcome whose RS results were prospectively included in the treatment planning and detailed knowledge of pathologic and treatment variables. Its main limitations are the retrospective study design, the low number of LRR events, and the follow-up time interval of less than five years in some patients. In addition, our tertiary academic institution predominantly treats women with screen-detected breast cancer and women from a specific

geographic region. Nonetheless, our findings are in agreement with the results of other studies discussed above.

In conclusion, we report low LRR rates and a significant association between the RS risk categories (low, intermediate, high) and the risk of LRR in a cohort of 2326 lymph node negative, ER+/HER2– breast cancer patients by univariate and multivariate analyses. Our findings suggest that in addition to its value for prognostic stage grouping and decision making regarding adjuvant systemic therapy, the role of the RS in identifying patients not requiring radiotherapy should be studied.

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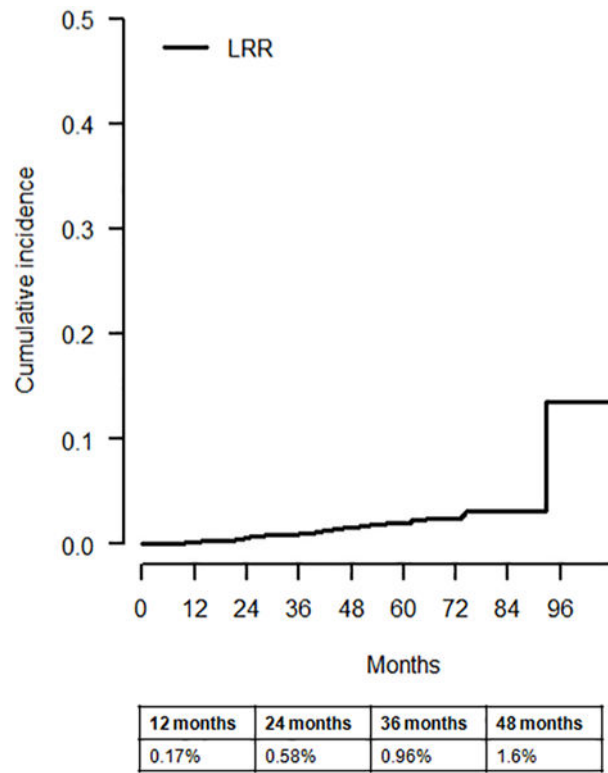


Fig. 1.
Cumulative incidence of locoregional recurrence (LRR)

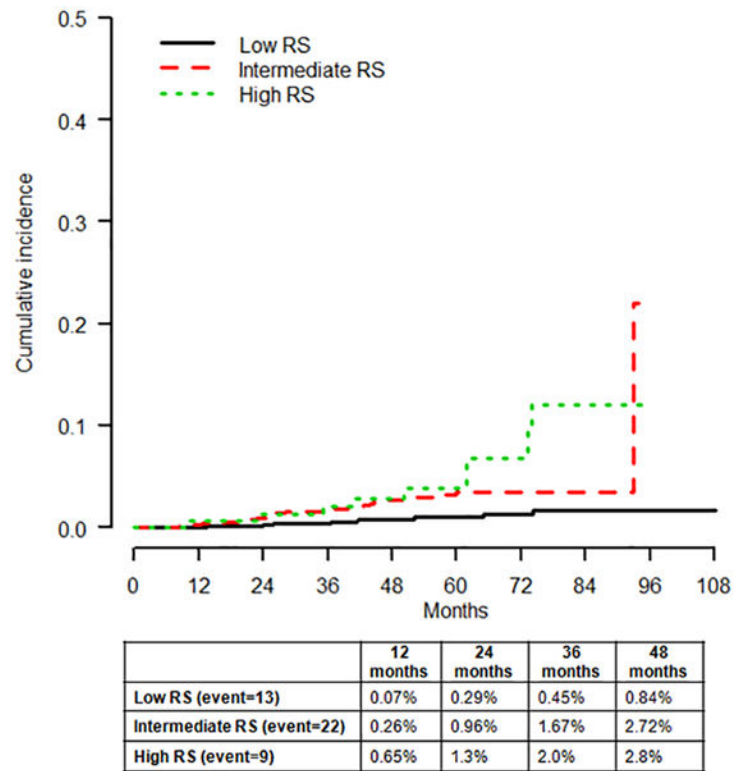


Fig. 2.
Cumulative incidence of locoregional recurrence (LRR) by recurrence score (RS) categories (Gray's test $p < 0.01$)

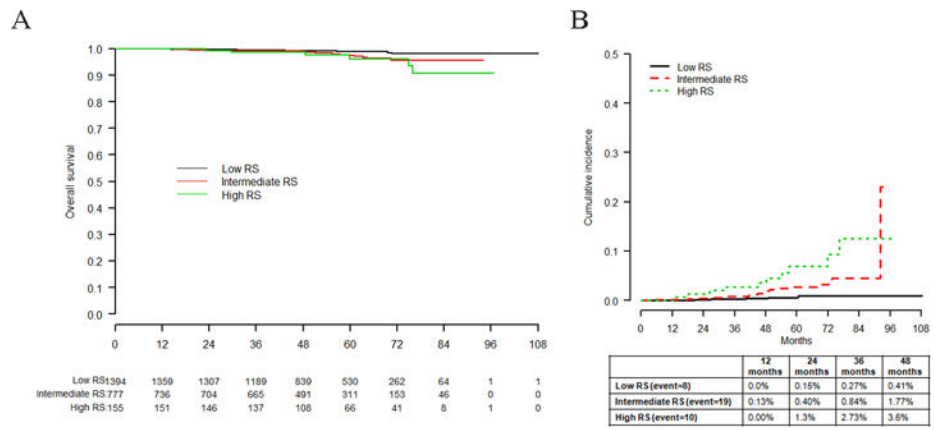


Fig. 3. A Analysis of overall survival by recurrence score (RS) categories (log-rank $p = 0.01$). B Cumulative incidence of distance recurrence by RS categories (Gray's test p value < 0.01)

Table 1

Clinicopathologic characteristics of 2326 patients by RS results

	Total (n=2326)	Low RS (n=1394)	Intermediate RS (n=777)	High RS (n=155)	p-value
Age at diagnosis, years					
<40 years	128 (6%)	63 (5%)	47 (6%)	18 (12%)	<0.01
40 years	2198 (94%)	1331 (95%)	730 (94%)	137 (88%)	
Median (range)	57 (22–90)	57.0 (22–90)	57 (24–85)	57 (27–83)	
Tumor size, median (range), cm	1.2 (0.3–5.9)	1.2 (0.3–5.8)	1.2 (0.3–5.9)	1.5 (0.35–5)	<0.01
Multifocality/multicentricity	420 (18%)	297 (21%)	105 (14%)	18 (12%)	<0.01
LVI	486 (21%)	265 (19%)	176 (23%)	45 (29%)	<0.01
Local Treatment					
BCS	89 (3.8%)	48 (3.4%)	33 (4.2%)	8 (5.2%)	
BCS + radiation	1539 (66.2%)	929 (66.6%)	510 (65.6%)	100 (64.5%)	0.73
Total mastectomy	682 (29.3%)	417 (29.9%)	220 (28.3%)	45 (29%)	
BCS, radiation unknown	16 (0.7%)	0	14 (1.8%)	2 (1.3%)	
Systemic therapy					
Endocrine therapy only	1505 (64.7%)	1182 (84.8%)	309 (39.8%)	14 (9%)	N/A*
Endocrine + chemotherapy	699 (30.1%)	168 (12.1%)	398 (51.2%)	133 (85.8%)	
Chemotherapy only	22 (0.9%)	1 (0.1%)	19 (2.4%)	2 (1.3%)	
None	78 (3.4%)	43 (3.1%)	32 (4.1%)	3 (1.9%)	
Unknown	22 (0.9%)	0	19 (2.4%)	3 (1.9%)	

BCS, breast-conserving surgery; LVI, lymphovascular invasion; RS, recurrence score;

* Not provided as systemic treatment was guided by the RS results.

Table 2

Clinicopathologic characteristics of 44 patients with LRR

	Total (n=44)	Low RS (n=13)	Intermediate RS (n=22)	High RS (n=9)
Age at diagnosis, years				
<40 years	39 (88.6%)	11 (84.6%)	2 (9.1%)	1 (11.1%)
40 years	5 (11.4%)	2 (15.4%)	20 (90.9%)	8 (88.9%)
Median (range)	54 (34–79)	54 (35–79)	51 (38–75)	57 (34–69)
Tumor size, median (range), cm	1.6 (0.5–3.5)	1.3 (0.5–3.5)	1.7 (0.5–3.4)	1.6 (0.6–2.6)
Multifocality/multicentricity	9 (20.5%)	6 (46.2%)	3 (13.6%)	0
LVI	17 (38.6%)	4 (30.8%)	11 (50%)	2 (22.2%)
Local Treatment				
BCS	3 (6.8%)	2 (15.4%)	0	1 (11.1%)
BCS + radiation	27 (61.4%)	7 (53.8%)	14 (63.6%)	6 (66.7%)
Total mastectomy	14 (31.8%)	4 (30.8%)	8 (36.4%)	2 (22.2%)
Systemic therapy				
Endocrine therapy only	16 (36.4%)	8 (61.5%)	6 (27.3%)	2 (22.2%)
Endocrine + chemotherapy	25 (56.8%)	4 (30.8%)	15 (68.2%)	6 (66.7%)
Chemotherapy only	2 (4.5%)	0	1 (4.5%)	1 (11.1%)
None	1 (2.3%)	1 (7.7%)	0	0

BCS, breast-conserving surgery; LVI, lymphovascular invasion; LRR, locoregional recurrence; RS, recurrence score.

Table 3

Association of clinicopathologic variables with LRR by univariate analysis

	HR	95% CI	p-value
RS score			
Intermediate vs. low	2.94	(1.48–5.84)	<0.01
High vs. low	5.51	(2.36–12.87)	<0.01
T stage (T2-3 vs. T1)	2.83	(1.48–5.40)	<0.01
LVI (yes vs. no)	2.28	(1.23–4.17)	0.009
Multifocal/multicentral (yes vs. no)	1.16	(0.56–2.42)	0.693
Age 40 vs. <40 years	0.44	(0.17–1.11)	0.08

CI, confidence interval; HR, hazard ratio; LVI, lymphovascular invasion; LRR, locoregional recurrence; RS, recurrence score.

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Table 4

Multivariable competing risk analysis of predictors of LRR in 2326 patients

	HR	95%CI	p-value
RS score			
High vs. low	4.61	(1.90–11.19)	<0.01
Intermediate vs. low	2.81	(1.41–5.56)	<0.01
Intermediate vs. high	0.61	(0.27–1.35)	0.23
T stage (T2-3 vs T1)	2.10	(1.02–4.31)	0.04
LVI (yes vs. no)	1.81	(0.96–3.42)	0.06

CI, confidence interval; HR, hazard ratio; LVI, lymphovascular invasion; LRR, locoregional recurrence; RS, recurrence score.