Impact of Different Modules of 21-Gene Assay in Early Breast Cancer Patients

Mengdi Chen
Shanghai Jiao Tong University School of Medicine
Deyue Liu
Shanghai Jiao Tong University School of Medicine
Weilin Chen
Shanghai Jiao Tong University School of Medicine
Weiguo Chen
Shanghai Jiao Tong University School of Medicine
Kunwei Shen
Shanghai Jiao Tong University School of Medicine
Jiayi Wu
Shanghai Jiao Tong University School of Medicine
Li Zhu (zhuli8@yeah.net)
Shanghai Jiao Tong University School of Medicine https://orcid.org/0000-0002-9294-5727

Research article

Keywords: breast cancer, recurrence score, hormone receptor positive, adjuvant therapy.

Posted Date: July 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-652397/v1

Abstract

Background: Young patients were under-evaluated in the construction and validation of the 21-gene Assay Recurrence Score (RS). Previous evidence suggested that RS performed differently according the ages of patients. Our study aimed to explore the molecular driving patterns in patients of different ages.

Methods: A total of 1,078 estrogen receptor (ER)-positive breast cancer patients between Jan 2009 and Mar 2017 from Shanghai Jiao Tong University Breast Cancer Data Base were divided into three subgroups: Group A, ≤40y and premenopausal (n=97); Group B, >40y and premenopausal (n=284); Group C, postmenopausal (n=697). The correlation of RS and its modules and the variance of RS modules was explored.

Results: Estrogen module had a stronger correlation with RS in patients >40y (ρ = -0.76 in Group B and -0.79 in Group C) compared with patients ≤40y (ρ = -0.64). Contrarily, the correlation between RS and invasion group was weaker in patients >40y (ρ = 0.29 in Group B and 0.25 in Group C) than in patients ≤40y (ρ = 0.44). The proliferation module contributed most to the variance in young patients (37.3%) while ER module contributed most in old patients (54.1% in Group B and 53.4% in Group C). For RS >25, proliferation module was the leading driver in all three subgroups (ρ = 0.38, 0.53 and 0.52 in Group A, B and C) while estrogen module had a weaker association with RS. The negative impact of ER related features on RS was stronger in clinical low-risk patients while the positive effect of proliferation module was stronger in clinical high-risk patients.

Conclusions: RS was primarily driven by estrogen module in patients regardless of age, but the proliferation module had a stronger impact on RS in patients ≤40y than in those >40y. The impact of modules varied in patients with different genetic and clinical risk.

1. Background

Estrogen receptor (ER) is a significant biomarker for breast cancer and ER-positive subtype constitutes about 70% of invasive breast cancers[1]. Endocrine therapy is essential for all ER + breast cancer patients, while chemotherapy can improve the prognosis of some[2]. To identify ER + breast cancer patients who can benefit from chemotherapy, several multiparameter molecular profiling tests were developed. The 21-gene Recurrence Score (RS) is the most widely used profile worldwide, comprised of 16 cancer-related genes and 5 reference genes[3]. Calculated using fixed coefficients which are predefined according to the regression analysis of gene expression and recurrence in the three training studies, the 21-gene RS categorizes patients into low-, intermediate- or high-risk groups. The use of RS score makes clinicians informed of the prognosis of patients and allows them to make adjuvant treatment decisions objectively.

Young patients (≤ 40 years old) compose approximately 2–6% of patients in clinical trials about 21-gene[4–6]. Previous data suggested that luminal tumors in young women have different clinicopathological pattern compared with those in old patients[7–9]. They tend to be more aggressive and thus may benefit

from enhanced therapy such as chemotherapy[10]. Correspondingly, the RS score was found to show different values of predicting the benefit of adding chemotherapy in subsets of different ages. The Trial Assigning Individualized Options for Treatment (TAILORx) categorized RS ranges as low- (< 11), intermediate- (11–25) or high- (> 25) risk subgroups and discovered that for the majority of population with RS < 25, endocrine therapy alone was noninferior to combined chemo-endocrine therapy. Of note, the interaction between age and RS was obvious. For instance, for patients < 50 years, RS 11–25 might predict some benefit derived from chemotherapy, whereas in patients ≥ 50 years with a RS 11–25, chemotherapy-derived benefit was absent[11].

Refined ranges of RS will inform clinicians of recurrence and survival outcomes more accurately than traditional clinicopathological features and allow to selecting patients avoid chemotherapy. Thus, it's important to understand the biological features as well as molecular drivers behind the RS. A previous study was performed to investigate the utility of RS in postmenopausal patients and discovered that, in contrast to the weight of coefficient in the formula for calculating RS, the molecular drivers of RS was mainly estrogen-related features instead of proliferation markers[12]. However, experience in patients with different ages was scarce, especially in premenopausal young women. Given the different prognostic and predictive value of RS in people with different ages, it is necessary to explore the internal molecular mechanisms of RS, especially in younger patients.

In this study, we tried to explore the association of RS with its modules and identify the discordance of molecular drivers in patients of different ages.

2. Patients And Methods

2.1 Patients

Clinical data of a total of 1,078 unilateral ER-positive female breast cancer patients between Jan 2009 and Mar 2017 was derived from the prospectively-maintained Shanghai Jiao Tong University Breast Cancer Data Base (SJTU-BCDB). The use of data was approved by SJTU-BCDB for clinical research. Patients were collected if meeting the following criteria: (1) ER positivity with ≥1% immunoreactive tumor cell nuclei determined by immunohistochemical (IHC) test[13]; (2) human epidermal growth factor receptor 2 (HER2) negativity defined as IHC score 0/1+ or 2+ combined with non-amplification of HER2 gene on fluorescence in situ hybridization (HER2/CEP17 ratio < 2.0 with average HER2 gene copy number <6.0 signals/cell, or average HER2 gene copy number <4.0 signals/cell regardless of the ratio)[14]; (3) intact information of a 21-gene test report. Menopause was determined if meeting any of the following: (1) prior bilateral oophorectomy; (2) age ≥60 years old; (3) age <60 years old, amenorrheic for more than 12 or more months and the follicle-stimulating hormone and estradiol in the postmenopausal range.

2.2 The 21-gene RS Assay

The 21-gene tests were performed using formalin-fixed, paraffin-embedded tissue. Hematoxylin and eosin-stained slides were deparaffinized into two 10μm unstained sections using xylene followed by ethanol as we did before[15]. RNA was extracted using the RNeasy FFPE kit (QIAGEN, Hilden, Germany). Total RNA content was quantified after confirmed the absence of DNA contamination. Then, gene-specific reverse transcription was conducted followed by standardized quantitative reverse transcriptase-polymerase chain reactions (RT-PCR) in 96-well plates with Applied Biosystems (Foster City, CA) 7500 Real-Time PCR system. The PCR cycling went as follows: 95◦C for 10 min for one cycle, 95◦C for 20 s, and 60◦C

2.3 Genetic and Clinical Risk Stratification

As defined in the TAILORx trial[11], we categorized patients into genetic high- and low-risk with a cut-off RS value of 25. In addition, patients with tumors of (1) ≤3cm and Grade 1; (2) ≤2cm and Grade 2; (2) ≤1cm and Grade 3 were classified as clinical low-risk while others were clinical high-risk[4,11].

2.4 Statistical Analysis

Spearman's rank correlation was performed to analyze the correlation of RS and RS modules. Variance of components of the RS was studied in different subgroups. All tests were performed using R Studio version 1.2.5019 based on R version 4.0.3.

3. Results

3.1 Baseline characteristics

According to the 4th International Consensus Conference for Breast Cancer in Young Women (BCY4) international consensus guidelines[16] as well as patients' menopausal status, we divided patients into three subgroups: (1) Group A, ≤40y and premenopausal; (2) Group B, >40y and premenopausal; (3) Group C, postmenopausal. Of 1,078 cases, 9.0%, 26.3%, 64.7% were group A, B and C. The median ages were 37 (range 27-40), 47 (range 41-56) and 63 (range 45-93), respectively. A total of 31.5% of the tumors were luminal-A subtype and the majority (86.4%) was invasive ductal cancer. Approximately half of the patients had grade II tumors. For AJCC stage, 67.9% of tumors were pT1 and 93.4% were node-negative. A total of 638 (59.2%) patients had RS scores ≤25 and 440 (40.8%) had RS scores >25. We determined clinical risk as in the MINDACT (Microarray in Node Negative Disease May Avoid Chemotherapy) and TAILORx trial. The patients of high or low clinical risk had almost equal proportions (49.5% vs 50.5%). The distribution of clinicopathologic features in each subgroup was summarized in Table 1.

3. 2 Correlation between RS score and individual modules

We analyzed the relevance of each separate modules and the total score (Figure 1). For HER2 module and proliferation module, the thresholds of 8 and 6.5 were applied. In terms of the estrogen module, RS scores had a stronger correlation with ER group in patients >40y (ρ = -0.76 in Group B and -0.79 in Group

C) compared with patients ≤40y (ρ = -0.64 in Group A). Contrarily, the correlation between RS score and invasion group was weaker in patients >40y (ρ = 0.29 in Group B and 0.25 in Group C) than in patients ≤40y (ρ = 0.44 in Group A). The coefficients of HER2 modules also showed difference between patients >40y (ρ = 0.14 in Group B and 0.15 in Group C) and patients <40y (ρ = 0.23 in Group A). Interestingly, the correlation between proliferation group and RS score was similar in premenopausal population (ρ = 0.54 in Group A and 0.56 in Group B), while it was a little weaker in postmenopausal patients (ρ = 0.39 in Group C). Notably, a total of 15.1% patients in our study had the unthresholded proliferation module (19.6%, 12.3%, and 15.6% in Group A, B, and C).

3.3 Variance of RS score accounted for by individual modules

Analysis of variance tests were applied to evaluate the ratio of every module that explained the variance of RS score. The distribution of variance of Group B and C was similar and showed a different pattern compared with that of Group A (Figure 2). In young patients, the proliferation module contributed most to the variance (37.3% in Group A). Meanwhile, ER module was the group contributed most in old patients (54.1% in Group B and 53.4% in Group C). In all three groups, the invasion and HER2 group explained little part of the variance of RS score, shown in Table 2.

3.4 Correlation in genetic high- and low-risk subgroups

We explored the correlation of RS with its modules in genetic high- and low-risk subgroups (RS>25 and RS≤25, Figure 3-5). Obvious difference was observed between patients with genetic high- and low-risk. For ER module, the negative impact was much stronger in low-risk patients compared with high-risk ones. Similarly with the results in total population, the impact of ER module in genetic low-risk subgroup also became stronger with age (ρ = -0.68, -0.77 and -0.84 in Group A, B and C). For proliferation module, the positive correlation presented in total population only occurred in genetic high-risk subgroups. Different with the tendency in total population (ρ = 0.54, 0.56, 0.39 in Group A, B and C), the correlations reversed between young and old subgroups (ρ = 0.38, 0.53 and 0.52 in Group A, B and C). In terms of invasion module, though it showed stronger impact on RS in patients ≤40y than >40y when analyzing the total population, the coefficient was actually strongest in genetic low-risk <40y patients (ρ = 0.55) while the difference was not obvious between the left five subgroups.

3.5 Correlation in clinical high- and low-risk subgroups

We further compared the correlations between patients with different clinical risks. The tendency of the correlations between RS and its individual modules was constant on the whole. Meanwhile, small difference was observed between clinical high- and low-risk subgroups. As for ER module, its negative impact on RS was stronger in patients with low clinical risk compared with those of the same age but with higher risk. In terms of proliferation module, the positive impact on RS was stronger in high-risk patients regardless of age. For invasion module, the coefficient was stronger in patients ≤40 years old. Detailed data was shown in Figure 6-8. The relationships between RS and its estrogen/proliferation module were summarized in Figure 9.

4. Discussion

21-gene RS was an important tool to help clinicians predict survival outcome and make treatment strategies. Clinical data showed that patients with the same 21-gene RS but different ages had different benefit from adjuvant chemotherapy[11], thus, it was necessary to understand the internal molecular drivers of RS. A recent study uncovered the discordance of the primary coefficient in the Cox model of RS and the unique molecular features of RS in postmenopausal patients[12], leading the attention to RS usage. However, data in premenopausal women was insufficient. Here, we discussed the difference of molecular drivers of 21-gene RS score between young and old patients and found that RS was primarily driven by estrogen module in patients regardless of the age, while the proliferation module had a stronger impact on RS in patients ≤ 40y than in those > 40y.

Despite the similar RS, patients with different ages might respond differently to the addition of chemotherapy. The result of TAILORx[11] and RxPONDER[17] trial suggested that premenopausal patients with RS ≤ 25 gained a survival improvement from the addiction of chemotherapy while the postmenopausal counterparts didn't. Consistently, the MINDACT trial[4] showed that for the population with clinical high risk and genetic low risk, a 5.4% reduction of distant metastasis from chemotherapy was observed in patients ≤ 50y but not in those > 50y. Based on these results, we divided the patients according to their menopausal status. To explore the mechanisms of RS in patients with different ages, we further categorized patients as young or aged with a cut-off of 40 years old according to BCY4 guidelines.

The results of our study were consistent with the recent study based on patients from the ATAC trial[12], in which RS was found to be mainly driven by estrogen-related features in postmenopausal women. Our study confirmed that this phenomenon also occurred in premenopausal patients > 40 year of age. However, in patients ≤ 40y, the link of estrogen module and RS became weak and in contrast, the proliferation module had a strong impact on RS, as well as explained the most of RS's variance. Given the crescendo of estrogen module's impact of RS, a rational inference might be that in the majority of patients > 40y, estrogen module played a leading role in RS, resulting in the loss of prediction value of RS after 5 years due to the withdrawal of endocrine therapy[18]. Second, the estrogen module had a weak impact in patients less than 40y probably due to the relatively lower ER related gene expression. As for the proliferation module, its strong correlation with RS in young patients was in accordance with the previous retrospective studies that young patients were more likely to have tumors with higher grade[9] and higher expression of proliferation related genes[19]. Consequently, a higher proportion of patients ≤ 40y (19.6%) had unthresholded proliferation module score than patients > 40y did (12.3% and 15.6% in Group B and C). To some extent, the application of threshold distinctly narrowed the gap of proliferation modules' contribution to RS between patients < 40y and ≥ 40y.

In our exploratory analysis in subgroups with different genetic risk, the trend was similar that the association between the RS and its estrogen module was weaker among younger patients, especially in low genetic risk groups. In terms of proliferation related features, no statistically significant relationship

was found between RS and its proliferation in patients with RS < 25, suggesting that proliferation related features might affect very little in patients with low-to-immediate gene risk. Evidence from TAILORx showed that if the RS was 11–25, chemotherapy associated benefit only occurred in patients 41–50 years of age[11]. In our study, the stronger correlation between RS and ER module existed in premenopausal patients > 40y, among whom no significant association between RS and proliferation module was observed. Therefore, a probable presumption was that in addition to cell cytotoxicity, the chemotherapy benefit for these patients was mainly derived from chemotherapy induced amenorrhea which was common in women 40 years of age or older[20]. For these patients, ovarian function suppression incorporated into endocrine therapy might be an effective choice for omitting adjuvant chemotherapy as well as achieving the reduction of the rate of recurrence and death[21,22].

Clinicopathological features were considered as important prognostic factors[23] and we further investigated the molecular drivers in subgroups with different clinical risk determined by the standard of TAILORx[11]. The negative impact of ER related features on RS was stronger in clinical low-risk patients than in high-risk ones. Meanwhile, the proliferation module had a stronger impact on RS in clinical high-risk patients. These results were in accordance with previous evidence and suggested that with the same RS, the internal molecular mechanisms might be quite different. For instance, for a 60y postmenopausal low clinical risk patient, a RS of 30 might be driven primarily by the strong expression of estrogen module along with the weak proliferation module. Meanwhile, for a patient with high clinical risk, a RS of 30 might be attributed to the positive impact of proliferation related genes and negative impact of estrogen module. Undoubtedly, our results supported the conclusion of the secondary analyses of TAILORx in which clinical-risk stratification[20] (tumor size and tumor grade used) was incorporated into RS to provide better prognostic information. Additionally, it also explained the better performance of RSClin tool[24] than that of RS alone.

Our study has several strengths. First, we explored the molecular drivers of RS in young patients and compared them with that in elder patients, which had never illuminated before. Second, the previous study based on samples from ATAC trial and the majority of patients were at clinical low risk and able to receive tamoxifen or anastrozole alone[25]. Instead, patients studied in our study derived from real-world data thus might be more representative of clinical practice. Thirdly, we used a cut off age of 40y to substitute for 50y to divide customized risk groups. We found distinct patterns of molecular drivers between patients ≤ 40y and those > 40y. Thus, it might be necessary to further categorized the ranges of ages in addition to the cut-off of 50y used by TAILORx and recommended by the ASCO Clinical Practice Guideline[26] and NCCN[27] guideline.

5. Conclusions

In conclusion, RS was primarily driven by estrogen module in patients regardless of the age, while the proliferation module had a stronger impact on RS in patients ≤ 40y than in those > 40y. The chemo-related benefit in young patients might be largely derived from CIA because the proliferation module had

no obvious association with the RS when it was ≤ 25. For RS > 25, proliferation module was the leading driver while estrogen module had a weaker association with RS. The negative impact of ER related features on RS was stronger in clinical low-risk patients while the positive effect of proliferation module was stronger in clinical high-risk patients. Future analysis might pay more attention to the difference between patients ≤ 40y and > 40y when using the RS score to determine the addition of chemotherapy to endocrine therapy.

Declarations

Ethics approval and consent to participate: NA

Funding: NA

Consent for publication: Written informed consent for publication was obtained from all participants.

Acknowledgements: We appreciate all the patients for their participation and study coordinators, nurses, and physicians for their assistance.

Author Contributions:

LZ, JY and MC made study design. DL, WC and KS participated in data acquisition. JW and MC conducted statistical analysis and manuscript preparation. KS and LZ helped to review the manuscript. All authors read and approved the final manuscript.

Competing Interests statement: The authors disclose that no financial support has been received from any person or organization for this study.

Availability of supporting data: The data used to support the findings of this study are available from the corresponding author upon request.

References

1. Howlader N, Altekruse SF, Li CI, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. *J Natl Cancer Inst*. 2014;106(5).doi:10.1093/jnci/dju055

2. Burstein HJ. Systemic Therapy for Estrogen Receptor-Positive, HER2-Negative Breast Cancer. *N Engl J Med*. 2020;383(26):2557-2570.doi:10.1056/NEJMra1307118

3. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004;351(27):2817-2826.doi:10.1056/NEJMoa041588

4. Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med*. 2016;375(8):717-729.doi:10.1056/NEJMoa1602253

5. Sparano JA, Gray RJ, Makower DF, et al. Prospective Validation of a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med*. 2015;373(21):2005-2014.doi:10.1056/NEJMoa1510764

6. Dowsett M, Cuzick J, Wale C, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. *J Clin Oncol*. 2010;28(11):1829-1834.doi:10.1200/JCO.2009.24.4798

7. Partridge AH, Hughes ME, Warner ET, et al. Subtype-Dependent Relationship Between Young Age at Diagnosis and Breast Cancer Survival. *J Clin Oncol*. 2016;34(27):3308-3314.doi:10.1200/JCO.2015.65.8013

8. Copson E, Eccles B, Maishman T, et al. Prospective observational study of breast cancer treatment outcomes for UK women aged 18-40 years at diagnosis: the POSH study. *J Natl Cancer Inst*. 2013;105(13):978-988.doi:10.1093/jnci/djt134

9. Collins LC, Marotti JD, Gelber S, et al. Pathologic features and molecular phenotype by patient age in a large cohort of young women with breast cancer. *Breast Cancer Res Treat*. 2012;131(3):1061-1066.doi:10.1007/s10549-011-1872-9

10. Early Breast Cancer Trialists' Collaborative G. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet*. 2005;365(9472):1687-1717.doi:10.1016/S0140-6736(05)66544-0

11. Sparano JA, Gray RJ, Makower DF, et al. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med*. 2018;379(2):111-121.doi:10.1056/NEJMoa1804710

12. Buus R, Sestak I, Kronenwett R, et al. Molecular Drivers of Oncotype DX, Prosigna, EndoPredict, and the Breast Cancer Index: A TransATAC Study. *J Clin Oncol*. 2021;39(2):126-135.doi:10.1200/JCO.20.00853

13. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol*. 2010;28(16):2784-2795.doi:10.1200/JCO.2009.25.6529

14. Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*. 2018;36(20):2105-2122.doi:10.1200/JCO.2018.77.8738

15. Lin C, Wu J, Lin L, et al. A Novel Prognostic Scoring System Integrating Gene Expressions and Clinicopathological Characteristics to Predict Very Early Relapse in Node-Negative Estrogen Receptor-Positive/HER2-Negative Breast Cancer. *Front Oncol*. 2020;10:1335.doi:10.3389/fonc.2020.01335

16. Paluch-Shimon S, Cardoso F, Partridge AH, et al. ESO-ESMO 4th International Consensus Guidelines for Breast Cancer in Young Women (BCY4). *Ann Oncol*. 2020;31(6):674-696.doi:10.1016/j.annonc.2020.03.284

17. K K. First results from a phase III randomized clinical trial of standard adjuvant endocrine therapy (ET) +/- chemotherapy (CT) in patients (pts) with 1-3 positive nodes, hormone receptor positive (HR+) and HER2-negative (HER2-) breast cancer (BC) with recurrence score (RS) <25: SWOGS1007 (RxPONDER). the virtual San Antonio Breast Cancer Symposium; 2020.

18. Dowsett M, Sestak I, Buus R, et al. Estrogen Receptor Expression in 21-Gene Recurrence Score Predicts Increased Late Recurrence for Estrogen-Positive/HER2-Negative Breast Cancer. *Clin Cancer Res*. 2015;21(12):2763-2770.doi:10.1158/1078-0432.CCR-14-2842

19. Azim HA, Jr., Michiels S, Bedard PL, et al. Elucidating prognosis and biology of breast cancer arising in young women using gene expression profiling. *Clin Cancer Res*. 2012;18(5):1341-1351.doi:10.1158/1078-0432.CCR-11-2599

20. Sparano JA, Gray RJ, Ravdin PM, et al. Clinical and Genomic Risk to Guide the Use of Adjuvant Therapy for Breast Cancer. *N Engl J Med*. 2019;380(25):2395-2405.doi:10.1056/NEJMoa1904819

21. Francis PA, Regan MM, Fleming GF, et al. Adjuvant ovarian suppression in premenopausal breast cancer. *N Engl J Med*. 2015;372(5):436-446.doi:10.1056/NEJMoa1412379

22. Pagani O, Regan MM, Walley BA, et al. Adjuvant exemestane with ovarian suppression in premenopausal breast cancer. *N Engl J Med*. 2014;371(2):107-118.doi:10.1056/NEJMoa1404037

23. Weiss A, King TA, Hunt KK, Mittendorf EA. Incorporating Biologic Factors into the American Joint Committee on Cancer Breast Cancer Staging System: Review of the Supporting Evidence. *Surg Clin North Am*. 2018;98(4):687-702.doi:10.1016/j.suc.2018.03.005

24. Sparano JA, Crager MR, Tang G, Gray RJ, Stemmer SM, Shak S. Development and Validation of a Tool Integrating the 21-Gene Recurrence Score and Clinical-Pathological Features to Individualize Prognosis and Prediction of Chemotherapy Benefit in Early Breast Cancer. *J Clin Oncol*. 2021;39(6):557-564.doi:10.1200/JCO.20.03007

25. Cuzick J, Sestak I, Baum M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. *Lancet Oncol*. 2010;11(12):1135-1141.doi:10.1016/S1470-2045(10)70257-6

26. Andre F, Ismaila N, Henry NL, et al. Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: ASCO Clinical Practice Guideline Update-Integration of Results From TAILORx. *J Clin Oncol*. 2019;37(22):1956-1964.doi:10.1200/JCO.19.00945

27. Gradishar WJ, Anderson BO, Abraham J, et al. Breast Cancer, Version 3.2020, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*. 2020;18(4):452-478.doi:10.6004/jnccn.2020.0016

Tables

Abbreviations: HR, hormone receptor; HER2, human epidermal growth factor receptor 2; IDC, invasive ductal cancer; ILC, invasive lobular cancer; RS, recurrence score.

Table 2 Variance of RS Scores as Accounted for by Individual Modules

	Group A		Group B		Group C	
RS module	Sum of Squares	Variance Explained (%)	Sum of Squares	Variance Explained (%)	Sum of Squares	Variance Explained (%)
Proliferation (unthresholded)	3430	37.3	6125	19.5	16681	18.2
ER	1968	21.4	17025	54.1	48958	53.4
Invasion	541	5.9	614	2.0	2779	3.0
HER2 (unthresholded)	24	0.3	170	0.5	81	0
Residuals	3235	35.2	7541	23.9	23113	25.2

Abbreviations: RS, recurrence score; ER, hormone receptor; HER2, human epidermal growth factor receptor 2.

Figures

Table 1 Basic features of HR+/HER2- early breast cancer patients from SJTU-BCDC

Characteristics	Total(%) n=1,078	Premenopause ≤40y n=97	Premenopause >40y n=284	Postmenopause n=697
Median Age	58(24-93)	37(27-40)	47(41-56)	63(45-93)
Subtype				
Luminal-A	340(31.5)	28(28.8)	101(35.6)	211(30.3)
Luminal-B(HER2-)	738(68.5)	69(71.2)	183(64.4)	486(69.7)
Pathology				
IDC	932(86.4)	88(90.7)	243(85.6)	601(86.3)
ILC	46(4.3)	2(2.1)	13(4.6)	31(4.4)
Others	100(9.3)	7(7.2)	28(9.8)	65(9.3)
Histologic grade				
1	103(9.6)	8(8.2)	31(10.9)	64(9.2)
2	559(51.9)	51(52.6)	161(56.7)	398(57.1)
3	224(20.8)	28(28.9)	53(18.7)	143(20.5)
Undifferentiated	141(13.1)	10(10.3)	39(13.7)	92(13.2)
pT				
1	732(67.9)	64(66.0)	212(74.6)	456(65.4)
2	335(31.1)	29(29.9)	71(26.1)	235(33.7)
3	11(0.1)	4(4.1)	1(0.3)	6(0.8)
pN				
0	1,007(93.4)	94(96.9)	278(97.9)	635(91.1)
1	71(6.6)	3(3.1)	6(2.1)	62(8.9)
RS score				
≤25	638(59.2)	58(59.8)	179(63.0)	401(57.5)
>25	440(40.8)	39(40.2)	105(37.0)	296(42.5)
Clinical Risk				
Low	544(50.5)	42(43.3)	165(58.1)	350(50.2)
High	534(49.5)	55(56.7)	119(41.9)	347(49.8)

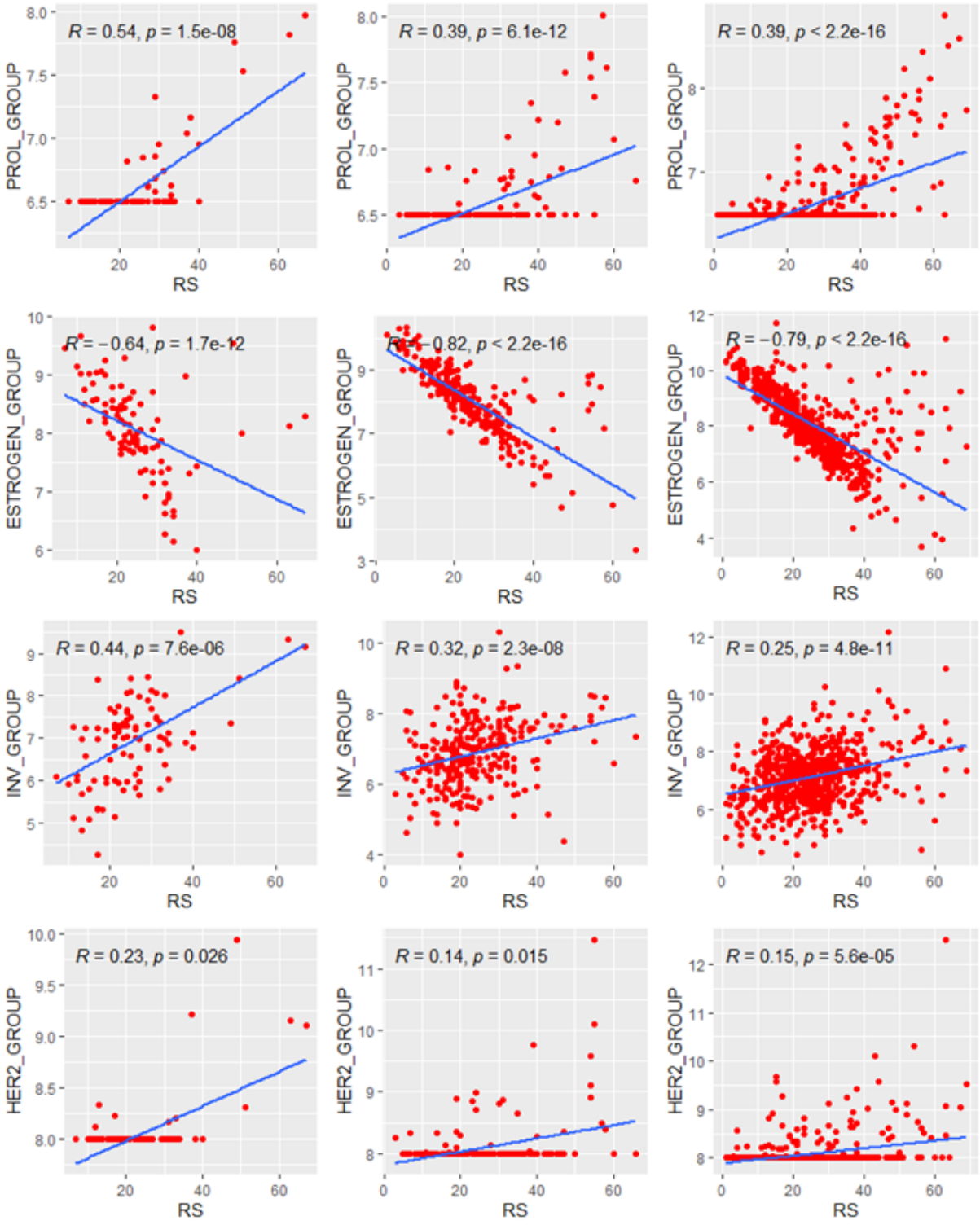

Figure 1

Relationships of the RS with its proliferation module and estrogen module. Group A, B, C were presented from left to right. Abbreviations: RS, recurrence score.

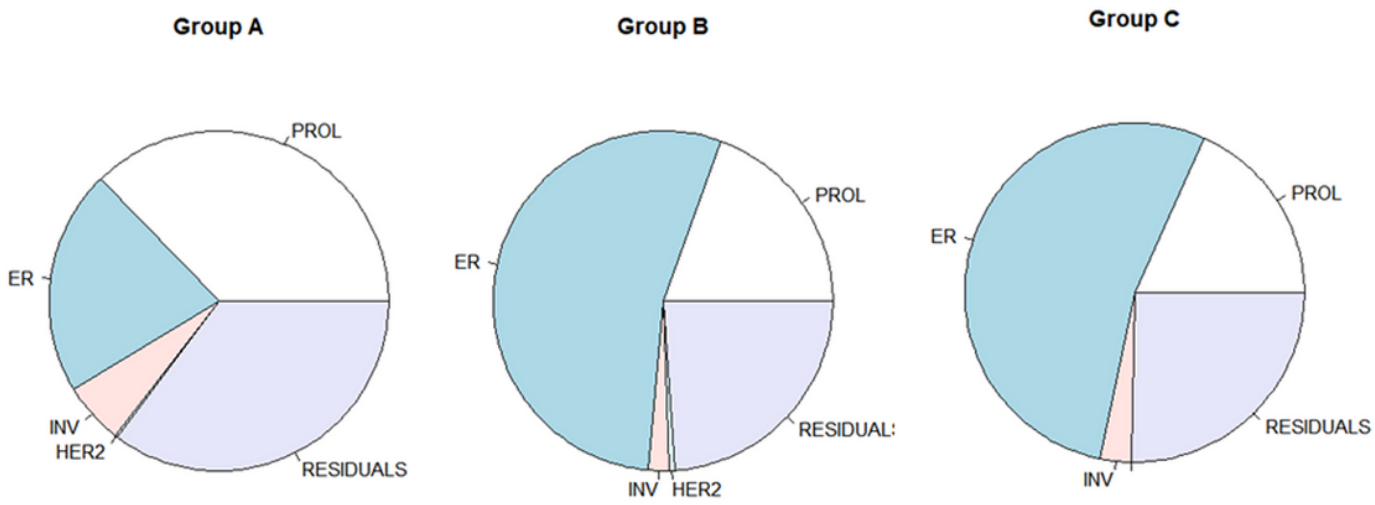

Figure 2

Variance of RS scores as accounted for by individual modules. Abbreviations: RS, recurrence score.

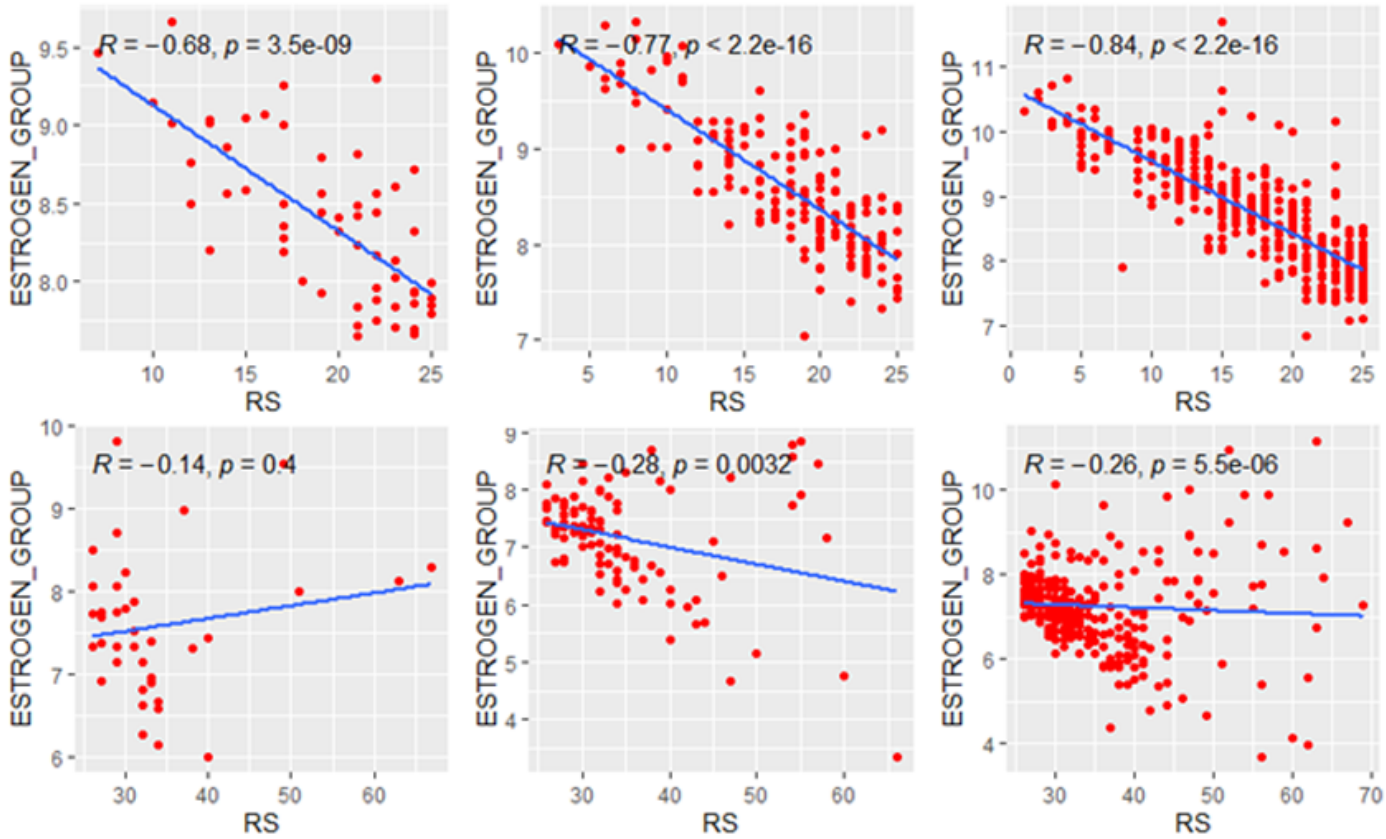

Figure 3

Relationships of the RS with its estrogen module. The upside and downside ranks showed the relationship in patients with RS ≤25 and RS >25 respectively. Group A, B, C were presented from left to

right. Abbreviations: RS, recurrence score.

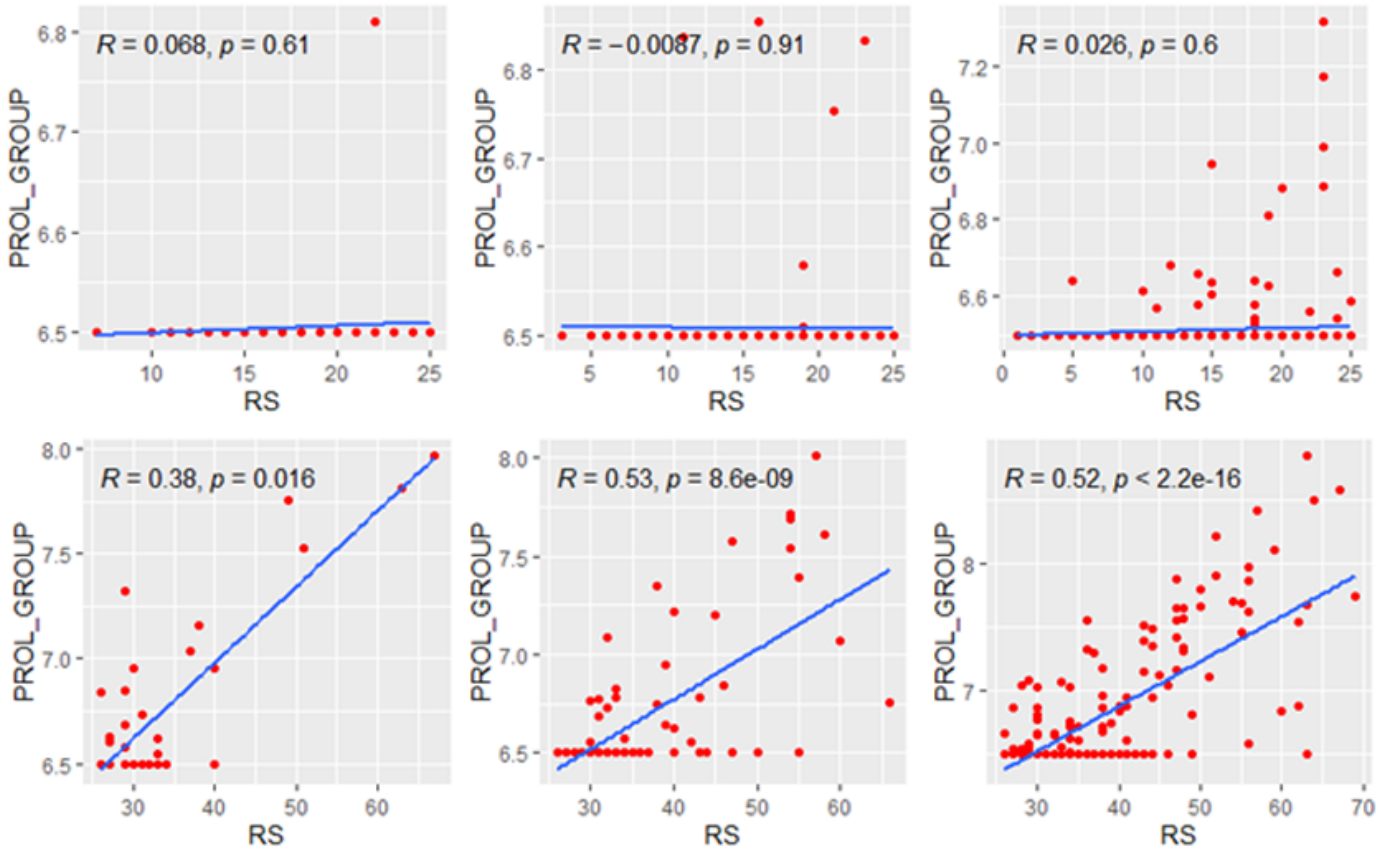

Figure 4

Relationships of the RS with its proliferation module. The upside and downside ranks showed the relationship in patients with RS ≤25 and RS >25 respectively. Group A, B, C were presented from left to right. Abbreviations: RS, recurrence score; PROL, proliferation.

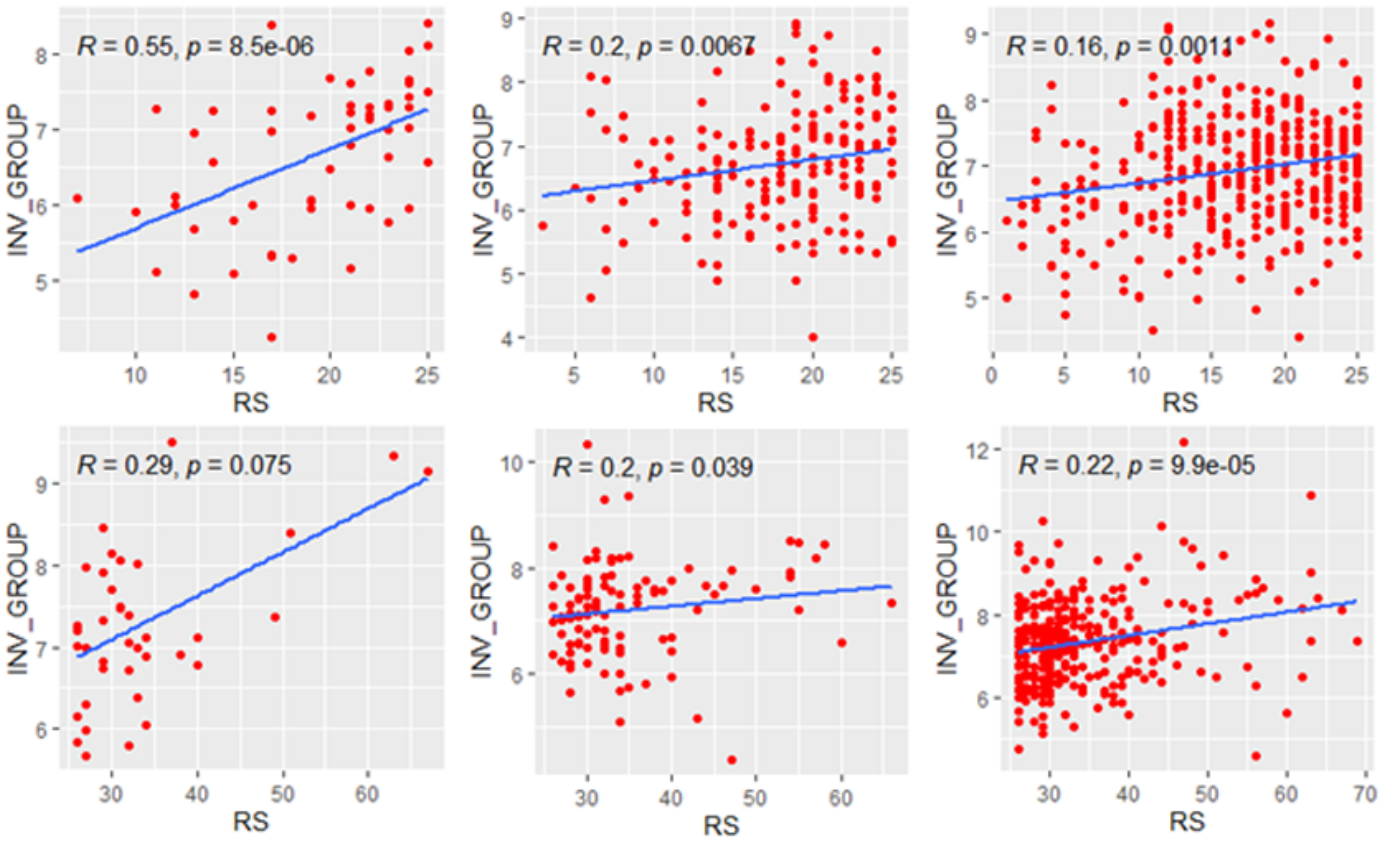

Figure 5

Relationships of the RS with its invasion module. The upside and downside ranks showed the relationship in patients with RS ≤25 and RS >25 respectively. Group A, B, C were presented from left to right. Abbreviations: RS, recurrence score; INV, invasion.

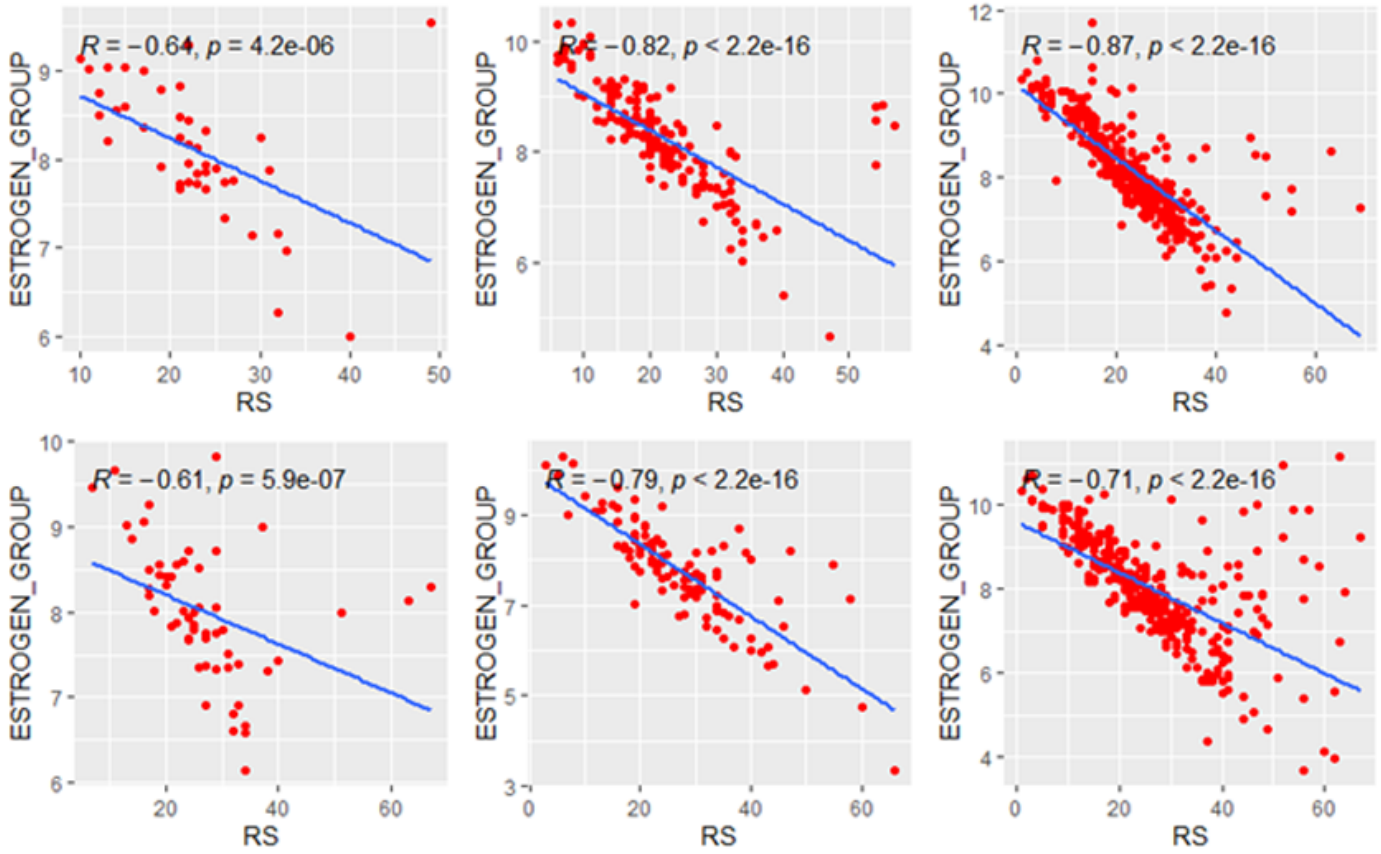

Figure 6

Relationships of the RS with its estrogen module. The upside and downside ranks showed the relationship in patients with low and high clinical risk respectively. Group A, B, C were presented from left to right. Abbreviations: RS, recurrence score.

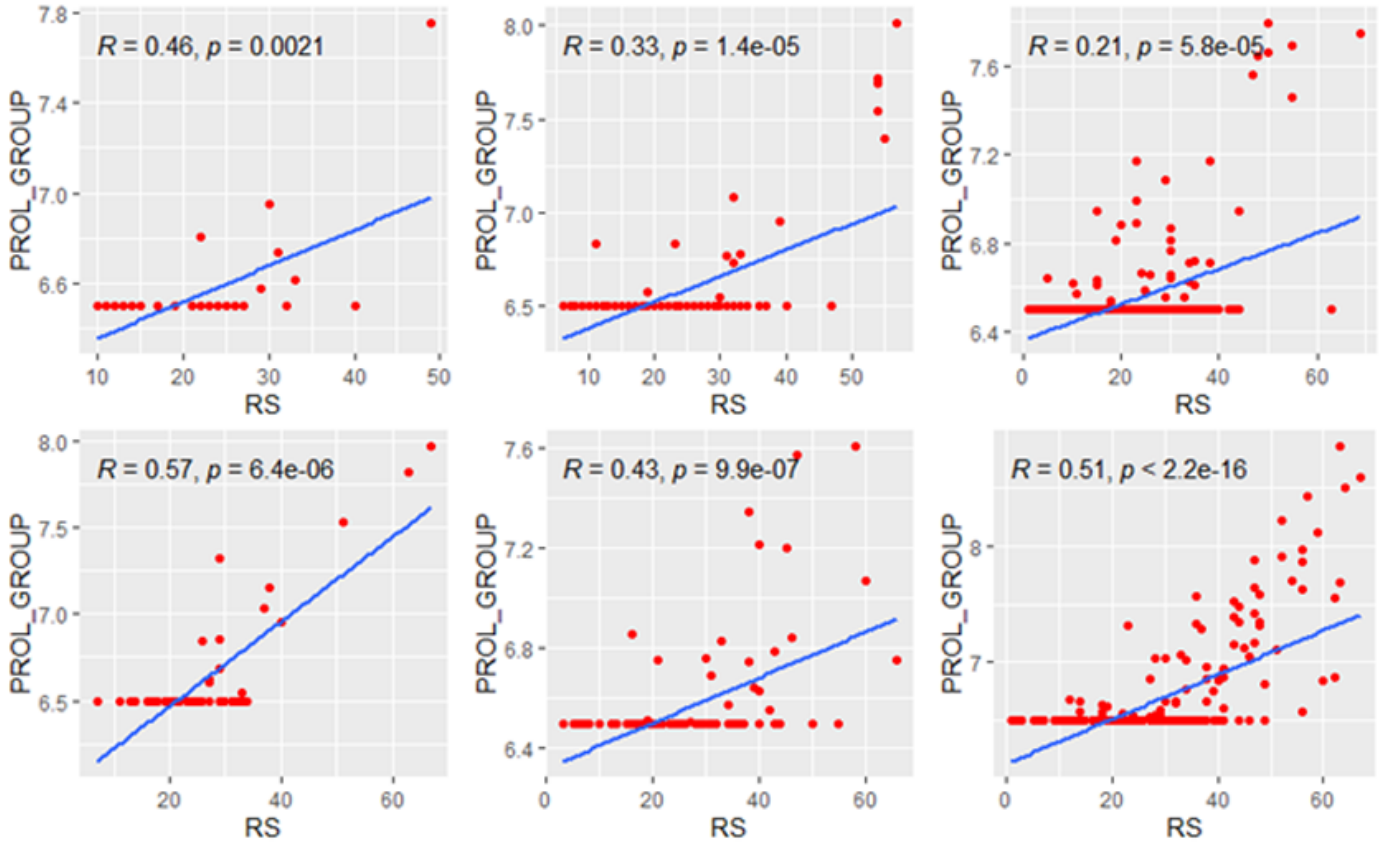

Figure 7

Relationships of the RS with its proliferation module. The upside and downside ranks showed the relationship in patients with low and high clinical risk respectively. Group A, B, C were presented from left to right. Abbreviations: RS, recurrence score; PROL, proliferation.

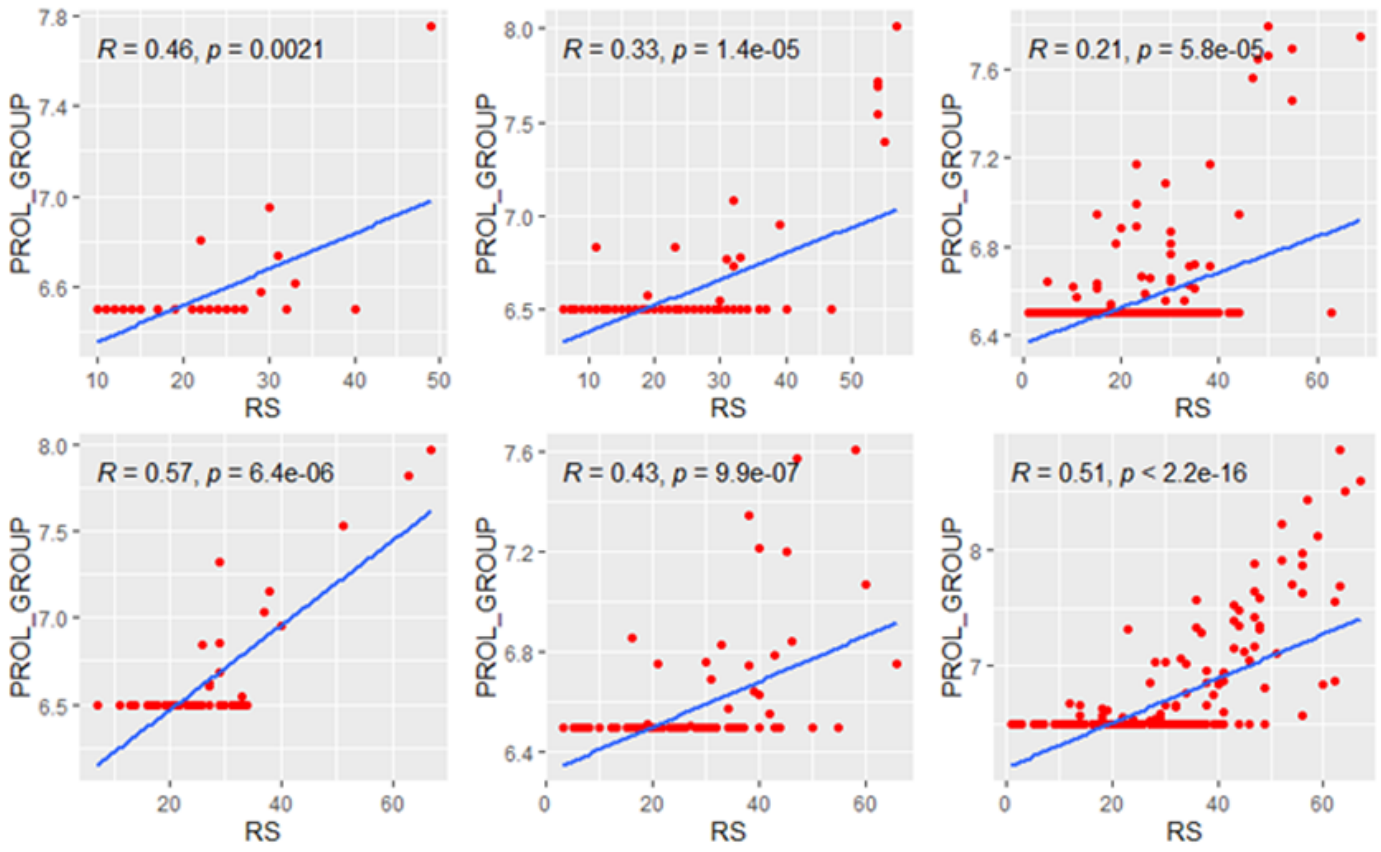

Figure 8

Relationships of the RS with its invasion module. The upside and downside ranks showed the relationship in patients with low and high clinical risk respectively. Group A, B, C were presented from left to right. Abbreviations: RS, recurrence score; INV, invasion.

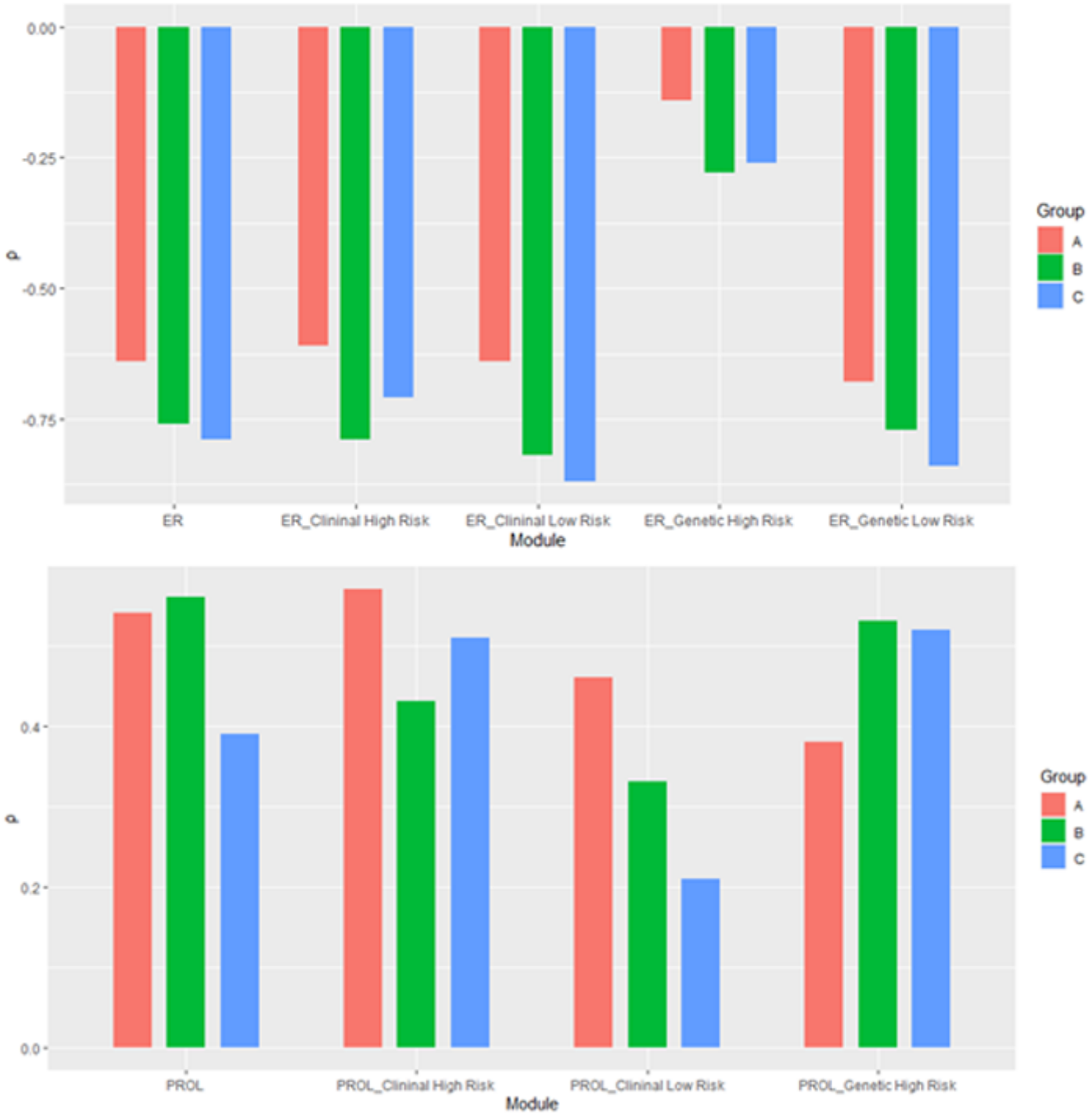

Figure 9

The histogram of relationships of the RS with its (A) estrogen and (B) proliferation module. The subgroup of proliferation module with genetic low risk was omitted due to non-significance. Abbreviations: ER, estrogen receptor; PROL, proliferation.