Clinicopathological characteristics, adjuvant chemotherapy decision and disease outcome in patients with breast cancer with a 21-gene recurrence score of 26-30

JING YU, JIAYI WU, OU HUANG, JIANRONG HE, LI ZHU, WEIGUO CHEN, YAFEN LI, XIAOSONG CHEN and KUNWEI SHEN

Department of General Surgery, Comprehensive Breast Health Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, P.R. China

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Abstract. Recurrence score (RS) could be used to predict clinical outcomes and chemotherapy efficacy in patients with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative and lymph node-negative breast cancer. However, the clinical features and management of patients with an RS of 26-30 are not completely understood. In the present study, 783 patients with $HR^+/HER2^-$, lymph node-negative early breast cancer and RS ≥18 were included and categorized into RS=18-25 (47.8%), 26-30 (25.5%) or ≥31 (26.7%) groups. Clinicopathological characteristics, adjuvant chemotherapy usage and disease outcomes were compared. Alterations in the adjuvant chemotherapy recommendation after 21-gene RS testing were also analyzed. The results indicated that patients with RS=26-30 had higher progesterone receptor (PR) expression [odds ratio (OR)=2.84; P<0.001] and lower Ki-67 index (OR, 1.88; P=0.032) compared with patients with RS ≥31. Multivariate analysis demonstrated that age ≤50 years (OR, 5.75; P=0.001) and luminal-B subtype (OR, 7.75; P<0.001) were factors that were independently associated with chemotherapy usage in the RS=26-30 group. Among 104 patients who were not recommended chemotherapy before 21-gene RS testing, the treatment decision for 52 patients was changed to recommend chemotherapy once an RS of 26-30 was identified. The patient adherence rate to the treatment recommendation was 95.0% (190/200). After a median follow-up of 21.5 months, 6 patients displayed disease recurrence in the RS=26-30 group, and there was no significant difference between patients receiving chemotherapy and patients not receiving chemotherapy. In conclusion, patients with RS=26-30 had tumors with higher PR expression and lower Ki-67 index compared with those of patients with RS ≥31. Age, luminal subtype and RS testing influenced chemotherapy usage in patients with RS=26-30; however, no significant benefit from adjuvant chemotherapy was observed in a short term of 2 years.

Correspondence to: Dr Xiaosong Chen or Dr Kunwei Shen, Department of General Surgery, Comprehensive Breast Health Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, 197 Ruijin Er Road, Shanghai 200025, P.R. China
E-mail: chenxiaosong0156@hotmail.com
E-mail: kwshen@medmail.com.cn

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Introduction

In women, breast cancer is the most frequently diagnosed malignant disease, accounting for 25% of all cancer cases, and presenting the highest mortality rate worldwide (1). Chemotherapy can reduce the risk of 10-year mortality by a third in patients with breast cancer (2). Approximately 60-75% of patients have hormone receptor (HR)-positive breast cancer, for whom adjuvant endocrine therapy alone can significantly improve clinical outcomes (3). Traditionally, physicians make treatment decisions based on clinicopathological characteristics; however, in the past decades, several multigene signatures have been developed, which can provide more precise prognostic and predictive information for early-stage HR-positive breast cancer. Multigene signatures facilitate individualized treatment and decrease adjuvant chemotherapy usage in patients with breast cancer (4).

Among the identified multigene assays, the 21-gene recurrence score (RS) assay, which is composed of 16 cancer-related genes and 5 reference genes, is the most widely studied and used in the clinic (5). The 21-gene RS assay is performed on fixed, paraffin-embedded tumor tissues using reverse transcription-quantitative PCR (RT-qPCR). The 21-gene RS assay scores, ranging from 0 to 100, are classified into low-(RS <18), intermediate-(18≤ RS <31) and high-(RS ≥31) risk groups (5). Data from National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 trial showed that RS predicted distant recurrence for patients with HR-positive, human epidermal growth factor receptor 2 (HER2)-negative and lymph node-negative breast cancer who were treated with tamoxifen (5). The NSABP B-20 trial further demonstrated that patients in the high-risk group displayed a 27.6% decrease

in the 10-year distant recurrence rate due to chemotherapy, which confirmed the necessity of adjuvant chemotherapy for patients with RS ≥31 (6). The Southwest Oncology Group-8814 and Eastern Cooperative Oncology Group E2197 studies extended the application of the 21-gene RS assay to the lymph node-positive population (7,8). With robust prognostic and predictive value in breast cancer, the 21-gene RS assay has been recommended by clinical practice guidelines, including those published by the National Comprehensive Cancer Network (NCCN) (9), and has been increasingly used in the clinic worldwide.

To lower the risk of undertreatment, which was defined as controlling the 10-year recurrence risk of breast cancer at a distant site to 10 and 20% for each cutoff, respectively, the prospective trials TAILORx and West Germany Study Plan-B used the 21-gene RS assay with a different cutoff value compared with previous studies (10,11). In the two aforementioned trials, the risk group classification criteria were as follows: Low (RS <11), intermediate (11≤ RS <26) and high (RS ≥26). The TAILORx trial reported that endocrine therapy alone resulted in improved disease-free survival for patients in the low-risk group (10). For patients with RS=11-25, adjuvant chemotherapy did not provide additional survival benefits, especially for patients aged >50 years (12). Furthermore, for patients with RS=26-100 receiving adjuvant chemotherapy, the clinical outcomes were improved compared with those of patients treated with endocrine therapy alone (13).

In the TAILORx study, patients with RS ≥26 received adjuvant chemotherapy (10); however, the NSABP B-20 study demonstrated that only patients with RS ≥31 tumors benefited the most from adjuvant chemotherapy (6). At present, there are limited data available on the clinicopathological features and treatment patterns of patients with RS=26-30.

The present study evaluated the clinicopathological characteristics, adjuvant chemotherapy usage and disease outcomes of patients with $HR^+/HER2^-$/lymph $node^-$ breast cancer with RS=26-30 in comparison with those of patients with RS=18-25 and RS ≥31. Furthermore, whether 21-gene RS testing lead to a treatment recommendation alteration was investigated. The aim of the present study was to demonstrate the clinical features and to identify the appropriate treatment decision for patients with RS=26-30.

Materials and methods

Study population. Female patients diagnosed with invasive breast cancer who received surgery between January 2014 and December 2017 at Ruijin Hospital, Shanghai Jiaotong University School of Medicine (Shanghai, China) were retrospectively included in the present study. Data regarding clinicopathological characteristics, treatment decisions and survival events were retrieved from Shanghai Jiaotong University Breast Cancer Database. The present study was reviewed and approved by the Ethical Committee of Ruijin Hospital. The inclusion criteria were as follows: i) Primary invasive breast cancer; ii) HR positive and HER2 negative; iii) lymph node-negative; and iv) RS ≥18. The exclusion criteria were as follows: i) Male patients with breast cancer; and ii) patients with incomplete data for immunohistochemical (IHC) results, chemotherapy usage or survival.

Pathological, IHC analysis and 21-gene RS assay testing. Histopathological examination of tumor histological subtype, grade and presence of lymphovascular invasion was conducted by experienced pathologists at the Department of Pathology of Ruijin Hospital in concordance with the World Health Organization classification (14). The tumor tissue was fixed by 10% neutral buffered formalin at room temperature overnight before embedding in paraffin. The 4-μm-thick, formalin-fixed, paraffin-embedded (FFPE) tissue sections were incubated with the peroxidase-blocking solution (cat. no. S2023; Dako; Agilent Technologies, Inc.) for 3 min and blocked with the blocking solution (cat. no. X0909; Dako; Agilent Technologies, Inc.) for 10 min after dewaxing in xylene for 60 min and rehydration in a descending alcohol series (100, 95 and 75%), all at room temperature. Subsequently, IHC staining of estrogen receptor (ER), progesterone receptor (PR) and Ki-67 index was automatically performed using a Ventana BenchMark XT system (Ventana Medical Systems, Inc.). Briefly, the FFPE tissue sections were incubated for 32 min at 42˚C with primary antibodies targeted against ER (cat. no. IR657; clone 1D5; 1:100; rabbit monoclonal), PR (cat. no. IR068; clone PR636; 1:100; mouse monoclonal) and Ki-67 (cat. no. IR626; clone MIB-1; 1:100; mouse monoclonal) (all Dako; Agilent Technologies, Inc.). Subsequently, tissue sections were incubated with secondary goat anti-mouse (cat. no. P0447) or goat anti-rabbit (cat. no. P0448) (both 1:100; Dako; Agilent Technologies, Inc.) antibodies for 30 min at room temperature. A Dako automated immunohistochemistry system (Dako; Agilent Technologies, Inc.) was used to interpret the IHC results, which were checked by two experienced pathologists using a light microscope (magnification, x100). ER^+ and PR^+ tumors were defined as tumors with nuclear staining in ≥1% of tumor cells. Ki-67 index was assessed in ≥1,000 invasive tumor cells, and was characterized as the proportion of positively stained cells in the nucleus vs. the total number of cells in the field. Luminal subtype was determined according to the St Gallen 2013 expert panel (15). Luminal A-like subtype was defined as ER^+, PR ≥20% and Ki-67 <14%, while luminal B-like was defined as ER^+ and PR <20% or Ki-67 ≥14%. The 21-gene RS assay was performed on formalin-fixed, paraffin-embedded tissue sections as described in our previous study (16). The amount of tissue was determined by assessing the percentage of tumor on hematoxylin and eosin-stained slides. Briefly, the tissue slides were stained with Harris hematoxylin solution for 10 min and then differentiated in 1% acid alcohol, all at room temperature. Following bluing, eosin solution was used for counterstaining for 30 sec at room temperature, and then slides were dehydrated in 95 and 100% alcohol, washed in xylene and mounted using a neutral balsam (data not shown). Total RNA was extracted using the RNeasy FFPE RNA kit (Qiagen GmbH) from two 10-μm unstained sections and was measured after verifying the absence of DNA contamination, which was assessed by a quantitative (q)PCR assay for β-actin DNA. Gene-specific reverse transcription was performed at 65˚C for 5 min and 37˚C for 60 min using the Omniscript RT kit (Qiagen GmbH). Subsequently, standardized qPCR was performed using Premix Ex Taq™ (Takara Bio, Inc.) in 96-well plates using an Applied Biosystems 7500 Real-Time PCR system (Thermo Fisher Scientific, Inc.), with the following thermocycling conditions: 95˚C for 10 min, 95˚C

for 20 sec and 60˚C for 45 sec (for 40 cycles). The primers and probes used for qPCR are listed in Table SI. The expression levels of each cancer-associated gene were measured in triplicate and normalized to 5 reference genes, including ACTB, GAPD, GUSB, RPLP0 and TFRC. The RS was calculated using a specific algorithm as previously described (5). For patients with ipsilateral multifocal or bilateral invasive cancer, the highest RS was recorded.

Treatment decision and prognosis information. After surgery, the multidisciplinary team (MDT), which consisted of breast surgeons, medical oncologists, pathologists, radiation oncologists and specialized breast nurses, discussed and determined the appropriate adjuvant treatment recommendations for each patient. In the first-round of MDT voting, a primary chemotherapy recommendation was made based on standard clinicopathological and IHC results, which was recorded as the chemotherapy recommendation pre-RS assay. After obtaining the result of the 21-gene RS assay, the second-round of MDT voting was organized to determine the final decision, which was recorded as the chemotherapy recommendation post-RS assay. In both rounds of voting, if the vote was not unanimous, the decision of the attending physician who performed the surgery was recorded. The actual chemotherapy usage was confirmed during follow-up. The most frequently used chemotherapy regimens included docetaxel plus cyclophosphamide, epirubicin plus cyclophosphamide and epirubicin plus cyclophosphamide followed by docetaxel.

Statistical analysis. All clinicopathological characteristics were presented as patient number and percentage [n, (%)] and analyzed as categorical variables. The χ^2 test was used to evaluate the RS distribution and chemotherapy usage in patients with different clinicopathological characteristics, and Fisher's exact test was performed when had expected values less than 5. Multiple logistic regression models were used to generate adjusted odds ratios (ORs) with 95% confidence intervals (CIs) to assess factors associated with RS distribution and chemotherapy. Kaplan-Meier with Tarone-Ware tests (17) was used to compare the recurrence-free survival rate, and pairwise comparisons were performed for the recurrence-free survival. Recurrence events included local, regional, distant and contralateral breast recurrence. P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed using SPSS software (version 22.0; IBM Corp.).

Results

Baseline clinicopathological characteristics. A total of 821 patients diagnosed between January 2014 and December 2017 were reviewed, of which, 38 were excluded and 783 were included in the present study. The number of patients in the RS=18-25, =26-30 and ≥31 groups was 374 (47.8%), 200 (25.5%) and 209 (26.7%), respectively. Baseline clinicopathological characteristics are presented in Table I. The mean age of the patients was 56.0±12.4 years, and 511 (65.3%) patients were aged >50 years. A total of 565 (72.2%) patients had tumors ≤2 cm in size. Additionally, 138 (17.6%) patients had grade-III tumors. The proportion of patients with ER ≥50%, PR ≥20% and Ki-67 ≥14% was 96.6, 63.9 and 53.0%, respectively. Furthermore, 562 (71.8%) patients had luminal B-like breast cancer.

Clinicopathological characteristics of the different RS groups. Univariate analysis indicated that age (P=0.030), tumor grade (P<0.001), ER status (P=0.001), PR status (P<0.001), Ki-67 index (P<0.001) and luminal subtype (P<0.001) were significantly different among the three RS groups (Table I). Grade-III tumors were present in 10.7, 17.0 and 30.6% of patients in the RS=18-25, =26-30 and ≥31 groups, respectively. Regarding luminal subtype, the RS=18-25, =26-30 and ≥31 groups contained 59.1, 75.5 and 90.9% luminal B-like tumors, respectively (Table I).

Multivariate analysis demonstrated that tumor grade, PR expression and Ki-67 index were independently associated with RS grouping (P<0.05). Compared with the RS ≥31 group, the RS=26-30 group was associated with higher PR expression (OR, 2.84; 95% CI, 1.69-4.79; P<0.001) and lower Ki-67 index (OR, 1.88; 95% CI, 1.06-3.34; P=0.032), and tended to display fewer grade-III tumors (OR, 1.63; 95% CI, 0.96-2.76; P=0.070). There was no significant difference between the RS=18-25 and =26-30 groups in terms of grade (P=0.133), PR expression (P=0.063) or Ki-67 index (P=0.924; Table II).

Adjuvant chemotherapy usage in the different RS groups. A total of 115 (30.7%), 140 (70.0%) and 186 (89.0%) patients in the RS=18-25, =26-30 and ≥31 groups, respectively, received chemotherapy (P<0.001). Table III presents the clinicopathological parameters associated with chemotherapy usage in the whole population.

Multivariate analysis indicated that menstruation (OR, 2.55; 95% CI, 1.62-4.02; P<0.001), larger tumor size (OR, 1.91; 95% CI, 1.23-2.98; P=0.004), histological grade III (OR, 2.35; 95% CI, 1.25-4.40; P=0.008), higher Ki-67 index (OR, 2.59; 95% CI, 1.40-4.81; P=0.002), luminal B-like tumor (OR, 2.29; 95% CI, 1.09-4.83; P=0.029) and RS category (P<0.001) were independently associated with chemotherapy usage. Compared with patients with RS=18-25, patients with RS=26-30 (OR, 7.20; 95% CI, 4.55-11.42; P<0.001) or RS ≥31 (OR, 16.08; 95% CI, 9.19-28.14; P<0.001) were more likely to receive adjuvant chemotherapy. Furthermore, patients with comorbidities received chemotherapy less often compared with patients without comorbidities (OR, 0.52; 95% CI, 0.34-0.79; P=0.002; Table IV).

In the RS=18-25 group, distribution of age, menstrual status, comorbidity, lymphovascular invasion, tumor size, histological type, tumor grade, PR status, Ki-67 index and luminal subtype were significantly different between patients receiving adjuvant chemotherapy and those not receiving it (Table SII). Multivariate analyses demonstrated that menstruation (OR, 2.53; 95% CI, 1.38-4.65; P=0.003), larger tumor size (OR, 2.21; 95% CI, 1.20-4.09; P=0.011), grade-III tumors (OR, 2.75; 95% CI, 1.18-6.44; P=0.019), PR <20% (OR, 4.36; 95% CI, 2.24-8.48; P<0.001) and Ki-67 ≥14% (OR, 6.90; 95% CI, 3.83-12.46; P<0.001) were factors independently associated with chemotherapy usage (Table SIII).

In the RS ≥31 group, univariate analysis indicated that younger age (97.0% vs. 85.2%; P=0.009), menstruation (97.0% vs. 85.2%; P=0.009), no comorbidity (94.6% vs. 79.7%;

Table I. Clinicopathological characteristics of patients according to 21-gene RS classification.

Variable	Total, n (%) (n=783)	RS=18-25, n (%) (n=374, 47.8%)	RS=26-30, n (%) (n=200, 25.5%)	RS ≥31, n (%) (n=209, 26.7%)	P-value
Age (years)					0.030
≤50	272 (34.7)	147 (39.3)	58 (29.0)	67 (32.1)	
>50	511 (65.3)	227 (60.7)	142 (71.0)	142 (67.9)	
BMI					0.740
<25	585 (74.7)	284 (75.9)	148 (74.0)	153 (73.2)	
≥25	198 (25.3)	90 (24.1)	52 (26.0)	56 (26.8)	
Comorbidity					0.286
No	456 (58.2)	217 (58.0)	91 (45.5)	130 (62.2)	
Yes	327 (41.8)	157 (42.0)	109 (54.5)	79 (37.8)	
Tumor size (cm)					0.206
≤2	565 (72.2)	281 (75.1)	139 (69.5)	145 (69.4)	
>2	218 (27.8)	93 (24.9)	61 (30.5)	64 (30.6)	
Histological type					0.123
IDC	650 (83.0)	301 (80.5)	167 (83.5)	182 (87.1)	
Non-IDC	133 (17.0)	73 (19.5)	33 (16.5)	27 (12.9)	
Tumor grade					<0.001
I/II	524 (66.9)	268 (71.7)	135 (67.5)	121 (57.9)	
III	138 (17.6)	40 (10.7)	34 (17.0)	64 (30.6)	
Unknown	121 (15.5)	66 (17.6)	31 (15.5)	24 (11.5)	
LVI					0.994
Yes	38 (4.9)	18 (4.8)	10 (5.0)	10 (4.8)	
No	745 (95.1)	356 (95.2)	190 (95.0)	199 (95.2)	
ER status (%)					0.001
<50	27 (3.4)	7 (1.9)	4 (2.0)	16 (7.7)	
≥50	756 (96.6)	367 (98.1)	196 (98.0)	193 (92.3)	
PR status (%)					<0.001
<20	283 (36.1)	82 (21.9)	75 (37.5)	126 (60.3)	
≥20	500 (63.9)	292 (78.1)	125 (62.5)	83 (39.7)	
Ki-67 index (%)					<0.001
<14	368 (47.0)	209 (55.9)	95 (47.5)	64 (30.6)	
≥14	415 (53.0)	165 (44.1)	105 (52.5)	145 (69.4)	
Luminal subtype					<0.001
Luminal A-like	221 (28.2)	153 (40.9)	49 (24.5)	19 (9.1)	
Luminal B-like	562 (71.8)	221 (59.1)	151 (75.5)	190 (90.9)	
Surgery type					0.346
BCS	371 (47.4)	172 (46.0)	91 (45.5)	108 (51.7)	
Mastectomy	412 (52.6)	202 (54.0)	109 (54.5)	101 (48.3)	

Data were analyzed using the χ^2 test. RS, recurrence score; BMI, body mass index; IDC, invasive ductal carcinoma; LVI, lymphovascular invasion; ER, estrogen receptor; PR, progesterone receptor; BCS, breast conserving surgery.

P<0.001) and infiltrating ductal carcinoma (91.8% vs. 70.4%; P=0.001) were associated with adjuvant chemotherapy administration (Table SIV). Multivariate analysis also indicated that the aforementioned factors were independently associated with chemotherapy usage (Table SV).

Adjuvant chemotherapy usage in patients with RS=26-30. The baseline characteristics of patients with RS=26-30 are presented in Table V. According to the results of the univariate analysis, younger age (P=0.012), menstruation (P=0.022), larger tumors (P=0.009), grade-III tumors (P<0.001), high-level Ki-67 index (P<0.001) and luminal B-like tumors (P<0.001) were associated with chemotherapy. Chemotherapy use was higher in patients aged ≤50 years (48/58; 82.5%) compared with patients aged >50-years (92/142; 64.8%; Fig. 1). Besides, among 151 patients with luminal B-like breast cancer, 120 (79.5%) patients received

Table II. Baseline characteristics stratified by 21-gene RS classification, with RS=26-30 as a reference.

	RS=18-25 (n=374)			RS ≥31 (n=209)			
Variable	OR	95% CI	P-value	OR	95% CI	P-value	P-value
Age (years)			0.111			0.142	0.209
≤50	1.37	0.93-2.02		1.41	0.89-2.23		
>50	1			1			
Tumor grade							0.007
I/II	1			1			
III	0.67	0.39-1.13	0.133	1.63	0.96-2.76	0.070	
Unknown	1.07	0.66-1.74	0.781	0.90	0.49-1.64	0.724	
ER status (%)			0.888			0.113	0.138
<50	1.10	0.31-3.94		2.54	0.80-8.04		
≥50	1			1			
PR status (%)			0.063			<0.001	<0.001
<20	0.61	0.37-1.03		2.84	1.69-4.79		
≥20	1			1			
Ki-67 index (%)			0.924			0.032	0.032
<14	1			1			
≥14	0.97	0.55-1.72		1.88	1.06-3.34		
Luminal subtype			0.212			0.950	0.342
Luminal A-like	1			1			
Luminal B-like	0.65	0.33-1.28		1.03	0.45-2.35		

RS, recurrence score; OR, odds ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor.

chemotherapy, which was higher compared with patients in the luminal-A cohort (40.8%; Fig. 2).

Multivariate analysis demonstrated that age and luminal subtype were independent factors associated with adjuvant chemotherapy usage. Patients aged ≤50 years were more likely to receive adjuvant chemotherapy compared with patients aged >50 years (OR, 5.75; 95% CI, 2.08-15.90; P=0.001). Compared with patients with luminal A-like tumors, a higher number of patients with luminal B-like tumors received adjuvant chemotherapy (OR, 7.75; 95% CI, 3.28-18.32; P<0.001; Table SVI).

Alteration to chemotherapy recommendation after 21-gene RS in patients with RS=26-30. A total of 200 patients with RS=26-30 underwent two rounds of MDT recommendations. Before 21-gene RS testing, endocrine therapy alone was recommended by the MDT for 104 (52.0%) patients, while chemoendocrine therapy was suggested for the remaining 96 (48.0%) patients. After RS testing, the adjuvant regimen of 54 (27.0%) patients was altered: 52 patients shifted from no chemotherapy to chemotherapy, and 2 patients shifted from chemotherapy to no chemotherapy (Table VI and Fig. 3).

Regarding the actual adjuvant treatment usage, 10 patients did not follow the treatment recommendation; therefore, the rate of adherence to MDT recommendations was 95.0% (190/200; Table VI). A total of 146 patients received a recommendation for adjuvant chemotherapy after the RS assay; however, only 138 (94.5%) patients received chemotherapy. The eight patients who did not adhere to the MDT recommendations displayed tumors with high ER expression and low Ki-67 index, which were primarily T1-stage and grade I/II tumors (Table SVII).

Adjuvant chemotherapy usage and disease outcomes. After a median follow-up of 21.5 months (range, 2-54 months), 13 (1.7%) patients experienced disease recurrence, including 6 locoregional, 4 distant and 3 contralateral breast recurrences. In the RS=18-25, =26-30 and ≥31 groups, there were 3, 6 and 4 patients, respectively (Table SVIII), with disease recurrence (P=0.183; Fig. 4). Pairwise comparison indicated that there were no significantly different clinical outcomes between each pair of groups (data not shown). In the RS=26-30 group, 4 patients with disease relapse received chemotherapy, and there was no significant difference compared with patients not receiving chemotherapy (2 patients; P=0.764; Table SVIII).

Discussion

The present study included 783 patients with $HR^+/HER2^-$/lymph $node^-$ breast cancer with RS ≥18, and indicated that patients with RS=26-30 displayed higher PR expression and lower Ki-67 index compared with patients with RS ≥31. Multivariate analysis suggested that age ≤50 years and luminal B-like tumors were independently associated with chemotherapy usage in the RS=26-30 group. After 21-gene RS testing and MDT discussion, the chemotherapy usage in patients with RS=26-30 increased, and a high adjuvant chemotherapy compliance rate of 95.0% was

Table III. Clinicopathological characteristics according to chemotherapy usage in the overall population.

Variable	Total, n (%) (n=783)	Chemo, n (%) (n=441)	Non-chemo, n (%) (n=342)	P-value
Age (years)				0.004
≤50	272 (34.7)	172 (63.2)	100 (36.8)	
>50	511 (65.3)	269 (61.0)	242 (70.8)	
Menstrual status				0.002
Premenopausal	287 (36.7)	182 (63.4)	105 (36.6)	
Postmenopausal	496 (63.3)	259 (52.2)	237 (47.8)	
Comorbidity				<0.001
No	456 (58.2)	287 (62.9)	169 (37.1)	
Yes	327 (41.8)	154 (47.1)	173 (52.9)	
Surgery type				0.891
BCS	371 (47.4)	208 (56.1)	163 (43.9)	
Mastectomy	412 (52.6)	233 (56.6)	179 (43.4)	
Tumor size (cm)				<0.001
≤2	565 (72.2)	296 (52.4)	269 (47.6)	
>2	218 (27.8)	145 (66.5)	73 (33.5)	
Histological type				<0.001
IDC	650 (83.0)	397 (61.1)	253 (38.9)	
Non-IDC	133 (17.0)	44 (33.1)	89 (66.9)	
Tumor grade				<0.001
I/II	524 (66.9)	280 (53.4)	244 (46.6)	
III	138 (17.6)	120 (87.0)	18 (13.0)	
Unknown	121 (15.5)	41 (33.9)	80 (66.1)	
LVI				0.027
Yes	38 (4.9)	28 (73.7)	10 (26.3)	
No	745 (95.1)	413 (55.4)	332 (44.6)	
ER status (%)				0.008
<50	27 (3.4)	22 (81.5)	5 (18.5)	
≥50	756 (96.6)	419 (55.9)	330 (44.1)	
PR status (%)				<0.001
<20	283 (36.1)	202 (71.4)	81 (28.6)	
≥20	500 (63.9)	198 (39.6)	302 (60.4)	
Ki-67 index (%)				<0.001
<14	368 (47.0)	135 (36.7)	233 (63.3)	
≥14	415 (53.0)	306 (73.7)	109 (26.3)	
Luminal subtype				<0.001
Luminal A-like	221 (28.2)	53 (24.0)	168 (76.0)	
Luminal B-like	562 (71.8)	388 (69.0)	174 (31.0)	
RS				<0.001
18-25	374 (47.8)	115 (30.7)	259 (69.3)	
26-30	200 (25.5)	140 (70.0)	60 (30.0)	
≥31	209 (26.7)	186 (89.0)	23 (11.0)	

Data were analyzed using the χ^2 test. Chemo, chemotherapy; BCS, breast conserving surgery; IDC, invasive ductal carcinoma; LVI, lymphovascular invasion; ER, estrogen receptor; PR, progesterone receptor; RS, recurrence score.

achieved. No survival difference was observed between patients receiving chemotherapy and patients not receiving chemotherapy in the RS=26-30 group, who displayed good prognoses after a short follow-up period.

Routine clinicopathological factors associated with RS have been widely studied, including age, tumor grade, PR expression, Ki-67 level and luminal subtype. Patients with high tumor grade (16,18,19) or low PR expression (16,19) are associated

Table IV. Multivariate analysis of factors associated with chemotherapy in the overall population.

Variable	OR	95% CI	P-value
Age (≤50 years vs. >50 years)	1.53	0.69-3.39	0.290
Menstrual status (premenopausal vs. postmenopausal)	2.55	1.62-4.02	<0.001
Comorbidity (yes vs. no)	0.52	0.34-0.79	0.002
Tumor size (>2 cm vs. ≤2 cm)	1.91	1.23-2.98	0.004
Histologic type (non-IDC vs. IDC)	0.33	0.10-1.12	0.076
Tumor grade			0.027
III vs. I/II	2.35	1.25-4.40	0.008
Unknown vs. I/II	0.92	0.27-3.18	0.899
ER status (<50% vs. ≥50%)	1.60	0.43-5.95	0.487
PR status (<20% vs. ≥20%)	1.78	1.00-3.18	0.052
Ki-67 index (≥14% vs. <14%)	2.59	1.40-4.81	0.002
Luminal subtype (luminal B-like vs. luminal A-like)	2.29	1.09-4.83	0.029
RS			<0.001
26-30 vs. 18-25	7.20	4.55-11.42	<0.001
≥31 vs. 18-25	16.08	9.19-28.14	<0.001

OR, odds ratio; CI, confidence interval; IDC, invasive ductal carcinoma; ER, estrogen receptor; PR, progesterone receptor; RS, recurrence score.

with high-risk RS. The similarities and differences between the RS=26-30 and RS ≥31 groups have attracted increased attention, which may guide further adjuvant chemotherapy selection (20). Park *et al* (20) reported that, compared with patients with RS=18-25, patients with RS=26-30 displayed more aggressive tumor characteristics. The present study suggested that patients in the RS=26-30 group displayed higher PR expression (OR, 2.84) and lower Ki-67 index (OR, 1.88) compared with those of patients in the RS ≥31 group. Moreover, there was no significant difference between the RS=18-25 and =26-30 groups, indicating that patients with RS=26-30 may display similar biological behavior to patients with RS=18-25, and cannot be managed in the same way as patients in the RS ≥31 group.

RS has been reported to be the most important independent factor associated with adjuvant chemotherapy usage in patients with $HR^+/HER2^-/node^-$ breast cancer (21). The usage of the 21-gene RS testing has significantly reduced chemotherapy administration (22,23). Based on the standard RS risk classification (5), the adjuvant usage rates are 4-7, 30-40 and >80% in patients with low-, intermediate- and high-risk RS, respectively (24,25). In the present study, the rates of chemotherapy were 30.7, 70.0 and 89.0% in the RS=18-25, =26-30 and ≥31 groups, respectively. In patients with RS ≥18, RS displayed the highest adjusting OR value in adjuvant chemotherapy selection (7.20 for RS=26-30 and 16.08 for RS ≥31 vs. patients in the RS=18-25 group) compared with the OR values of other clinicopathological parameters; this could reflect the importance of the 21-gene RS assay over routine clinical parameters. In the TAILORx trial, patients with RS=26-30 were typically categorized into the intermediate-risk RS group, but were recommended chemotherapy, which may have resulted in the high rate of chemotherapy usage in these patients (10).

According to the NCCN guideline, adjuvant endocrine therapy and adjuvant chemotherapy followed by endocrine therapy can be considered for patients in the RS=26-30 group (9). Park *et al* (20) reported that, in the RS=26-30 group, patients who were younger and displayed grade-III tumors could gain survival benefit from adjuvant chemotherapy. Tsai *et al* (26) reported that the 70-gene signature could guide adjuvant chemotherapy in patients with RS=18-30. Moreover, a previous study indicated that a nomogram based on routine clinicopathological factors could also predict the probability of chemotherapy recommendation (27). The present study conducted a univariate analysis, which indicated that age, menstrual status, comorbidity, tumor size, histological type, tumor grade, Ki-67 index and luminal subtype were associated with chemotherapy usage in patients with RS 26-30, whereas only age and luminal subtype remained significant in the multivariate analysis. The TAILORx trial observed that patients aged ≤50 years with RS=16-25 could benefit from chemotherapy (12). Williams *et al* (28) reported that patients aged <50 years were more likely to receive adjuvant chemotherapy compared with those aged >50 years, regardless of their RS. The Danish Breast Cancer Cooperative Group-77B clinical trial demonstrated that patients with luminal A-like breast cancer did not benefit from adjuvant chemotherapy (hazard ratio=1.06; P=0.86) (29). Luminal subtype was included in the nomogram model construction that could predict the usage of adjuvant chemotherapy in patients with RS=18-30 (27). The results of the present study suggested that chemotherapy usage was more common in patients aged ≤50 years vs. >50 years, or with luminal B-like vs. luminal A-like tumors in the RS=26-30 group. The effect of clinicopathological parameters on treatment decision had also been confirmed by the updated results of the TAILORx trial, which demonstrated that RS combined with clinical-risk stratification helped to optimize treatment selection (30).

Table V. Clinicopathological characteristics according to chemotherapy usage in patients with a 21-gene recurrence score of 26-30.

Variable	Total, n (%) (n=200)	Chemo, n (%) (n=140)	Non-chemo, n (%) (n=60)	P-value
Age (years)				0.012
≤50	58 (29.0)	48 (82.8)	10 (17.2)	
>50	142 (71.0)	92 (64.8)	50 (35.2)	
Menstrual status				0.022
Premenopausal	63 (31.5)	51 (81.0)	12 (19.0)	
Postmenopausal	137 (68.5)	89 (65.0)	48 (35.0)	
Comorbidity				0.017
No	109 (54.5)	84 (77.1)	25 (22.9)	
Yes	91 (45.5)	56 (61.5)	35 (38.5)	
Surgery type				0.173
BCS	91 (45.5)	61 (43.6)	30 (56.4)	
Mastectomy	109 (54.5)	79 (72.5)	30 (27.5)	
Tumor size (cm)				0.009
≤2	139 (69.5)	90 (64.7)	49 (35.3)	
>2	61 (30.5)	50 (82.0)	11 (18.0)	
LVI				0.479
Yes	10 (5.0)	6 (60.0)	4 (40.0)	
No	190 (95.0)	134 (70.5)	56 (29.5)	
Histological type				0.001
IDC	167 (83.5)	126 (75.4)	41 (24.6)	
Non-IDC	33 (16.5)	14 (42.4)	19 (57.6)	
Tumor grade				<0.001
I/II	135 (67.4)	95 (70.4)	40 (29.6)	
III	34 (17.0)	32 (94.1)	2 (5.9)	
Unknown	31 (15.5)	13 (41.9)	18 (58.1)	
ER status (%)				0.319
<50	196 (98.0)	136 (69.4)	60 (30.6)	
≥50	4 (2.0)	4 (100.0)	0 (0.00)	
PR status (%)				0.151
<20	75 (37.5)	57 (76.0)	18 (24.0)	
≥20	125 (62.5)	83 (66.4)	42 (33.6)	
Ki-67 index (%)				<0.001
<14	95 (47.5)	52 (54.7)	43 (45.3)	
≥14	105 (52.5)	88 (83.8)	17 (16.2)	
Luminal subtype				<0.001
Luminal A-like	49 (24.5)	20 (40.8)	29 (59.2)	
Luminal B-like	151 (75.5)	120 (79.5)	31 (20.5)	

Data were analyzed using the Fisher's exact test or the χ^2 test. Chemo, chemotherapy; BCS, breast conserving surgery; LVI, lymphovascular invasion; IDC, invasive ductal carcinoma; ER, estrogen receptor; PR, progesterone receptor.

Previous studies reported that the adjuvant chemotherapy recommendation in ~30% of cases, irrespective of RS risk stratification, would be modified after the 21-gene assay (23,25,31). In the present study, the treatment recommendation of 54 (27.0%) patients was altered once an RS of 26-30 was identified; among them, 52 patients changed from being chemotherapy not recommended prior to multigene testing to chemotherapy recommended afterwards. A possible explanation may be that RS=26-30 is close to high-risk RS (≥31), and therefore, physicians considered the patients to be at risk in the present study. Furthermore, a high compliance rate of 95.0% (190/200 patients) was achieved in the cohort of patients included in the present study after 21-gene RS testing and MDT discussion. Furthermore, the acceptance rate in the

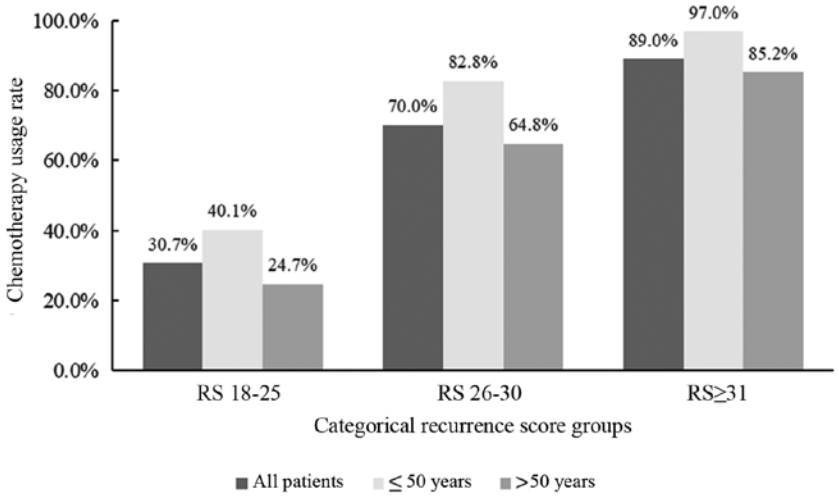

Figure 1. Proportion of chemotherapy usage according to 21-gene recurrence score classification and age.

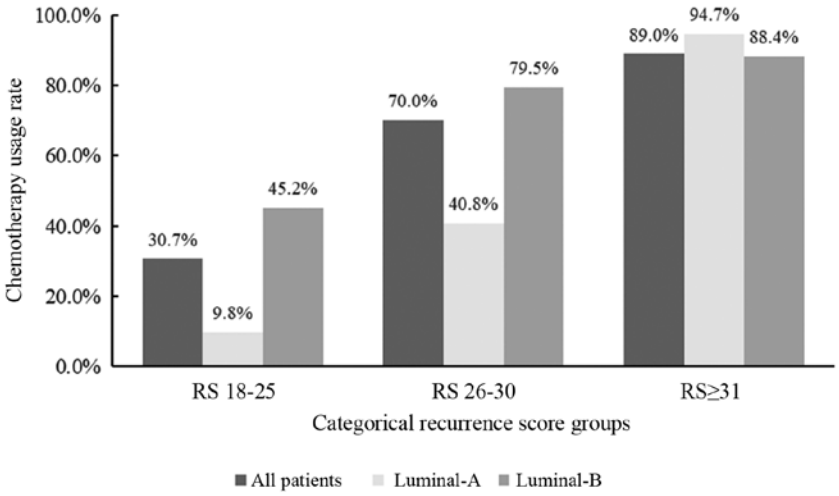

Figure 2. Proportion of chemotherapy usage according to 21-gene recurrence score classification and luminal subtype.

post-RS assay chemotherapy-recommended cohort was 94.5% (138/146). Kuchel *et al* (32) also reported that patient decision conflicts decreased after 21-gene RS testing. Additionally, for patients receiving 70-gene testing, a high compliance rate was reported (91%) in terms of adjuvant chemotherapy application (33).

The results from the Surveillance, Epidemiology and End Results (SEER) database revealed a 5-year breast cancer-specific mortality of 2.4 and 4.4% in RS=25-30 and >30 cohorts, respectively (34). The TAILORx study indicated that, for patients with RS=11-25, =26-30 and ≥31 who received chemotherapy, the estimated rate of invasive disease-free survival was 97.0, 90.5 and 78.0% at 5 years, and 92.9, 86.3 and 74.8% at 9 years, respectively (13). There was no significant survival difference between the three RS groups within the short follow-up period of the present study. However, the SEER data demonstrated that patients with RS=26-30 had inferior breast cancer-specific survival (BCSS; hazard ratio=1.81) and overall survival (OS; hazard ratio=1.37) compared with those of patients with RS=18-25 (20). When the survival outcome was analyzed according to chemotherapy usage, no benefit from chemotherapy was observed in patients with RS=26-30 in the present study, which was similar to the result reported for the Israeli population (35). Nevertheless, data from the National Cancer Database indicated that there was a 1.8% absolute decrease in the 5-year mortality risk by chemotherapy in the RS=26-30 population with lymph node-negative disease (hazard ratio=0.68; P=0.029) (36). SEER data also suggested that adjuvant chemotherapy was associated with a decreased risk in BCSS (hazard ratio=0.68) and OS (hazard ratio=0.58) (20). The relatively short follow-up time and the small number of recurrence events in the present study may have underestimated not only the long-term real survival difference in different RS stratifications, but also the influence of adjuvant chemotherapy in patients with RS=26-30.

The present study was designed to evaluate the role of 21-gene RS testing in patients with RS 26-30, for whom adjuvant treatment has been unanimous until now. Additionally, the treatment decision change due to 21-gene RS testing was evaluated in these patients, which has been scarcely investigated in previous literature. However, the present study had a number of limitations. Firstly, the follow-up period was short;

Table VI. Pre- and post-assay chemotherapy decision and actual usage in patients with a 21-gene recurrence score of 26-30.

Post-assay		Pre-assay		Actual chemo usage		
Decision	N (%)	Chemo	Non-chemo	Chemo	Non-chemo	Adherence to MDT decision
Chemo	146 (73.0)	94	52	138	8	190/200=95.0%
Non-chemo	54 (27.0)	2	52	2	52	

Chemo, chemotherapy; MDT, multidisciplinary team.

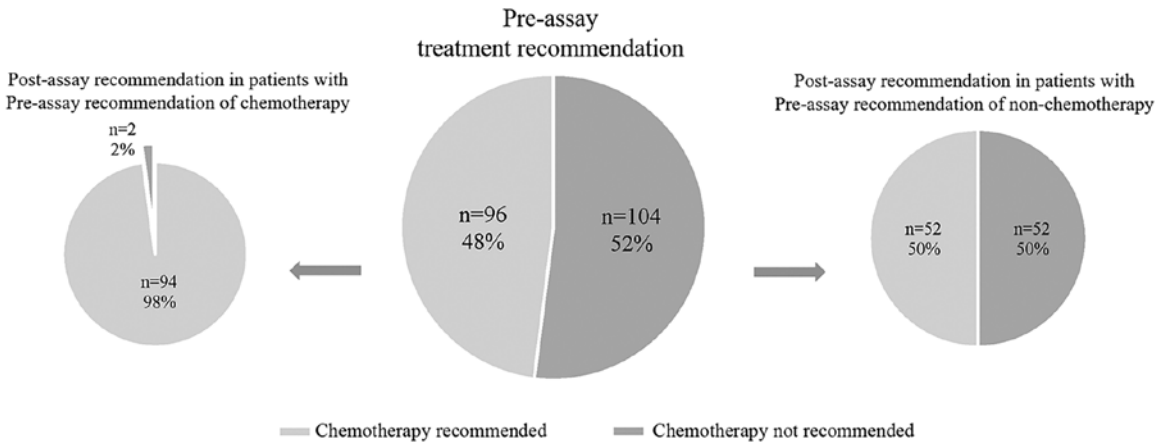

Figure 3. Treatment recommendations before and after the 21-gene assay in patients with a recurrence score of 26-30.

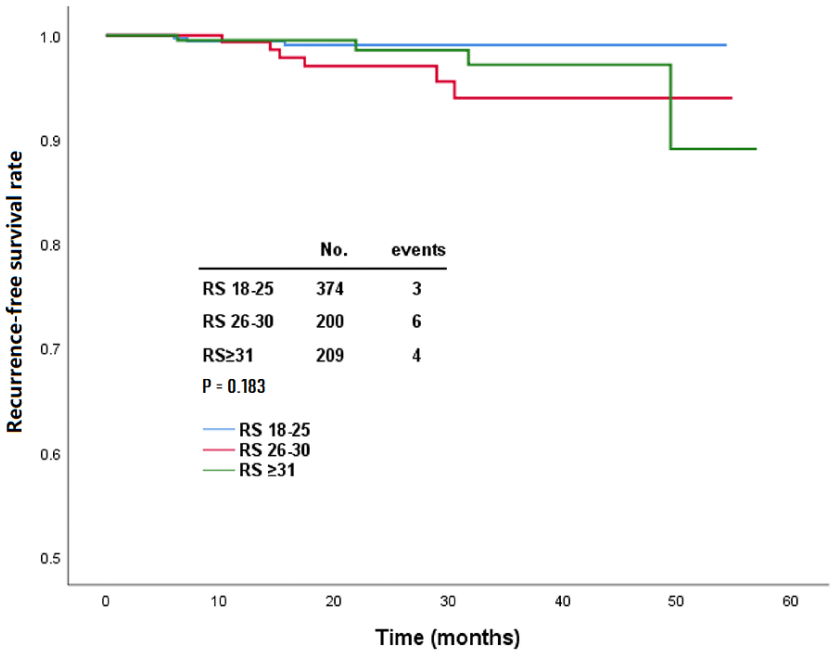

Figure 4. Kaplan-Meier curve of the recurrence-free survival rate according to 21-gene recurrence score classification.

therefore, although the survival benefits due to chemotherapy were greater in the early years and the prognostic effect of the 21-gene RS assay was more robust in the short term, further follow-up is required. Secondly, the similarities and differences in regard to molecular biological features of the RS=26-30 and other risk groups remain unknown. The expression levels of every single gene in the 21-gene RS panel need to be further compared across the three RS groups. Finally, the retrospective design was subjected to confounding factors, and although multivariate analysis was used to eliminate the

confounding effect, selection bias may still exist; therefore, the results require cautious interpretation and further validation.

In conclusion, the present study suggested that PR expression was higher and Ki-67 index was lower in the RS=26-30 group compared with those in the RS ≥31 group, and there was no significant clinicopathological difference between the RS=18-25 and =26-30 groups. For patients with RS=26-30, age ≤50 years and luminal B subtype were independently associated with increased chemotherapy usage. The results suggested that the 21-gene RS testing could influence chemotherapy administration and improve the adherence rate of adjuvant treatment. The short follow-up period demonstrated that patients with RS=26-30 displayed promising disease outcomes, and may receive little benefit from adjuvant chemotherapy; however, further evaluation is required.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JY, XC and KS conceived the study. JY performed the data analysis. JY wrote the manuscript. XC reviewed and edited the manuscript. KS revised the manuscript critically for important intellectual content. JW, OH, JH, LZ, WC and YL collected and interpreted the data. KS and XC acquired the funding. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures were performed in accordance with the ethical standards of the Ethical Committees of Ruijin Hospital and the Declaration of Helsinki of 1964. The present study was reviewed and approved by the Ethical Committee of Ruijin Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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