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# Clinicopathological characteristics of breast cancer patients underwent radiotherapy with different genotypes in relation to the risk of recurrence

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► ***Original article***

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***Keywords:*** *Gene expression level, breast cancer, estrogen receptor, progesterone receptor, recurrence risk score.*

## ABSTRACT

***Background*:** This study aims to analyze the clinicopathological characteristics of breast cancer (BC) patients with different genotypes who underwent radiotherapy. The goal is to explore the relationship between these characteristics and the risk of recurrence, providing valuable insights for clinical adjuvant therapy. ***Materials and Methods:*** A retrospective analysis was conducted on pathological data of 256 BC patients who underwent surgical resection and radiotherapy. Data included age structure, tumor diameter and grading, estrogen receptor (ER) and progesterone receptor (PR) indicators, human epidermal growth factor receptor 2 (HER2), and the cell proliferation antigen marker (Ki-67). Multifactorial analysis was employed to assess correlations. ***Results:*** The distribution of BC patients in the low, medium-high, and high-risk groups was 70.9%, 23.2%, and 5.6%, respectively. Multifactorial analysis revealed that PR, Ki-67 expression, and histological grading were independent factors influencing the RS score, with corresponding P values less than 0.05. They were positively correlated ($P < 0.001$) with Ki-67 expression levels and tumor tissue grading, and negatively correlated with hormonal indicators. The short-term probability of survival for patients with the four staged BC in the low-risk group was 82.34%, 76.12%, 62.13%, and 60.23%, and 23.69%, respectively. Triple negative breast cancer (TNBC) patients and those with Luminal B BC exhibited a higher risk of metastasis ($P < 0.05$). ***Conclusion:*** The pathological characteristics of BC patients with different genotypes showed significant differences. TNBC patients and those with Luminal B BC should be particularly vigilant about their risk of recurrence and metastasis, and strengthen prognostic considerations.

## INTRODUCTION

Breast cancer (BC) is one of the most prevalent malignancies affecting women. The incidence rate and mortality rate increase by more than 1% every year. The pervasive impact of this disease makes it a prominent global public health concern [1]. The treatment methods for BC encompass clinical intervention, radiotherapy, and "biological missile" treatment, all of which demonstrate potential in diminishing recurrence and metastasis rates. However, the efficacy of these interventions remains uncertain due to tumor heterogeneity and individual variations in diseases [2,3].

The inconspicuous nature of BC in its early stages often affects diagnoses and treatments during the advanced phases, imposing physical and psychological distress upon patients. BC encompasses heterogeneous subtypes, including Luminal A, Luminal B, human epidermal growth factor receptor 2 (HER2) over expression, and triple-negative breast cancer (TNBC) [4, 5]. TNBC, characterized by negativity for estrogen receptor (ER) and progesterone receptor (PR) and the absence of HER2 over expression, exhibits a bleaker prognosis compared to other subtypes [6]. Although there is a moderate to strong correlation between the cell proliferation antigen marker (Ki-67) and RS, they are not interchangeable [7,8]. The 21-gene assay indicates that high Ki-67 levels are often associated with an elevated high RS [9]. However, it should be noted that Ki-67 alone is insufficient for determining RS. In cases where patients exhibit low RS but high Ki-67 level, there may be a consideration to omit chemotherapy based on guidelines that prioritize RS as the ultimate determinant, despite a discordance between these two biomarkers [10, 11].

The discernible molecular variations among patients sharing common pathological features in BC contribute to clinical intervention challenges and variable prognoses. Consequently, minimizing unwarranted chemotherapeutic harm and delivering precise medical interventions represent crucial steps in effectively mitigating the risk of recurrence. The

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Recurrence Score (RS) is a valuable prognostic tool, aiding in predicting recurrence and metastasis in BC patients over the past decade and guiding the formulation of tailored treatment plans [12].

Advancements in molecular biology techniques have replaced conventional pathology indicators with genetic tests related to recurrence risk, which can design tailored auxiliary interventions. The 21-gene recurrence hazard score, employing fluorescence technology, assesses hormone receptor-positive, HER2-negative, and lymph node metastasis-negative BC patients, facilitating personalized and precise treatment. This risk classification enhances treatment recommendations for patients with varied conditions, minimizing adverse effects and material burdens [13]. TNBC individuals utilizing genetic and clinicopathological characteristics demonstrated improved survival rates with radiotherapy, which can activate immunotherapy to some extent [14]. The recurrence hazard is combined with patient clinicopathological data, providing a novel approach to adjuvant therapy, and compensating for traditional clinicopathological factors' limitations [15]. The impact of ER expression status on BC pathology is evaluated using immunostaining, enhancing the chemical staining assessment tool for a more effective prognostic tool. BC is a highly heterogeneous disease. The therapeutic efficacy and prognosis are closely related to patients' genotypes. By studying the relationship between different genotypes and clinicopathological features and recurrence risk, the development of personalized medicine can be promoted, making medical treatment options more targeted and effective [16,17].

This study introduces a novel exploration into the clinicopathological characteristics of BC patients, focusing on distinct genotypes and their associated risk of recurrence. By investigating the interplay between different genotypes and recurrence risk, the research aims to contribute unique insights into the existing knowledge system. This study emphasizes understanding the heterogeneity within BC subtypes and their implications for recurrence, providing a valuable perspective that may guide more precise and targeted interventions. This nuanced approach addresses a critical gap in the current understanding of BC management, offering innovative perspectives for enhancing patient outcomes and refining treatment strategies. The development of genomics has made some multigene markers more useful in predicting clinical tumor detection, which can provide assistance in the formulation of treatment plans. However, the previous studies on 21-gene research in the BC population are relatively few. The innovation of this study is to analyze the correlation between pathological indicators and different genotype expressions of BC patients with the help of the 21-gene tool. Multivariate analysis is used to assess the risk of BC recurrence, providing reference value for judging the prognosis of the disease and optimizing the clinical treatment of cancer.

## MATERIALS AND METHODS

### *General information*

A total of 256 BC patients who underwent surgical resection and pathology diagnoses between June 2021 and June 2023 at a provincial cancer hospital were included in this study. All patients provided written consent forms. A retrospective analysis was conducted to collect data including patient demographic information, pathological diagnostic features, follow-up details, and quality of life indicators. Patients included in the study received a standardized adjuvant radiotherapy protocol following surgical resection. The radiotherapy regimen (True Beam Radiotherapy System, Varian Medical Systems, US) consisted of external beam radiation delivered to the affected breast and regional lymph nodes. A total dose of 50 Gy was administered over 6 weeks, with a daily fractionation of 2 Gy per fraction. Advanced techniques, such as intensity-modulated radiation therapy (IMRT) or three-dimensional conformal radiotherapy (3DCRT), were employed to optimize dose distribution and minimize exposure to surrounding healthy tissues [18].

Immunohistochemical testing data encompassed assessments for ER, PR, human epidermal HER2, and Ki-67.

### *Inclusion and exclusion criteria*

Inclusion criteria: ① All BC patients were treated with radiotherapy or endocrine therapy, ② There were no contraindications to surgery, ③ The patients were cooperative and compliant. Exclusion criteria: ① Elderly and frail patients who are not amenable to long-term observation, ② Patients with surgical complications, ③ Patients with distant metastases, ④ Patients with incomplete clinical information and refusal to affix the informed consent form. This study was approved by the Ethics Committee of Cancer Hospitals.

### *Main drugs and reagents*

Immunohistochemistry (IHC) related reagents: Two portions each of 0.01M citrate buffer (Phygene, Yunke Biotechnology Co., Ltd.) with hydrogen ion concentration indexes of 6 and 7.34, 10% formalin (Millonig, Shanghai Sangbao Biotechnology Co., Ltd.); cell permeabilisation solution (Saponin, Beijing Huaxia Yuanyang Science and Technology Co., Ltd.); 100% concentration difference alcohol (Zhengyu Chemical, Shandong Zhengyu Chemical Science and Technology Co., Ltd.). Xylene (Coward, Hubei Chemical Co., Ltd.); neutral gum (Biosharp, Fuzhou Aoyan Experimental Equipment Co., Ltd.); and

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Hematoxylin basic dyes (Clariant brand, Handan Seying Chemical Trading Co., Ltd.). The study was carried out with the help of Streptomyces avidin protein-peroxidase (SP) method for immunohistochemical indexes. The paraffin (Jialong, Lichang Chemical Co., Ltd., Dongli District, Tianjin, China) sectioned tissues of breast carcinoma were deparaffinised and hydrated. Then it was subjected to cellular antigenic analysis in the presence of peroxidase (Guren, Shanghai Yingshen Laboratory Equipment Co. Ltd.) for cellular antigen repair. Subsequently, primary and secondary antibodies were incubated with closed non-specific proteins, and SP chromogenic reaction was performed [19].

### *Observational indicators and clinicopathological data*

Observation indicators: The study compared and analyzed the clinical features of BC patients with different molecular staging. Patients were categorized into distinct stages based on the expression of immunohistochemical detection indexes. Molecular staging followed the 2019 guidelines for ER and PR testing published by the American Society of Clinical Oncology [14]. The staging indicators used are as follows.

a. Luminal Type A: ER and/or PR positive, HER2 negative, PR high expression (≥ 20%), and Ki-67 low expression < 14%.

b. Luminal B: ER and/or PR positive, HER2 negative, PR low expression (<20%), Ki-67 high expression ≥ 14%, ER and/or PR positive, HER2 positive, and Ki-67 any level.

c. HER2 over expression: ER and PR negative, and HER2 positive.

d. Triple Negative: ER and PR negative, and HER2 positive.

Clinicopathological features included tumor size and diameter, pathological type, tumor stage status, and survival status. Immunodiagnosis criteria defined positive hormone receptors and antigenic cells with nuclear staining in more than 10% of cells. PR cells with a threshold of 20% served as the basis for differentiation between Lumina A and Lumina B. HER2 IHC scores of (+) or 0 were considered negative. Otherwise, they were deemed positive. Prognostic intervention element regression analysis in BC patients was performed [20].

Gene recurrence risk detection and scoring involved sectioning paraffin-embedded tissue specimens from BC patients. Complementary Deoxyribonucleic Acid (complementary DNA, cDNA) was reverse transcribed using specific primers and a gene detection kit. The cDNA reverse transcription process, assisted by specific primers, followed the following steps. Firstly, total Ribonucleic Acid (RNA) or messenger Ribonucleic Acid (mRNA) were extracted from cells or tissues, and prior to the reverse transcription reaction. The extracted RNA usually was mixed with reverse transcription-specific primers, which could be gene-specific primers, random primers or gene-specific primers. If specific primers were used, initial annealing was achieved by allowing the primers to bind to their complementary RNA template sequences at a low temperature (usually around 37°C). Once the primers were mixed with the template RNA, the reverse transcription mixture was heated to the optimal working temperature of the reverse transcriptase (usually between 42°C and 50°C). It was attached to the template RNA. The reverse transcriptase was guided along the RNA template to match the corresponding nucleotides and synthesis the cDNA strand to ensure adequate reverse transcription [21,22]. The genes analysed and their corresponding primers are GRB7 (CCATCTGCATCCATCTTGTT), HER2 (CGGTGTGAGA AGTGCAGCAA), ESR1 (CGTGGTGCCCCCCTCTATGAC), PGR (CATCAGGCTGTCATTATGG), CCNB1 (TTCAGG TTGTTGCAGGAGAC), MKI-67 (CGGACTTTGGGGTG CGACTT), MYBL2 (GCCGAGATCGCCAAGATG), and STK15 (CATCTTCCAGGAGGACCACT). Reference genes, and their corresponding primers for ACTB (CAGCAGATGTGGATCAGCAAG), GAPDH (ATTCCACCC ATGGCAAATTC), RPLP0 (CCATTCTATCATCAACGGGT ACAA), GUS (CCCACTCAGTAGCCAAGTCA), TFRC (GCCAACTGCTTTCATTTGTG). The polymerase chain reaction (PCR) system included 3 wells and 4 reaction zones (16). Cycle Threshold Value (Ct) values of reference genes in the 21 genes were averaged. This mean value was used to calculate the Ct values of the 16 genes associated with BC gene expression. The recurrence risk score (RS) was then determined using the following formulas.

Hormone group: ([0.8 × ESR1] + [1.2 × PGR] + BCL-2 + SCUBE2)/4
Value-added group: (BIRC5 + MKI67 + MYBL2 + CCNB1 + STK15)/5
HER2 group: (0.9 × GRB7) + (0.1 × HER2)

The uncorrected RS was calculated as 20 × ([0.47 × HER2 group - 0.34 × hormone group + 1.04 × proliferation group score + 0.05 × CD68 - 0.08 × GSTM1 - 0.07 × BAG1] - 6.7). Based on the RS score, patients were classified into low-risk, medium-risk, and high-risk groups with corresponding scores of <18, 18 to 31 and >31, respectively [17].

### *Statistical methods*

The study employed SPSS 23.0 statistical software for data collation and analysis. For variables conforming to a normal distribution, mean and standard deviation were computed. Group comparisons between two groups were conducted using the t-test. One-way ANOVA was employed for multiple group comparisons. In cases where variables deviated from a normal distribution, quartiles were utilized. Group differences were assessed through

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non-parametric tests. Count information expressed as columns or percentages were analyzed by the chi-square test. Spearman correlation coefficients were applied for non-normally distributed data.

Furthermore, the correlation between genotyping and the clinicopathological characteristics of patients was investigated using multifactorial logistic regression. Statistically significant differences were considered at a threshold of $P < 0.05$.

## RESULTS

### *Clinicopathology of patients with distinct staging of BC*

The outcomes presented in table 1 revealed the distribution of BC subtypes among the experimental subjects. Specifically, there were 136 patients with Lumina A BC, 36 patients with Lumina B BC, 58 individuals with HER2 over expression BC and 26 patients with triple-negative BC. Significantly distinct data were observed for patients with various stages of BC concerning tumor diameter, tumor stage, and tissue grading ($P < 0.05$).

**Table 1.** Clinical characteristics of patients with multifarious form of BC (Number of cases/%).

| Tumor classification | | Lumina A (n=136) | Lumina B (n=36) | HER2 overexpression (n=58) | Triple yin type (n=26) |
|---|---|---|---|---|---|
| Pathological type | Infiltrating lobular carcinoma | 18 (13.23) | 3 (8.33) | 8 (13.79) | 3 (11.53) |
| | Invasive ductal carcinoma | 67 (49.26) | 31 (86.11) | 29 (50.00) | 15 (57.69) |
| | Mixed type | 33 (24.26) | 2 (5.56) | 13 (22.41) | 5 (19.23) |
| | Other types | 18 (13.24) | 0 (0.00) | 8 (13.79) | 3 (11.54) |
| Tumor diameter | ≤2 cm | 75 (55.15) | 16 (44.4) | 20 (34.48) | 13 (50.00) |
| | 2~5cm | 59 (43.38) | 18 (50) | 35 (60.34) | 11 (42.31) |
| | > 5cm | 2 (1.47) | 2 (5.56) | 3 (5.17) | 2 (7.69) |
| Tumor staging | I | 54 (39.71) | 4 (11.11) | 12 (20.69) | 8 (30.77) |
| | II | 63 (46.32) | 30 (83.33) | 38 (65.51) | 14 (53.85) |
| | III | 17 (12.5) | 2 (5.56) | 8 (13.79) | 2 (7.69) |
| | IV | 2 (1.47) | 2 (5.56) | 0 (0.00) | 2 (7.69) |
| Organization classification | I | 8 (5.88) | 13 (36.11) | 3 (5.17) | 2 (7.69) |
| | II | 109 (80.15) | 21 (58.33) | 34 (58.62) | 19 (73.08) |
| | III | 20 (14.70) | 2 (5.56) | 21 (13.79) | 5 (15.23) |

### *Distribution of RS scores for pathological conditions in breast cancer patients*

The findings in table 2 illustrated that BC patients were classified into three risk levels based on the recurrence risk score, including low, medium, and high. The distribution among these groups was 70.9%, 23.2%, and 5.6% of patients, respectively. Significant associations were observed between the RS-score and the tissue grading status as well as the Ki-67 expression status of BC patients ($P < 0.05$). However, no significant associations were found concerning age, tumor diameter, and menstrual status ($P > 0.05$).

**Table 2.** RS score of pathological characteristics of breast cancer patients (Number of cases/%).

| Tumor classification | | Low danger group | Medium danger group | High danger group | $X^2/Z$ | *P* value |
|---|---|---|---|---|---|---|
| Pathological type | Infiltrating lobular carcinoma | 12 (4.68) | 6 (2.34) | 12 (4.68) | 0.609 | 0.438 |
| | Invasive ductal carcinoma | 126 (49.21) | 24 (9.37) | 17 (6.64) | | |
| | Mixed type | 6 (2.34) | 12 (4.68) | 0 (0.00) | | |
| | Other types | 23 (8.98) | 6 (2.34) | 12 (4.68) | | |
| Age/year | <49 | 103 (40.23) | 14 (5.47) | 14 (5.47) | 0.723 | 0.521 |
| | ≥49 | 80 (31.25) | 23 (8.98) | 22 (8.59) | | |
| Organization classification | I | 45 (17.58) | 0 (0.00) | 0 (0.00) | -2.346 | 0.016 |
| | II | 156 (60.93) | 36 (14.06) | 19 (7.42) | | |
| | III | 0 (0.00) | 0 (0.00) | 0 (0.00) | | |
| ER status | Positive | 183 (71.48) | 37 (14.45) | 36 (14.06) | - | 0.327 |
| | Negative | 0 (0.00) | 0 (0.00) | 0 (0.00) | | |
| PR status (%) | >20 | 170 (66.41) | 36 (14.06) | 28 (10.94) | - | 0.485 |
| | ≤20 | 8 (3.12) | 7 (2.73) | 7 (2.73) | | |
| Ki-67 status (%) | <14 | 125 (48.82) | 15 (5.86) | 14 (5.46) | 4.218 | 0.004 |
| | ≥14 | 58 (22.65) | 29 (11.33) | 15 (5.85) | | |
| Whether menstruation stops | Yes | 132 (51.56) | 22 (8.59) | 22 (8.59) | 0.000 | 0.471 |
| | No | 58 (22.65) | 14 (5.46) | 8 (3.12) | | |

Note: Regression analysis of factors influencing RS scores in BC patients.

A univariate analysis of factors distinguishing the pathological presentation of BC patients revealed statistically significant differences in the tissue grade of the tumor, ER and PR hormone levels and Ki-67 expression, in relation to the RS score ($P < 0.05$). Conversely, the high-risk scores for pathological class type, age, and lymphatic metastasis were only 8.20%, 24.42% and 25.19%, respectively. Their significant relationship with the RS score was not observed ($P > 0.05$) (table 3).

Subsequently, a multi factor analysis was conducted on the influencing indicators. There was a

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significant correlation ($P$ < 0.05) with RS scores. Table-to-table analysis revealed that PR status and Ki-67 expression level were independent factors influencing RS scores. The proportion of PR indicators with a threshold of 20% on the high-risk RS score was 7.97% (11/138) and 8.47% (10/118), respectively with a mean RS score of 26 (OR = 3.88, 95% CI=2.01-7.31, $P$ = 0.000 < 0.05). Additionally, the proportion of high-risk patients in the Ki-67≥19% category was 17.78% (24/138) (table 4).

**Table 3.** Single factor analysis of influencing RS score of breast cancer patients.

| Tumor classification | | Number of cases (n) | RS score | | | Single factor analysis |
|---|---|---|---|---|---|---|
| | | | Low danger group | Medium danger group | High danger group | *P* value |
| Pathological type | Infiltrating lobular carcinoma | 217 | 154 | 45 | 18 | 0.432 |
| | Invasive ductal carcinoma | 18 | 11 | 5 | 2 | |
| | Mixed type | 17 | 10 | 6 | 1 | |
| | Other types | 4 | 3 | 1 | 0 | |
| Age/year | <49 | 125 | 95 | 12 | 18 | 0.654 |
| | ≥49 | 131 | 85 | 11 | 35 | |
| Organization classification | I | 74 | 36 | 34 | 4 | 0.000 |
| | II | 106 | 50 | 28 | 28 | |
| | III | 76 | 34 | 29 | 12 | |
| ER state | Positive | 167 | 115 | 45 | 7 | <0.001 |
| | Negative | 89 | 60 | 19 | 10 | |
| PR state (%) | <20 | 138 | 106 | 21 | 11 | <0.001 |
| | ≥20 | 118 | 62 | 46 | 10 | |
| Ki-67 state (%) | <14 | 112 | 81 | 25 | 26 | <0.001 |
| | 14-19 | 27 | 17 | 6 | 4 | |
| | ≥19 | 117 | 50 | 16 | 6 | |
| Whether there is lymphatic metastasis | Yes | 213 | 146 | 67 | 29 | 0.316 |
| | No | 43 | 28 | 11 | 4 | |

**Table 4.** Multi factor analysis of RS score of breast cancer patients.

| Tumor classification | | Number of cases (n) | RS score | | | OR | 95% *CI* | P |
|---|---|---|---|---|---|---|---|---|
| | | | Low danger group | Medium danger group | High danger group | | | |
| Organization classification | I | 74 | 36 | 34 | 4 | 1 | - | - |
| | II | 106 | 50 | 28 | 28 | 1.48 | 0.35 - 5.64 | 0.623 |
| | III | 76 | 34 | 29 | 12 | 3.46 | 0.72 - 13.89 | 0.084 |
| ER state | Positive | 167 | 115 | 45 | 7 | 2.46 | 0.78 - 7.69 | 0.105 |
| | Negative | 89 | 60 | 19 | 10 | 1 | - | - |
| PR state (%) | <20 | 138 | 106 | 21 | 11 | 3.88 | 2.01 - 7.31 | 0.000 |
| | ≥20 | 118 | 62 | 46 | 10 | 1 | - | - |
| Ki-67 state (%) | <14 | 112 | 81 | 25 | 26 | 1 | - | - |
| | 14-19 | 27 | 17 | 6 | 4 | 2.92 | 1.23-6.54 | 0.017 |
| | ≥19 | 117 | 61 | 32 | 24 | 4.48 | 1.63 - 12.44 | 0.003 |

***Correlation analysis between pathological indicators of breast cancer and RS score***

The pathological indicators strongly correlated with the RS score of BC underwent Spearman analysis, with results depicted in figures 1 and 2. Figure 1 revealed a positive correlation between the RS score and the Ki-67 expression level as well as tumor tissue grading. However, the correlation values, corresponding to R values of 0.305 and 0.218, respectively, indicate a low but statistically significant correlation ($P$ < 0.001). The distribution of tumor cell samples was more concentrated in the lower RS score range.

In figure 2, RS scores exhibited a negative correlation with hormonal indicators, specifically PR, with values in the lower range being statistically significant ($P$ < 0.05). Particularly noteworthy is the negative correlation with the PR indicator (R = -0.264, $P$ < 0.001).

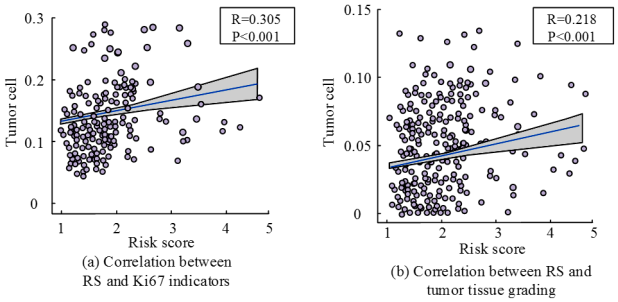


**Figure 1.** Correlation between RS and pathological index Ki-67 and tumor tissue grade.

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***Pathological indicators for breast cancer patients with different genotypes under RS score***

In table 5, the risk of RS recurrence was examined in relation to the tumor length, tissue grade, hormone receptor status and Ki-67 status of BC patients. The *P* values for the four indicators in patients with Lumina A BC were 0.022, 0.017, 0.014 and 0.011 respectively. For patients with Lumina B BC, the *P* values for the four indicators were 0.026, 0.004, 0.023 and 0.010. Those with HER2 over expressed BC showed *P* values of 0.0147, 0.020, 0.004 and 0.000 for the four indicators. Similarly, for individuals with triple-negative BC, the *P* values for the four indicators were 0.019, 0.034, 0.002 and 0.000. These results suggest that the higher the tumor grade and expression level, the more significant the recurrence hazard in BC patients.

***Survival analysis***

The study analyzed four genotypes of BC for the recurrence risk of metastasis, as depicted in figure 3. Patients with Luminal A, Luminal B, HER2 over expression, and triple-negative BC corresponded to ROC curve areas (AUC) of 0.751, 0.546, 0.689 and 0.613 respectively. These values suggest that the pathological characteristics provides better predictive capabilities for the recurrence of BC patients.

The BC patients selected for the study were categorized into high and low-risk groups. The survival curves for individuals under various genotypes were analyzed in Figure 4. Among those in the low-risk of recurrence group, the survival probability for individuals with Luminal A BC was 82.34% and 63.12% at 1 and 2 years respectively. For those with Luminal B BC, it was 76.12% at 1 year, 35.44% at 2 years and 62.13% at 3 years. Patients with HER2 over expressed BC had a survival probability of 62.13% at 1 year and 47.36% at 2 years, while for those with TNBC, it was 60.23% at 1 year and 23.69% at 2 years. In the higher risk group, the survival probability at 1-2 years was 76.12%, 75.09%, 77.23% and 74.25% for patients with TNBC and Luminal B BC, respectively.

Patients with TNBC and Luminal B BC in the lower risk group were more likely to develop metastases, indicating a higher risk ($P < 0.05$). The difference in the data for patients with Luminal A and HER2 over expressed BC was not statistically significant ($P > 0.05$).

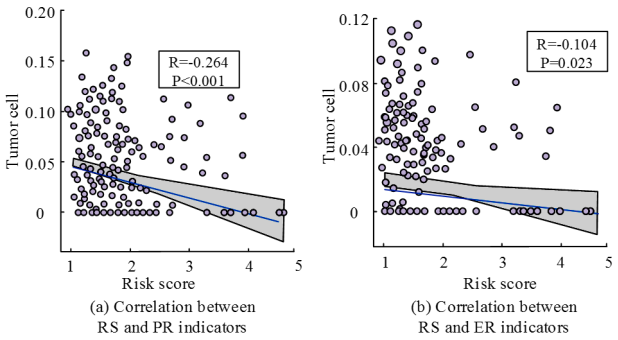


**Figure 2.** Correlation between RS and pathological indexes PR and PS.

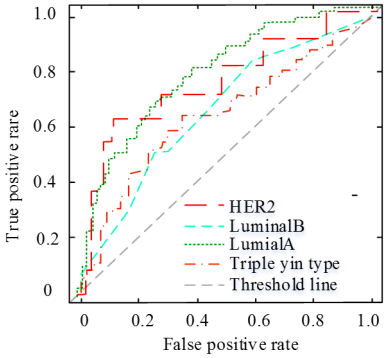


**Figure 3.** Risk ROC curve of four genotypes of BC.

**Table 5.** Pathological indicators of BC person with different genotypes under RS score.

| Tumor classification | | Lumina A (n=136) | | | Lumina B (n=36) | | | HER2 over expression (n=58) | | | Triple yin type (n=26) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Low recurrence | Moderately high recurrence | P | Low recurrence | Moderately high recurrence | P | Low recurrence | Moderately high recurrence | P | Low recurrence | Moderately high recurrence | P |
| Tumor diameter | ≤2 cm | 16 | 8 | 0.022 | 3 | 1 | 0.026 | 4 | 1 | 0.014 | 1 | 1 | 0.019 |
| | >2cm | 0 | 6 | | 1 | 2 | | 0 | 4 | | 2 | 2 | |
| Organization classification | I | 14 | 5 | 0.017 | 4 | 1 | 0.004 | 5 | 1 | 0.020 | 1 | 1 | 0.034 |
| | II~III | 8 | 12 | | 4 | 4 | | 6 | 8 | | 2 | 3 | |
| Hormone receptor status (<20%) | | 16 | 14 | 0.014 | 5 | 2 | 0.023 | 8 | 5 | 0.004 | 3 | 1 | 0.002 |
| Ki-67 status (%) | <14 | 17 | 9 | 0.011 | 6 | 1 | 0.010 | 5 | 3 | 0.000 | 2 | 3 | 0.000 |
| | ≥14 | 2 | 9 | | 1 | 1 | | 2 | 6 | | 1 | 3 | |

## DISSCUSION

The surge in living standards and shifts in healthy lifestyle habits have led to an increased incidence of BC, particularly prevalent in middle-aged and older women. The initial concealment of symptoms and individual variances in BC treatment significantly impact post-operative outcomes [23]. Various factors, including demographic characteristics, dietary habits, family genetics, and reproductive patterns, expand the hazard of BC. Differences in breast gland density also contribute. Compared to women within the normal density range, women who exhibit high-density glands have a higher risk of cancer [24]. Common treatments include breast-conserving surgery and excision, complemented by radiotherapy, targeted therapy, and endocrine therapy. However, inaccurate risk assessment due to inappropriate

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prognostic interventions and incomplete pathological detection challenges postoperative adjuvant therapy decisions [25,26]. Hu *et al.* found the side effect of pulmonary cryptococcosis in BC patients on tamoxifen endocrine therapy after radical surgery and in the identification. It was more pronounced in patients on weight-bearing oestrogen receptor inhibitors [27]. Rakici *et al.* used a rotating treatment bed in the radiotherapy treatment plan for unilateral cancer patients. It could be performed without compromising the radiation dose coverage of the target area, effectively reducing the risk of organ damage in patients [28]. In this paper, the RS score was employed to analyze different BC genotypes, aiming to offer a precise reference for postoperative interventions.

Despite the introduction of personalized precision therapy controlling BC morbidity and mortality, differences in molecular structures among different genotypes result in varying responses to interventions, leading to distinct recurrence risks [29]. A retrospective analysis by Fragomeni investigated recurrence mechanisms based on biological subtypes, revealing a higher rate of recent recurrence and distant metastasis risk in HER2-positive cases. The Luminal BC exhibited a lower recurrence risk and prolonged postoperative survival [30]. Wang *et al.* associated higher recurrence risk with staging differences, age, and genetic profiles of BC, highlighting correlations between basal-like subtypes, Endothelial transcription factor 3 (GATA3) mutations, and reduced recurrence hazards in younger patients [31].

ER and PR, serving as biomarkers for BC detection, are proteins binding to hormone receptors, determining the need for endocrine therapy and guiding postoperative prognostic treatment options. The study found significant correlations in tumor diameter, tumor stage, tissue grading, RS score, and tissue grading status, as well as Ki-67 expression status of BC patients, but not in age, tumor diameter, and menstrual status ($P < 0.05$). Ki-67, a crucial cell cycle regulator, reflects cell growth. It is widely employed in prognostic interventions for tumor malignancy, recurrence, and metastasis. Positive correlations were observed between RS scores and Ki -67 expression and tumor tissue grading, albeit with low R values of 0.305 and 0.218, which were statistically significant ($P<0.001$). Negative correlations were found with hormonal indicators ($P < 0.05$), aligning with findings from Wang J W's study [12]. Univariate results showed a significant association between tumour grade, ER and PR hormone levels, Ki-67 expression and RS scores ($P < 0.05$), with high risk scores of only 8.20%, 24.42% and 25.19% for pathological grading type conditions, age differences and lymphatic metastases. The relationship between BC characteristics and ER and PR expression was analyzed with the help of binomial classification proposed by Wei *et al*. The quantitative expression analysis and retrospective review were performed for pathogenic variants of cancer gene predisposition. The results found that breast cancer patients carrying predisposition genes and pathogenic variants had significantly lower ER and PR expression. The cell cycle regulatory site kinase gene 2, which is associated with an increased risk of BC, showed higher ER and PR expression. Fu *et al.* showed that patients with TNBC had a higher incidence of mutations in susceptibility genes, which were statistically different from each other. The relationship between this finding and the BC recurrence risk is highly agreement with previous studies [32-34]. A high expression level of Ki-67 indicates a higher recurrence risk. Celepli analyzed the Ki-67 expression level of BC patients with the help of retrospective analysis. The Ki-67 expression of the patients was mostly positive, which was significantly correlated with the tumour grade, necrosis, and the oestrogen receptor expression. The marker was directly correlated with the tumour aggressiveness. These two studies were highly similar [35].

Variability in BC treatment outcomes can lead to changes in physical appearance, high treatment costs, and low self-esteem, thus affecting patients' quality of life. Differences in survival exhibited by breast cancer patients with different genotypes were found. Notably, TNBC patients and Luminal B BC patients had higher survival probability at 1-2 years at high risk (76.12%, 75.09%, 77.23% and 74.25%, respectively) ($P < 0.05$). BC patients with over expression of Luminal A and HER2 showed no significant difference in the data ($P > 0.05$). The different survival exhibited by patients with different genotypes of BC is associated with the biological characteristics of the tumor, treatment responsiveness, and the pattern of disease progression. Different genotypes have different biological characteristics. For example, triple-negative BC usually grows rapidly with high invasiveness, while Luminal A breast cancer usually grows slower with less invasiveness [36]. Triple-negative BC types may be more prone to early metastasis, which in turn leads to changes in their long-term survival. This result has similarity with the study of Fawzy *et al.* [37]. Fawzy *et al.* suggested that genetic variations in mechanistic genes can have an impact on BC risk. Computer calculations found that this mechanistic gene variant had a significant relationship with BC risk in stealth models.

From the above studies, it is evident that the clinicopathological characteristics of BC patients under different genotypes vary significantly, with a correlation between these characteristics and the recurrence risk. Luminal B and triple-negative BC exhibit poor prognosis and survival. Follow-up clinical research can further strengthen the exploration of these findings, providing valuable reference materials for the formulation of

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postoperative auxiliary programs.

## CONCLUSION

The pathologic features of BC patients with different genotypes are significantly different. The attention should be paid to the recurrence risk exhibited by patients with different genotypes of BC. TNBC patients and tubulointerstitial B-type BC should be particularly alert to the recurrence risk and metastasis, emphasizing the need for enhanced prognostic considerations. Studying the relationship between clinicopathological characteristics and recurrence risk in patients with different genotypes of BC can provide reference value for the development of precise treatment and intervention programs, improving their quality of life. This study deepens the understanding of the BC complex biology, providing important data and theoretical foundations for future basic science and clinical research.

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## REFERENCES


1. Waks AG and Winer EP (2019) Breast Cancer Treatment: A Review. *Jama,* ***321(3):*** *288-300.*
2. Trayes KP and Cokenakes SE (2021) Breast cancer treatment. *Am Fam Physician,* ***104(2):*** *171-8.*
3. Du Z, Wang L, Zhou Y, Wan H, Liang F, Lyu Q (2018) Association of CYP19A1 gene rs7176005 single nucleotide polymorphism with breast cancer risk and clinicopathologic features of tumor. *Zhonghua Yu Fang Yi Xue Za Zhi (Chinese Journal of Preventive Medicine),* ***52(8):*** *827-832.*
4. Tsang JY, Gary MT (2020) Molecular classification of breast cancer. *Adv Anat Pathol,* ***27(1):*** *27-35.*
5. Al-Eitan LN, Rababa'h DM, Alghamdi MA, Khasawneh RH (2019) Correlation between candidate single nucleotide variants and several clinicopathological risk factors related to breast cancer in Jordanian women: a genotype-phenotype study. *J Cancer,* ***10(19):*** *4647.*
6. Ivashkiv LB (2018) IFNγ: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy. *Nat Rev Immunol,* ***18(9):*** *545-58.*
7. Gluz O, Nitz UA, Christgen M, Kates RE, Shak S, Clemens M, *et al*. (2016) West German Study Group Phase III PlanB Trial: First Prospective Outcome Data for the 21-Gene Recurrence Score Assay and Concordance of Prognostic Markers by Central and Local Pathology Assessment. *J Clin Oncol,* ***34(20):*** *2341-2349.*
8. Crager M, Wijayawardana SR, Gruver AM, Blacklock A, Russell C, Baehner FL, *et al*. (2022) Population-based estimate for the correlation of the Oncotype Dx Breast Recurrence Score® result and Ki-67 IHC MIB-1 pharmDx in HR+, HER2−, node-positive early breast cancer. *BREAST Cancer Res,* ***24(1):*** *1-7.*
9. Sahebjam S, Aloyz R, Pilavdzic D, Brisson M, Ferrario C, Bouganim N, *et al*. (2011) Ki 67 is a major, but not the sole determinant of Oncotype Dx recurrence score. *Brit J Cancer 8.0,* ***105(9):*** *1342-5.*
10. Andre F, Ismaila N, Allison KH, Barlow WE, Collyar DE, Damodaran S, *et al*. (2022) Biomarkers for adjuvant endocrine and chemotherapy in early-stage breast cancer: ASCO guideline update. *J Clin Oncol,* ***40(16):*** *1816-1837.*
11. Gradishar WJ, Moran MS, Abraham J, Aft R, Agnese D, Allison KH, *et al*. (2022) Breast cancer, version 3.2022, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Ne,* ***20(6):*** *691-722.*
12. Wang JW, Wei XL, Dou XW, Huang WH, Du CW, Zhang GJ (2018) The association between Notch4 expression, and clinicopathological characteristics and clinical outcomes in patients with breast cancer. *Oncol Lett,* ***15(6):*** *8749-8755.*
13. Kim JO, Schaid DJ, Vachon CM, *et al*. (2021) Impact of Personalized Genetic Breast Cancer Risk Estimation with Polygenic Risk Scores on Preventive Endocrine Therapy Intention and Uptake.*Cancer Prev Res,* ***14(2):*** *canprevres.0154.*
14. Sessler DI, Pei L, Huang Y, Fleischmann E, Marhofer P, Kurz A, *et al*. (2019) Recurrence of breast cancer after regional or general anaesthesia: a randomised controlled trial. *The Lancet,* ***394(10211):*** *1807-1815.*
15. Dowsett M, Sestak I, Regan MM, Dodson A, Viale G, Thürlimann B, *et al*. (2018) Integration of clinical variables for the prediction of late distant recurrence in patients with estrogen receptor–positive breast cancer treated with 5 years of endocrine therapy: CTS5. *J Clin Oncol,* ***36(19):*** *1941.*
16. Jayaseelan VP, Ramesh A, Arumugam P (2021) Breast cancer and DDT: putative interactions, associated gene alterations, and molecular pathways. *Environ Sci Pollut R,* ***28(21):*** *27162-27173.*
17. Rugo HS and Huppert L (2021) Answers are in the Blood: cfDNA to Enhance Precision Medicine for Breast Cancer.*Clin Cancer Res,* ***27(12):*** *clincanres.0353.*
18. Zhao S, Ma D, Xiao Y, Jiang YZ, Shao ZM (2018) Clinicopathologic features and prognoses of different histologic types of triple-negative breast cancer: a large population-based analysis. *Eur J Surg Oncol,* ***44(4):*** *420-428.*
19. Li Y, Yang D, Yin X, Zhang X, Huang J, Wu Y, *et al*. (2020) Clinicopathological characteristics and breast cancer–specific survival of patients with single hormone receptor–positive breast cancer. *Jama Netw Open,* ***3(1):*** *e1918160.*
20. Heil J, Kuerer H, Pfob A, Rauch G, Sinn H, Golatta M, *et al*. (2020) Eliminating the breast cancer surgery paradigm after neoadjuvant systemic therapy: current evidence and future challenges. *Ann Oncol,* ***31(1):*** *61-71.*
21. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio I, *et al*. (2019) Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol,* ***30(8):*** *1194-220.*
22. Schmid P, Cortes J, Dent R, Pusztai L, McArthur H, Kümmel S, *et al*. (2022) Event-free survival with pembrolizumab in early triple-negative breast cancer. *N Engl J Med,* ***386(6):*** *556-67.*
25. Abubakar M, Figueroa J, Ali HR, Blows F, Lissowska J, Caldas C, *et al*. (2019) Combined quantitative measures of ER, PR, HER2, and KI67 provide more prognostic information than categorical combinations in luminal breast cancer. *Mod Pathol,* ***32(9):*** *1244-1256.*
26. Wang K, Li HL, Xiong YF, Shi Y, Li ZY, Li J, *et al*. (2019) Development and validation of nomograms integrating immune-related genomic signatures with clinicopathologic features to improve prognosis and predictive value of triple-negative breast cancer: A gene expression-based retrospective study. *Cancer Med,* ***8(2):*** *686-700.*
27. Hu H, HU X, Li D, Cai J (2023) Cryptococcosis mimicking pulmonary metastasis during treatment with tamoxifen for breast cancer after surgery: A case report. *Int J Radiat Res,* ***21(4):*** *845-848.*
28. Rakici SY, Eren M (2023) Intensity-modulated radiation therapy (IMRT) with couch rotation in right unilateral breast cancer. *Int J Radiat Res,* ***21(2):*** *203-210*
29. Sporikova Z, Koudelakova V, Trojanec R, Hajduch M (2018) Genetic markers in triple-negative breast cancer. *Clin Breast Cancer,* ***18(5):*** *e841-e850.*
30. Fragomeni SM, Sciallis A, Jeruss JS (2018) Molecular subtypes and local-regional control of breast cancer. *Surg Oncol Clin,* ***27(1):*** *95-120.*
31. Wang MX, Ren JT, Tang LY, Ren ZF (2018) Molecular features in young vs elderly breast cancer patients and the impacts on survival disparities by age at diagnosis. *Cancer Medus,* ***7(7):*** *3269-3277.*
32. Wei G, Rosa M, Chang M, *et al*. (2021) Breast cancer ER, PR, and HER2 expression variance by germline cancer predisposition genes.*J Clin Oncol,* ***39(15_suppl):*** *10526-10526.*
33. Fu F, Zhang D, Hu L, *et al*. (2022) Association between 15 known or potential breast cancer susceptibility genes and breast cancer risks in Chinese women. *Cancer Biol Med,* ***19(2):*** *253-262.*
34. Bahaddin MM (2020) A comparative study between Ki67 positive versus Ki67 negative females with breast cancer: Cross sectional study. *Ann Med Surg,* ***60:*** *232-235.*
35. Celepli P, Karabulut S, Bigat R, *et al*. (2022) CD47 expression and tumor-associated immune cells in breast cancer and their correlation with molecular subtypes and prognostic factors. *Pathol Res Pract,* ***238:*** *154107.*
36. Ishii T and Nakamura Y (2020) Precision Medicine for Advanced Solid Malignancies Based on Circulating Tumor DNA Analysis.Gan to kagaku ryoho. *Cancer & chemotherapy,* ***47(12):*** *1645-1652.*
37. Fawzy MS, Toraih EA, Alelwani W, *et al*. (2020) The prognostic value of microRNA-biogenesis genes Argonaute 1 and 2 variants in breast cancer patients. *Am J Transl Res,* ***12(5):*** *1994-2006.*


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