Article

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Diagnostic Assessment of Breast Cancer with a Combination of the 21-Gene Recurrence Score, Clinicopathologic Parameters, and Biomarkers

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Background: A chemotherapy regimen based on biomarkers in patients might be more efficient than standard therapy. In this study, we evaluated the extensive correlation between the 21-recurrence score (RS), candidate genes, patient demographics, histopathologic factors, and prognosis. We identified the risk factors that affect breast cancer progression, providing evidence for the breast cancer treatment.

Methods: In this study, a total of 150 patients were analyzed, all of whom underwent a 21-gene RS score evaluation. The candidate genes evaluated in this study included thymidylate synthase (*TYMS*), ribonucleotide reductase M1 (*RRM1*), tubulin beta 3 class III (*TUBB3*), topoisomerase II alpha (*TOP2A*), and phosphatase and tensin homolog (*PTEN*) deleted on chromosome ten. Subsequently, we collected patient information regarding the types of endocrine therapy they received.

Results: The 21-gene RS score was significantly correlated with the sentinel lymph node status ($p = 0.045$). Further investigations into the *TYMS* and *RRM1* genes revealed that the genes are distinct factors involved in the progression of breast cancer. Additionally, human epidermal growth factor receptor 2 (*HER2*) staining was found to be a compelling indicator of disease progression. Specifically, grade $\geq$2 staining implies an advanced risk of disease progression. It was demonstrated that the combination of tumor node metastasis (TNM) stage, sentinel lymph node, and 21-gene RS data provides very convincing evidence for clinical application. Moreover, *TYMS*, *RRM1*, and *HER2* are all independent factors that separately affect the progression of breast cancer.

Conclusions: The 21-gene RS was closely associated with the sentinel lymph nodes status in breast cancer. Besides, *TYMS*, *RRM1*, and *HER2* were identified as independent factors affecting breast cancer progression.

Keywords: 21 gene recurrence score; breast cancer; diagnosis; treatment

Introduction

Breast cancer stands as the most prevalent cancer globally, being the leading cause of cancer-related deaths among women [1]. In 2018, approximately 2.1 million breast cancer patients were diagnosed [2]. Currently, the high level of the human epidermal growth factor receptor 2 (*HER2*) protein is an indicator for patients receiving *HER2*-targeted therapy, while patients with estrogen receptor (ER) and progesterone receptor receive endocrine therapy [3]. The administration of chemotherapy lowers the recurrence rate and greatly improves the survival rate of breast cancer patients; however, chemotherapy is not always necessary [4]. Thus, it is beneficial for breast cancer patients to explore effective molecular markers and start appropriate chemotherapy.

The 21-gene recurrence score (RS) evaluation (Oncotype DX, Genomic Health, DE, USA) can assist clinicians in assessing the risk of recurrence in estrogen receptor (ER)-positive breast cancer patients and in determining the potential benefit of chemotherapy following breast cancer surgery [5–9]. A score $<$10 indicates a low distant recurrence rate after 10 years, suggesting that adjuvant chemotherapy may not significantly impact the risk, while a score $>$30 means that the benefits of chemotherapy outweigh the associated risks [9,10].

In addition to the 21-gene RS, other potential molecular markers are also considered for treatment decisions or as information indicators. These include thymidylate synthase (*TYMS*), ribonucleotide reductase M1 (*RRM1*), topoisomerase II alpha (*TOP2A*), phosphatase and tensin homolog (*PTEN*), and tubulin beta 3 class III (*TUBB3*) [10,11].

This study evaluated and compared the levels of these genes, the relationship between 21-gene RS, clinicopathologic features, and progression-free survival (PFS) of patients.

Journal of
BIOLOGICAL REGULATORS
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Methods

Purpose of Research

Our study was conducted following the Declaration of Helsinki of the World Medical Association and was authorized by the Institutional Review Board of the First Affiliated Hospital of Xi'an Jiaotong University (No: XJTU1AF2020LSK-310). The patients were included if they provided informed consent, were ER-positive and *HER2*-negative and underwent 21-gene RS, *TYMS*, *RRM1*, *TUBB3*, *TOP2A*, and *PTEN* genes testing from June 2013 to September 2019. The gene testing of the patients was conducted by Mammafile® gene detection technology (Surexam Co., Ltd., Guangzhou, China). The detailed inclusion criteria were as follows: (1) diagnosed with breast cancer from June 2013 to September 2019; (2) unilateral primary invasive breast cancer diagnosed by biopsy; (3) between 18 and 70 years of age; (4) clinical stage II or III; and (5) met the requirements of the 2019 National Comprehensive Cancer Network (NCCN) guidelines for breast cancer [12]. The exclusion criteria were as follows: (1) incomplete pathologic results; (2) receiving non-standard chemotherapy or surgery; (3) declined to undergo gene testing; and (4) no signed informed consent.

Statistical Analysis

SAS software (version 9.3, SAS Institute Inc, Cary, NC, USA) was used for analyses. Comparisons of classified data were performed with a chi-square test or Fisher's exact test. Risk factors and the associated hazard ratio (HR) of PFS were determined using a Cox multivariate regression model. A p-value less than 0.05 was considered statistically significant.

Results

Overall Characteristics of Subjects

There were 150 patients with an average age of 50.8 years enrolled in our study (Table 1). The pathological types included invasive ductal carcinoma (IDC,72.0%), mixed IDC and ductal carcinoma *in situ* (DCIS) (20.0%), and invasive lobular carcinoma (ILC, 8.0%). Tumors with 21-gene RS of 62.3%, 32.0%, and 6.7% corresponded to low, medium, and high risk. The majority of patients were prescribed letrozole (46.0%) and tamoxifen (46.0%).

Table 1. The characteristics of included patients.

Classification		Number, n (%)
Age, years	<50	69 (42.0)
	≥50	81 (58.0)
Sentinel lymph node, n	<5	68 (45.3)
	≥5	82 (54.7)
Pathological types	IDC	108 (72.0)
	Mixed IDC and DOS	30 (20.0)
	ILC	12 (8.0)
Pathological grade	Grade 1–2	113 (75.3)
	Grade 3	37 (24.7)
TNM stage	T1N0M0	69 (46.0)
	T2N0M0	81 (54.0)
Ki-67 category (%)	<10	32 (21.3)
	>10, <20	47 (31.3)
	≥20	71 (47.3)
Endocrine therapy	Anastrozolc	7 (4.7)
	Letrozolc	69 (46.0)
	Tamoxifen	69 (46.0)
	Toremifene	1 (0.7)
	Exemestane	1(0.7)
	No endocrine therapy	3 (2.1)
21-gene RS	Low risk	92 (62.3)
	Media risk	48 (32.0)
	High risk	10 (6.7)
TYMS expression	Low	55 (36.7)
	Medium	75 (50.0)
	High	20 (13.3)
RRM1 expression	Low	47 (31.3)
	Medium	65 (43.3)
	High	38 (25.3)
TUBB3 expression	Low	41 (27.3)
	Medium	86 (57.3)
	High	23 (15.4)
TOP2A expression	Low	50 (33.3)
	Medium	71 (47.3)
	High	29 (19.4)
PTEN expression	Low	31 (20.6)
	Medium	65 (43.4)
	High	54 (36.0)

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; TNM, tumor node metastasis; RS, recurrence score; *TYMS*, thymidylate synthetase; *RRM*1, ribonucleotide reductase M1; *TUBB*3, tubulin beta 3 class III; *TOP2A*, topoisomerase II alpha; *PTEN*, phosphatase and tensin homolog.

Correlation between the 21-Gene RS Risk and Clinical Characteristics

The results showed that RS was closely related to the sentinel lymph nodes status ($p = 0.034$) in breast cancer patients. The correlations between the 21-gene RS and the clinical tumor node metastasis (TNM) stage ($p = 0.248$), tumor grade ($p = 0.184$), pathologic type ($p = 0.473$), and age ($p = 0.900$) were not significant (Table 2).

Risk Factors for Disease Progression

Cox regression analysis revealed that patients with a high-risk 21-gene RS had a worse PFS compared to those with low or medium risk. In high-risk 21-gene RS patients, the median PFS was 14 months (Fig. 1). In addition, potential molecular biomarkers were found to be correlated with disease progression (Table 3). The *TYMS* gene showed an HR of 0.113 [95% confidence interval (CI): 0.023–0.559; p

Table 2. The correlation between the 21-gene RS risk and clinical characteristics in breast cancer patients.

Clinical parameters	21-gene RS, n			Total	χ^2	*p*
	Low	Medium	High			
Age, years						
<50	41	23	5	69	0.212	0.900
≥50	51	25	5	81		
TNM stage						
T1N0M0	47	19	3	69	2.785	0.248
T2N0M0	45	29	7	81		
Pathological type						
IDC	63	37	8	108	1.499	0.473
Other	29	11	2	42		
Grade						
1–2	74	32	7	113	3.382	0.184
3	18	16	3	37		
Sentinel lymph node, n						
<5	36	29	3	68	6.784	0.034
≥5	56	19	7	82		

= 0.007], while the *RRM1* gene had an HR of 3.823 (95% CI: 1.079–13.544; p = 0.038), indicating that these two genes were potential risk factors affecting disease progression.

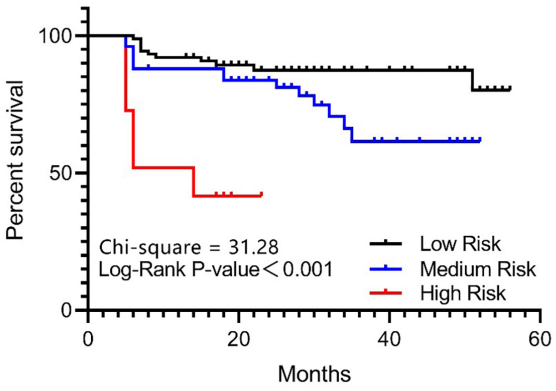

Fig. 1. Progression-free survival analysis of the breast cancer patients with different 21-recurrence scores.

Table 3. The results of correlation between potential molecular biomarkers and disease progression.

Biomarkers	HR	95% CI		*p*-value
		Lower	Upper	
TYMS	0.113	0.023	0.559	0.007
RRM1	3.823	1.079	13.544	0.038
TUBB3	1.907	0.639	5.692	0.247
TOP2A	1.417	0.384	5.227	0.601
PTEN	2.127	0.603	7.501	0.240

CI, confidence interval; HR, hazard ratio.

Discussion

The differences in gene expression give rise to variability in the response to chemotherapy among individuals. A chemotherapy regimen based on the biomarkers of patients will improve treatment. The advantages of using the 21-gene RS to make treatment decisions have been presented in many studies [9,10,13–15].

The 21-gene RS is considered a crucial factor in guiding the treatment of breast cancer. Research on its use in the Chinese population has shown that it increases clinicians' confidence and reduces the implement of chemotherapy [13–15]. In our study, we found a close association between the 21-gene RS risk and the sentinel lymph nodes status in breast cancer patients, and patients with a high-risk 21-gene RS had worse PFS than patients with low or medium risk, indicating that 21-gene RS risk could guide the chemotherapy application for breast cancer patients.

TYMS, one of the candidate genes in our study, was closely associated with breast cancer progression. *TYMS* is a gene related to deoxyribonucleic acid synthesis and serves as a target for 5-fluorouracil, the expression of which indicates a significant survival prognosis for prostate cancer patients [14,16]. Furthermore, the expression of *TYMS* is considered to be a predictor of treatment response. For example, the chemotherapeutic efficacy of pemetrexed was observed to be reflected by the expression of *TYMS* in lung respiratory and gastrointestinal tumors [17]. Additionally, the expression of *TYMS* is correlated with the sensitivity of advanced breast cancer patients to pemetrexed, suggesting that an analysis of *TYMS* expression or in combination with other molecular parameters of *TYMS* expression provides effective clinical information for breast cancer treatment [17]. In our study, we observed that *TYMS* was an independent factor of disease progression, indicating that *TYMS* can be used as an indicator for decision-making.

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BIOLOGICAL REGULATORS
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The *RRM1* gene is considered a potential biomarker for assessing breast cancer progression, although the result was not significant in our study. Ribonucleoside-diphosphate reductase is the enzyme producing deoxyribonucleotides ahead of deoxyribonucleic acid synthesis when the cell divides, and it contains a structural subunit of *RRM1*. Studies have shown that the content of *RRM1* in breast cancer is obviously elevated compare to that in adjacent non-cancerous tissues, and the expression of *RRM1* is closely related to lymph node metastasis, pathologic grade, and ER status [18,19]. An *RRM1* aberration, as revealed by fluorescence *in situ* hybridization, was found to be a risk factor, indicating a decreased overall survival rate of advanced breast cancer patients who received docetaxel/gemcitabine treatment. *RRM1* expression in lung and pancreatic cancers has been identified as a key factor for estimating the efficacy of gemcitabine treatment. Additionally, the accuracy of predicting the efficacy of gemcitabine in breast cancer using *RRM1* and 6 other genes was reported to be 85% [14]. In metastatic breast cancer patients, *RRM1* is closely related to deoxyribonucleic acid repair enzymes [breast cancer type 1 susceptibility protein (BRCA1) and excision repair cross-complementation group 1 (ERCC1)], suggesting that *RRM1* can be used as a marker for clinical decision-making [20]. Our results showed that *RRM1* was closely related to the PFS of breast cancer patients, which was consistent with the findings of previous study [21].

Our study revealed the significant involvement of the 21-gene RS, *TYMS*, and *RRM1* in breast cancer progression. However, there are limitations that cannot be overlooked. First, this research was designed as a single-center study with a small sample size. Second, it was a retrospective analysis with a limited number of indicators. Therefore, a follow-up study is necessary to address these limitations. It is essential to increase the sample size and conduct a prospective study to further validate our findings.

Conclusions

Our study confirmed that the 21-gene RS is closely associated with the sentinel lymph nodes status in breast cancer. *TYMS*, *RRM1*, and *HER2* were independent factors affecting breast cancer progression.

Clinical Significance

The tumor node metastasis (TNM) staging and sentinel lymph node status of estrogen receptor (ER)-positive and *HER2*-negative breast cancer patients, combined with the results of a 21-gene recurrence score, are crucial in treatment decision-making. Moreover, *RRM1* and *TYMS* are potential target genes in the clinical setting due to their close relationship to disease progression. Further research in this field could significantly enhance the effectiveness of specific treatments.

Availability of Data and Materials

All data included in this study are available upon request by contact with the corresponding author.

Author Contributions

BP and JW carried out the studies, participated in collecting data. YZ and XT performed the statistical analysis and participated in its design. All authors helped to draft the manuscript and performed the data analysis. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was conducted in according to the Declaration of Helsinki World Medical Association and authorized by the Institutional Review Board of the First Affiliated Hospital of Xi'an Jiaontong University (No: XJTU1AF2020LSK-310). Informed patient consent was obtained for this study.

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Conflict of Interest

All authors have completed the ICMJE uniform disclosure form. The authors declare no conflict of interest.

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