Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

The Ki67 index of preoperative breast cancer puncture is correlated with lymph node metastasis, ER, PR and AR expression: A retrospective study of real world data

Xiaotian Zhang

xiaotianzhang98@163.com

Xi'an Jiaotong University Affiliated Baoji Central Hospital

Xiang Liu

Xi'an Jiaotong University Affiliated Baoji Central Hospital

Lu Wang

Xi'an Jiaotong University Affiliated Baoji Central Hospital

Juan Jin

Xi'an Jiaotong University Affiliated Baoji Central Hospital

Yuan Cai

Xi'an Jiaotong University Affiliated Baoji Central Hospital

Qing She

Xi'an Jiaotong University Affiliated Baoji Central Hospital

Article

Keywords: Ki-67 index, Neoadjuvant therapy, Breast cancer

Posted Date: April 9th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9088621/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Objective

Breast cancer is a prevalent malignant disease among women. Currently, the primary clinical treatment strategies involve classifying breast cancer based on the expression of Ki67, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2), followed by endocrine or chemotherapy. Through the retrospective analysis of a real world data, this study explored the Ki67 index and clinicopathological features of breast cancer patients before operation and the clinical significance of neoadjuvant therapy.

Methods

Clinicopathological characteristics and preoperative immunohistochemistry (IHC) results for the Ki-67 index and ER, PR, Androgen receptor (AR), and HER-2, were collected from patients who underwent modified radical mastectomy at Baoji Central Hospital between June 2024 and June 2025. Using 30% as the cutoff value for the Ki-67 index, the chi-square test was used to compare differences in clinicopathological and immunohistochemical features between the low- and high-Ki-67 index expression groups.

Results

The results suggest that the preoperative puncture Ki67 index of breast cancer patients is significantly related to lymph node metastasis (LNM), ER, PR and AR expression. Univariate survival analysis showed that MKI67, ERS1, AR and ERBB2 had no significant effect on overall survival (OS) and progression-free survival (PFS) ($P > 0.05$), while PGR had significant statistical significance on PFI ($P < 0.05$) and no significant effect on OS ($P > 0.05$). Multivariate survival analysis showed that the gene sets of MKI67, ESR1, PGR, AR and ERBB2 had no significant effects on OS and PFS, but PGR might have marginal effects on OS ($P = 0.0523$). **C**

onclusion:

Ki67 stratification based on early breast cancer patients can predict the expression of LNM, HR and AR, and provide scientific basis for individualized neoadjuvant therapy. However, Ki-67 index does not have the potential to judge the survival prognosis, and more potential IHC characteristics should be explored as new biomarkers to predict the prognosis and long-term survival of neoadjuvant therapy.

Introduction

Breast cancer is an aggressive malignant tumor. The number of breast cancer patients is expected to reach 3 million per year by 2025, and the death toll is expected to increase to 1 million[1]. According to the National Cancer Center of China, breast cancer is the second most common cancer among Chinese women[2]. Moreover, the proportion of male breast cancer patients is approximately 1%[3].

The molecular subtypes of breast cancer can be classified into four categories based on ER, PR, and HER-2 status: luminal A type, luminal B type, HER-2 positive type, and triple-negative breast cancer. In addition to the above four molecular subtypes, several other rare molecular subtypes of breast cancer, such as variants of HER-2-positive and triple-negative breast cancer (TNBC), have been identified. These rare types usually have a high degree of malignancy and a poor prognosis[4]. Recently, a study classified the overall expression status of ER and PR in HER-2-positive breast cancer patients on the basis of hormone receptor (HR) status, dividing them into HR+/HER-2 + and HR-/HER-2 + groups. The results indicated that HR status significantly affects the clinicopathological characteristics, metastasis patterns and survival outcomes of breast cancer patients[5].

The preoperative puncture results of breast cancer patients support the choice of treatment scheme. Neoadjuvant therapy for breast cancer was originally introduced to treat locally advanced, borderline operable or inoperable diseases, so as to make it easier to operate and allow breast-conserving surgery[6]. However, due to the lack of specific biomarkers that can reliably predict the curative effect, the clinical decision-making of neoadjuvant therapy still faces great challenges and it is difficult to achieve real individualization. By circulating tumor DNA, circulating tumor cells, diffuse cancer cells and tumor infiltrating lymphocytes, early evidence about neoadjuvant therapy and its follow-up treatment can be provided[7]. It is clinically feasible to identify the response of early breast cancer patients to neoadjuvant therapy by IHC characteristics. The clinical retrospective analysis of 40711 patients with HER-2 positive shows that the IHC status of HER-2 can predict the benefit of breast cancer patients from neoadjuvant therapy, and the formulation of HER-2 stratification strategy may be helpful to improve clinical management[8].

The purpose of this study is to evaluate the biological relationship between Ki-67 index and biomarkers such as HR, AR and HER-2 by analyzing the characteristics of puncture IHC in breast cancer patients, and to explore its predictive effect on the efficacy of neoadjuvant chemotherapy for breast cancer. This study is helpful to provide evidence support for the clinical development of individualized treatment strategies, and is of great significance to deeply understand the stratified treatment under the guidance of Ki-67, which has high scientific value and clinical application prospects.

Materials and methods

Study design

From June 2024 to June 2025 in Baoji Central Hospital, we retrospectively analyzed the patients who were diagnosed with breast cancer by pathological puncture and underwent improved radical operation.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (i) breast cancer confirmed by pathological examination; (ii) received neoadjuvant chemotherapy; and (iii) after neoadjuvant chemotherapy met the surgical indications, modified radical resection was performed. The exclusion criteria were as follows: (i) patients who received other preoperative treatments and (ii) patients with other cancers. The pathology database of Baoji Central Hospital was retrieved, the data were organized, and abnormal and noncompliant data were excluded. A total of 116 patients were included in the study, and after screening according to the inclusion and exclusion criteria, 107 patients were ultimately included.

Specimen collection

Puncture specimen

For patients with breast ultrasound or mammography indicating a lump, the standard puncture procedure is performed. Under the guidance of ultrasound, a hollow puncture needle is used to pierce the edge of the lump, and the needle core is quickly cut to obtain a slender tissue strip. The specimen is obtained 3-5 times repeatedly, and immediately put into 10% neutral formalin solution for fixation, and the patient information label is attached. Immediately after pulling out the puncture needle, locally compress the puncture point with sterile gauze for 15-20 minutes, then cover it with dressing and apply pressure with elastic bandage.

Postoperative specimen

The surgeon or assistant should hand over the specimen to the visiting nurse or special person for treatment within 30 minutes after the specimen is isolated. The surgeon marks the surgical margin (top, bottom, inside, outside and base) with blue dye. The axillary lymph nodes and adipose tissue were collected and labeled with corresponding positions. In vitro samples were completely immersed in sufficient formalin solution and fixed for 24 hours before taking materials.

Specimen handling and fixation

The fixed tissue is checked by the pathologist, including the patient's name, gender, age, location, organization name and other information. After confirmation, standardized materials are taken, and the number, color, length and thickness of the punctured tissue are described, then photographed and wrapped in embedding paper. After the operation, the gross specimens were described and taken. First, the cutting edges (upper, lower, inner, outer, base and nipple) were taken, and then the page-like incision was made to find the tumor bed and divide it into 2×1.5×0.2cm tissue blocks, with emphasis on the parts bordering normal tissues or adjacent to the cutting edges. Carefully examine the axillary lymph nodes and adipose tissue, find out all the lymph nodes and put them in the embedding box.

Production and dyeing

After all tissues are placed in embedding boxes, they are processed in a tissue dehydrator (company: Leica, Germany, model: ASP300S) according to the set procedures. The dehydrated tissues are embedded in a biological tissue automatic embedding machine (company: China Hongye Medical Instrument Co., Ltd., model: BM-□□□), and after embedding, a rotary slicer (company: Leica, Germany, model: RM 22331) is used. HE dyeing uses a dyeing and sealing machine (company: Sakura Seiki Co., Ltd., model: DRS-Prisma-P-JCS) for dyeing and sealing. IHC uses ER (company: Roche Diagnostic Reagents, Switzerland, article number: M29468), PR (company: Roche Diagnostic Reagents, Switzerland, article number: M27931), AR (company: China Maixin Biotechnology Development Co., Ltd., article number: RMA-1055), Ki-67 antibody (company: Roche Diagnostic Reagents, Switzerland, ArticleNo.: N06786), HER-2 (company: China Maixin Biotechnology Development Co., Ltd., articleNo.: 2505280701d) and the corresponding secondary antibody were carried out on the all-active immunity histochemical staining platform (company: Roche Diagnostic Reagent Company, model: BenchMark ULTRA PLUS).

IHC classification

To detect the expression of tumor biomarkers in patients with breast cancer after neoadjuvant chemotherapy. ER and PR positivity were defined as staining of ≥ 1% of the cells. HER-2 status is evaluated by IHC, and HER2 negative is defined as IHC 0-1+, HER-2 positive is IHC 3+, and IHC 2+ is ambiguous (further evaluation is made by fluorescence in situ hybridization (FISH) if necessary)[9]. Wongmaneerung's ROC curve analysis of breast cancer patients shows that the best cut-off point of Ki-67 is ≥30%, its sensitivity is 75%, and its specificity is 48.85%, so this study takes 30% as the critical value[10].

Survival Analysis

GEPIA 3 integrates patient data from TCGA and is an open source bioinformatics analysis tool. Using the survival analysis function, the OS and PFS of univariate and multivariate are analyzed respectively, and the parameters of univariate analysis are set as follows: Time Unit is in months; Group Cutoff is divided by median.

Statistical analysis

The baseline clinical and pathological characteristics of the patients, including age, location of occurrence, tumor size, invasion status, LNM status, clinical TNM stage, and IHC detection of the ER, PR, AR, HER-2, and Ki-67 indices, were recorded. After diagnosis by two deputy chief physicians, the IHC score was evaluated. For cases with objections, multiple expert discussions were conducted to determine the final score. Categorical variables were compared via the chi-square test. A bilateral P value < 0.05 was considered statistically significant. SPSS 20.0 and GraphPad 8.0 were used for data processing and graph construction.

Results

Baseline characteristics

A total of 107 patients were included in the study, among whom 42 (39.25%) were under 50 years old and 65 (60.75%) were 50 years old or above. There were 3 males (2.80%) and 104 females (97.20%). Tumors occurred on the left side in 60 cases (56.07%) and on the right side in 47 cases (43.93%). There were 18 individuals (16.82%) with tumor sizes ≤2 cm, 72 individuals (67.29%) with tumor sizes ranging from 2 to 5 cm, and 17 individuals (15.89%) with tumor sizes > 5 cm. There were 93 patients (86.92%) without tumor invasion, 4 patients (3.74%) with papillary invasion, 7 patients (6.54%) with skin invasion, 7 patients (6.54%) with vascular tumor thrombus invasion, and 2 patients (1.87%) with nerve invasion. There were 38 patients (35.51%) (64.49%) with LNM and 69 patients (64.49%) without LNM. There were 5 patients (4.67%) in the T0 stage, 20 patients (18.69%) in the T1 stage, 61 patients (57.01%) in the T2 stage, 13 patients (12.15%) in the T3 stage, and 8 patients (7.48%) in the T4 stage. There were 37 people (34.58%) in the N0 stage, 55 people (51.40%) in the N1 stage, 10 people (9.35%) in the N2 stage, and 5 people (4.67%) in the N3 stage. There were 103 people (96.26%) in the M0 stage and 4 people (3.74%) in the M1 stage. As shown in Table 1 and Figure 1.

Table 1. Baseline characteristics (*n*=107)

Characteristic	Cases	Percentage□/□□
Age		
□50 years	42	39.25
≥50 years	65	60.75
Gender		
Male	3	2.80
Female	104	97.20
Tumor site		
Left	60	56.07
Right	47	43.93
Tumor size		
Grade1□□≤2 cm□	18	16.82
Grade2□□2 cm□□x≤5 cm□	72	67.29
Grade3□□□5 cm□	17	15.89
Tumor invasion		
None	93	86.92
Nipple	4	3.74
Skin	7	6.54
Blood vessel invasion	7	6.54
Neuron	2	1.87
LNM		
Yes	38	35.51
No	69	64.49
T stage		
0	5	4.67
1	20	18.69
2	61	57.01
3	13	12.15
4	8	7.48

N stage		
0	37	34.58
1	55	51.40
2	10	9.35
3	5	4.67
M stage		
0	103	96.26
1	4	3.74

IHC-related indicators

Immunohistochemical detection was performed on 107 patients with breast cancer by preoperative puncture, including Ki-67, ER, PR, AR and HER-2. The results revealed that 54 patients (50.47%) had a low Ki-67 index and that 53 patients (49.53%) had a high Ki-67 index. There were 33 (30.84%) ER-negative patients and 74 (69.16%) ER-positive patients. There were 38 patients (35.51%) with negative PRs and 69 patients (64.49%) with positive PRs. There were 9 patients (8.41%) negative for AR and 99 patients (91.59%) positive. There were 7 patients (6.54%) who were HER-2 negative and 100 patients (93.46%) who were HER-2 positive. As shown in Table 2 and Figure 2. Representative diagrams of various IHCs are shown in Figure 3.

Table 2. IHC-related indicators (*n*=107)

Indicators	Cases	Percentage□/□□
Ki-67 index		
Low expression□≤30□□	54	50.47
High expression□□30□□	53	49.53
ER		
Negative	33	30.84
Positive	74	69.16
PR		
Negative	38	35.51
Positive	69	64.49
AR		
Negative	9	8.41
Positive	99	91.59
HER-2		
Negative	7	6.54
Positive	100	93.46

Comparison of the Ki-67 index

Breast cancer patients were divided into two groups, namely, the low-expression group and the high-expression group, on the basis of a Ki-67 index of 30%. The baseline data and IHC indicators were subsequently classified, and the chi-square test was used to identify the factors related to the Ki-67 index. The results indicated that there were significant differences in LNM and ER, PR and AR expression between the low-expression and high-expression groups in terms of the Ki-67 index. The chi-square test results are shown in Tables 3 and 4, and the scatter plot of the Ki-67 index is illustrated in Figure 4.

Table 3. Analysis of factors related to the Ki-67 index and baseline data (*n*=107)

Characteristic	Ki-67 Low expression	Ki-67 High expression	χ^2	*P*
Age				
<50 years	19	23	1.052	0.305
≥50 years	36	29		
Gender				
Male	2	1	-	0.999
Female	52	52		
Tumor site				
Left	30	29	0.008	0.931
Right	24	24		
Tumor size				
Grade1（≤2 cm）	10	8	0.562	0.755
Grade2（2 cm＜x≤5 cm）	34	37		
Grade3（＞5 cm）	10	8		
Tumor invasion				
None	45	48	-	0.667
Nipple	2	2		
Skin	4	3		
Blood vessel invasion	3	4		
Neuron	2	0		
LNM				
Yes	25	13	5.534	0.019
No	29	40		
T stage				
0	1	4	3.599	0.463
1	13	7		
2	32	29		
3	6	7		
4	4	4		

N stage				
0	13	24	7.383	0.061
1	30	25		
2	6	3		
3	5	1		
M stage				
0	53	50	-	0.363
1	1	3		

Table 4. Analysis of the Ki-67 index and related IHC factors (*n*=107)

Characteristic	Ki-67 Low expression	Ki-67 High expression	χ^2	*P*
ER				
Negative	45	29	10.270	0.001
Positive	99	24		
PR				
Negative	13	25	6.230	0.013
Positive	41	28		
AR				
Negative	0	9	-	0.001
Positive	54	44		
HER-2				
Negative	1	6	-	0.060
Positive	53	47		

Survival Analysis

GEPIA 3 was used to analyze the survival of univariate and multivariate genes. Univariate gene analysis showed that MKI67, ERS1, AR and ERBB2 had no significant effect on OS and PFS ($P > 0.05$), while PGR had significant statistical significance on PFS ($P < 0.05$) and no significant effect on OS ($P > 0.05$). The results of multi-gene analysis showed that the gene sets of MKI67, ESR1, PGR, AR and ERBB2 had no

significant effects on OS and PFS ($P > 0.05$), but PGR may have marginal effects on OS ($P = 0.0523$), which may have certain clinical significance, but it is uncertain.

Discussion

The Ki-67 index, HR and HER-2 expression level play significant roles in the treatment and prognosis of patients with breast cancer[11]. For patients with positive HR, endocrine therapy can achieve better therapeutic effects[12], whereas for those with positive HER-2, targeted therapy can show significant efficacy[13]. Moreover, the efficacy of endocrine therapy and targeted therapy is closely related to the expression levels of HR and HER-2. Early neoadjuvant therapy on the basis of the levels and status of immune markers can effectively reduce the size of tumors, help patients progress from inoperable to operable, and improve the prognosis and therapeutic effect of patients[14,15].

The Ki-67 index was first reported in proliferating cancer cells in 1983 and has been confirmed to be closely related to tumor growth[16]. The evaluation of breast cancer based on the Ki-67 index can guide clinical decision-making and assess the prognosis of patients. A positive Ki-67 index indicates strong tumor invasiveness, LNM and a poor survival rate[17]. In 2011, the St. Gallen Conference proposed a critical value of 14% for the Ki-67 index to distinguish between type A and type B lumens[18], but this value was changed to 20% in 2013[9]. Some scholars also use 28.35% as the critical value[19]. Some scholars have set the critical value of the Ki-67 index at 30% as an independent prognostic factor for the overall survival and disease-free survival of breast cancer patients[20]. Patients with a high Ki-67 index (≥ 20%) are more likely to achieve pathological complete remission (pCR)[21]. The Ki-67 index of breast cancer had an impact on positive axillary lymph nodes in univariate analysis but not in multivariate analysis[22].

When considering the prognostic value of the Ki-67 index for patients, the influence of other clinicopathological features, such as histological grade, lymphatic metastasis, and tumor volume, should be taken into account[23]. Studies have shown that a high Ki-67 index is a predictive biomarker for pCR in early breast cancer patients[24]. When the Ki-67 index is between 46% and 68%, the mortality rate of breast cancer patients is gradually increasing, and the disease-free survival (DFS) period is significantly shortened[25]. The construction of a predictor model for the treatment effect in breast cancer patients indicates that the pCR factors of neoadjuvant chemotherapy in breast cancer patients include a Ki-67 index > 20% and positive expression of AR[26].

For breast cancers with different HR and HER-2 expression levels, the treatment and clinicopathological features also vary[27]. After neoadjuvant therapy for ER+/HER-2- breast cancer, the Ki-67 index decreases, and the tumor volume decreases[28]. Palbocicli is a CDK4/6 inhibitor[29]. When used in combination with endocrine therapy, it is a valuable treatment option for HR+/HER-2- advanced or metastatic breast cancer. With a Ki-67 index of 30% as the critical value, the PFS of breast cancer patients with a high Ki-67 index is significantly shorter. A PR higher than 20% is correlated with prolonged PFS. Breast cancer patients with a Ki-67 index < 30% and a PR ≥ 20% have longer PFS than those with a Ki-67 index ≥ 30%

and a PR < 20%[30]. Research on key molecular markers of tumors in Chinese women has shown that higher expression of AR and HR is significantly correlated and that there is a potential association with responsiveness to hormone therapy[31].

This study has the following limitations: First, the retrospective, single-center design may introduce inherent methodological biases such as selection bias. Second, the relatively limited sample size could affect statistical power and the reliability of subgroup analyses. Furthermore, the detection of biomarkers relied solely on immunohistochemistry, a method subject to inter-observer and inter-experimental variability, which may introduce certain biases. Future validation using complementary techniques such as Western blotting or immunofluorescence would help enhance the accuracy of the findings and the persuasiveness of the conclusions.

Conclusion

The preoperative pathological and clinical characteristics of patients who received neoadjuvant chemotherapy for breast cancer indicate that the Ki-67 index is significantly correlated with LNM, HR and AR expression. This finding reveals that for breast cancer patients with different Ki-67 indices, classification should be carried out in accordance with the guidelines on the expression status of HR and AR, and neoadjuvant chemotherapy strategies should be formulated to maximize pCR, reduce LNM in patients, and improve their survival status. At the same time, more high-quality clinical evidence is needed to confirm the role of polygenes in the long-term survival of breast cancer patients. The connection between the Ki-67 index and LNM, HR, and AR expression will provide a basis for the clinical diagnosis and treatment of breast cancer and is expected to lead to the formulation of personalized treatment plans on the basis of individual conditions to achieve precision medicine for breast cancer.

Declarations

Author contributions

ZXT conceived and designed the research, collected and analyzed the data, and wrote the manuscript. CY and JJ contributed to conceptualization, design and supervision of the research. LX and WL assisted in collecting data and conducted a preliminary organization. SQ received financial support and guided the writing of the thesis. All the authors have revised the manuscript and agreed to its final review and submission.

Data availability statement

The datasets utilized and/or analyzed in this study are available from the corresponding author upon reasonable request.

Funding

This research was supported by the Baoji health scientific research project (No:2024-043 and 2026-012).

Ethics declarations

Competing interests

The authors declare that they have no competing interests.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki of 2002. This study was approved by the Medical Ethics Committee of Baoji Central Hospital.

This study confirmed that informed consent had been obtained from all subjects and/or their legal guardians.

References

1. Elbasheer, M. M. A., Dodwell, D. & Gathani, T. Understanding global variation in breast cancer mortality. *Br J Radiol*, doi:10.1093/bjr/tqaf148 (2025).
2. Han, B. *et al.* Cancer incidence and mortality in China, 2022. *J Natl Cancer Cent* **4**, 47-53, doi:10.1016/j.jncc.2024.01.006 (2024).
3. Kong, X. *et al.* Mapping the Metastatic Landscape: A Population-Based Cohort Study for Prognostic Insights Into Newly Diagnosed Stage IV Breast Cancer Cases. *Cancer Innov* **4**, e70017, doi:10.1002/cai2.70017 (2025).
4. Luo, Q. *et al.* Erdafitinib inhibits the tumorigenicity of MDA-MB-231 triple-negative breast cancer cells by inducing TRIM25/ubiquitin-dependent degradation of FGFR4. *Breast Cancer Res* **27**, 128, doi:10.1186/s13058-025-02086-7 (2025).
5. Ran, R. *et al.* Clinicopathological characteristics, treatment patterns and outcomes in patients with HER2-positive breast cancer based on hormone receptor status: a retrospective study. *BMC Cancer* **24**, 1216, doi:10.1186/s12885-024-12974-4 (2024).
6. Valderrama, N. F. *et al.* Chemo-prAIdict Breast: A deep learning solution for predicting residual disease on biopsies of breast cancer patients treated with neoadjuvant chemotherapy. *Eur J Cancer* **234**, 116222, doi:10.1016/j.ejca.2026.116222 (2026).
7. Chen, L., Chen, Q. & Zhu, M. A retrospective analysis of clinicopathological characteristics and risk factors for recurrence in young patients with breast cancer. *Gland Surg* **13**, 1281-1290, doi:10.21037/gs-24-193 (2024).
8. Winer, L. *et al.* Immunohistochemical Status Predicts Pathologic Complete Response to Neoadjuvant Therapy in HER2-Overexpressing Breast Cancers. *Ann Surg Oncol* **32**, 931-943, doi:10.1245/s10434-024-16470-8 (2025).

9. Goldhirsch, A. *et al.* Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol* **24**, 2206-2223, doi:10.1093/annonc/mdt303 (2013).
10. Wongmaneerung, P. *et al.* The association between Ki-67 expression and survival in breast cancer subtypes: a cross-sectional study of Ki-67 cut-point in northern Thailand. *BMC Cancer* **25**, 346, doi:10.1186/s12885-025-13724-w (2025).
11. Okumuş, A. *et al.* The prognostic value of platelet count and lymphocyte-to-monocyte ratio, Ki-67, and Nottingham indexes in early-stage breast cancer. *Rev Assoc Med Bras (1992)* **71**, e20250067, doi:10.1590/1806-9282.20250067 (2025).
12. Nielsen, T. O. *et al.* Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group. *J Natl Cancer Inst* **113**, 808-819, doi:10.1093/jnci/djaa201 (2021).
13. Zhu, Z. *et al.* Real-World Efficacy of HLX02-Based Neoadjuvant Therapy in HER2-Positive Breast Cancer: Clinical Insights and Future Directions. *Breast J* **2025**, 1653319, doi:10.1155/tbj/1653319 (2025).
14. Civil, Y. A. *et al.* Impact of neoadjuvant systemic therapy on surgical and radiotherapy outcomes in patients with early-stage breast cancer: a cross-sectional retrospective single-center study. *BMC Cancer* **25**, 1046, doi:10.1186/s12885-025-14438-9 (2025).
15. Yang, Q., Xi, L., Huang, M., Zhang, H. & Zhou, F. Nomogram for the prediction of the prognosis of patients with triple-negative invasive ductal carcinoma of breast after neoadjuvant chemotherapy. *Sci Rep* **15**, 21666, doi:10.1038/s41598-025-05738-y (2025).
16. Gerdes, J., Schwab, U., Lemke, H. & Stein, H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer* **31**, 13-20, doi:10.1002/ijc.2910310104 (1983).
17. Aghebati, M., Hossieni, R., Makeh, A. S., Shirzadi, A. & Akbari, M. E. Ki-67 and 21-gene recurrence score assay in decision making for adjuvant chemotherapy in breast cancer patients. *Discov Oncol* **16**, 970, doi:10.1007/s12672-025-02233-8 (2025).
18. Goldhirsch, A. *et al.* Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Ann Oncol* **22**, 1736-1747, doi:10.1093/annonc/mdr304 (2011).
19. Shet, T. Ki-67 in breast cancer: Simulacra and simulation. *Indian J Cancer* **57**, 231-233, doi:10.4103/ijc.IJC_565_19 (2020).
20. Zhu, X. *et al.* The prognostic and predictive potential of Ki-67 in triple-negative breast cancer. *Sci Rep* **10**, 225, doi:10.1038/s41598-019-57094-3 (2020).
21. Jarząb, M. *et al.* Association of breast cancer grade with response to neoadjuvant chemotherapy assessed postoperatively. *Pol J Pathol* **70**, 91-99, doi:10.5114/pjp.2019.87101 (2019).
22. Kondov, B. *et al.* Prognostic Factors on the Positivity for Metastases of the Axillary Lymph Nodes from Primary Breast Cancer. *Pril (Makedon Akad Nauk Umet Odd Med Nauki)* **38**, 81-90,

doi:10.1515/prilozi-2017-0011 (2017).

23. Kadivar, M. & Aram, F. Assessment of Ki67 in Breast Cancer: A Comparison Between the Eye-10 Method, Stepwise Counting Strategy, and International System of Ki67 Evaluation. *Iran J Pathol* **15**, 13-18, doi:10.30699/ijp.2019.102290.2017 (2020).
24. Funasaka, C. *et al.* The utility of immunohistochemistry-based biomarkers in predicting the pathological complete response in early-stage triple-negative breast cancer. *Cancer Treat Res Commun* **44**, 100941, doi:10.1016/j.ctarc.2025.100941 (2025).
25. Wang, C. *et al.* Ki-67-Playing a key role in breast cancer but difficult to apply precisely in the real world. *BMC Cancer* **25**, 962, doi:10.1186/s12885-025-14374-8 (2025).
26. Liao, X. W. *et al.* Prediction of neoadjuvant chemotherapy efficacy and prognostic biomarker analysis in patients with triple-negative breast cancer. *Front Pharmacol* **16**, 1553831, doi:10.3389/fphar.2025.1553831 (2025).
27. Hu, X. *et al.* Expression changes of ER, PR, HER2, and Ki-67 in primary and metastatic breast cancer and its clinical significance. *Front Oncol* **13**, 1053125, doi:10.3389/fonc.2023.1053125 (2023).
28. López-Velazco, J. I. *et al.* Molecular characterisation of the residual disease after neoadjuvant endocrine therapy in ER+/HER2- breast cancer uncovers biomarkers of tumour response. *Transl Oncol* **57**, 102407, doi:10.1016/j.tranon.2025.102407 (2025).
29. Palmieri, C. *et al.* A real-world study of the first use of palbociclib for the treatment of advanced breast cancer within the UK National Health Service as part of the novel Ibrance® Patient Program. *Br J Cancer* **129**, 852-860, doi:10.1038/s41416-023-02352-5 (2023).
30. Oprean, C. M., Badau, L. M., Petrita, R., Median, M. D. & Dema, A. Real-World, National Study of Palbociclib in HR+/HER2- Metastatic Breast Cancer: A 2.5-Year Follow-Up PALBO01/2021. *Diagnostics (Basel)* **15**, doi:10.3390/diagnostics15091173 (2025).
31. Tu, J. *et al.* Androgen receptor expression distribution characteristics in young female breast cancer patients in China: a study of clinicopathological features. *Transl Cancer Res* **14**, 1388-1400, doi:10.21037/tcr-2025-147 (2025).

Figures

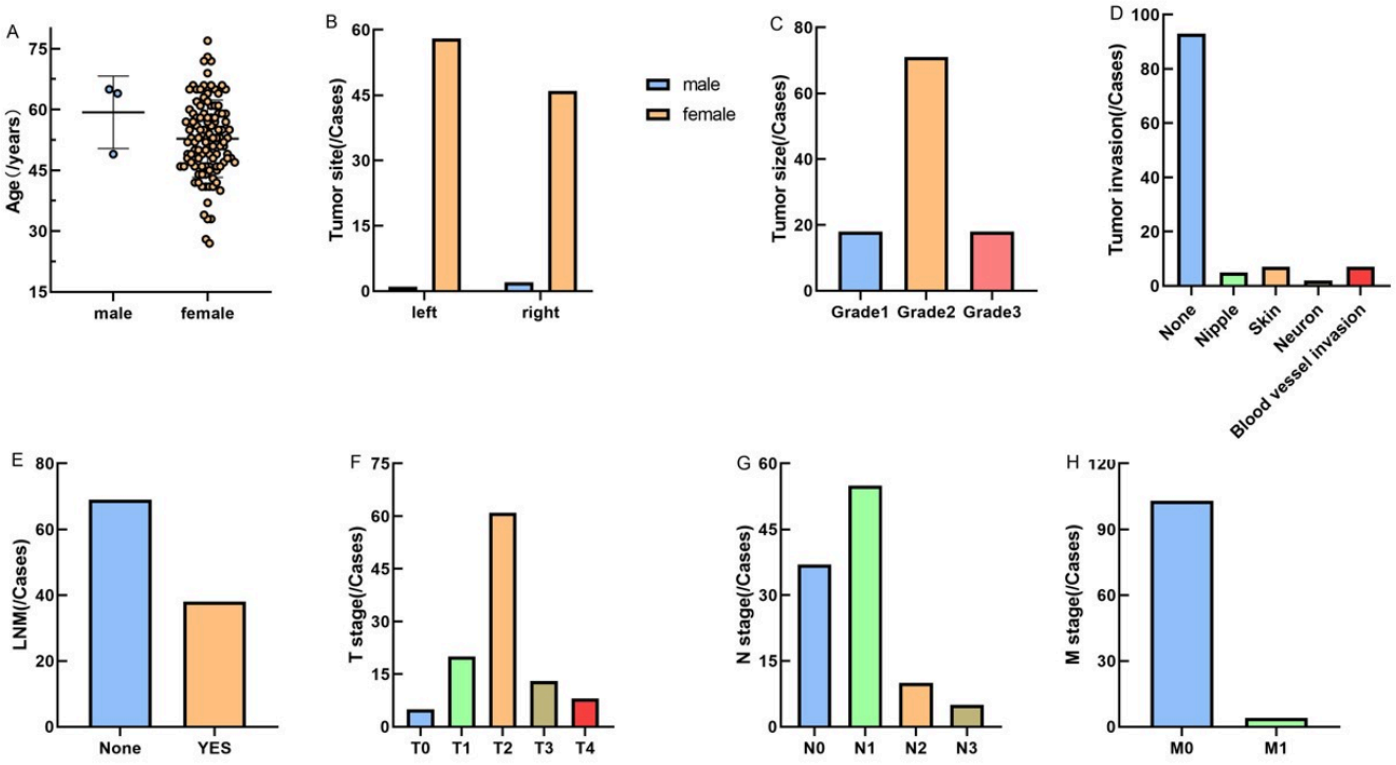


Figure 1

(A) Scatter plot of age and sex distributions. (B) Bar chart of the tumor site. (C) Bar chart of tumor size. (D) Bar chart of tumor invasion. (E) Bar chart of LNM. Bar chart of the T stage (F), N stage (G) and M stage (H) groups.

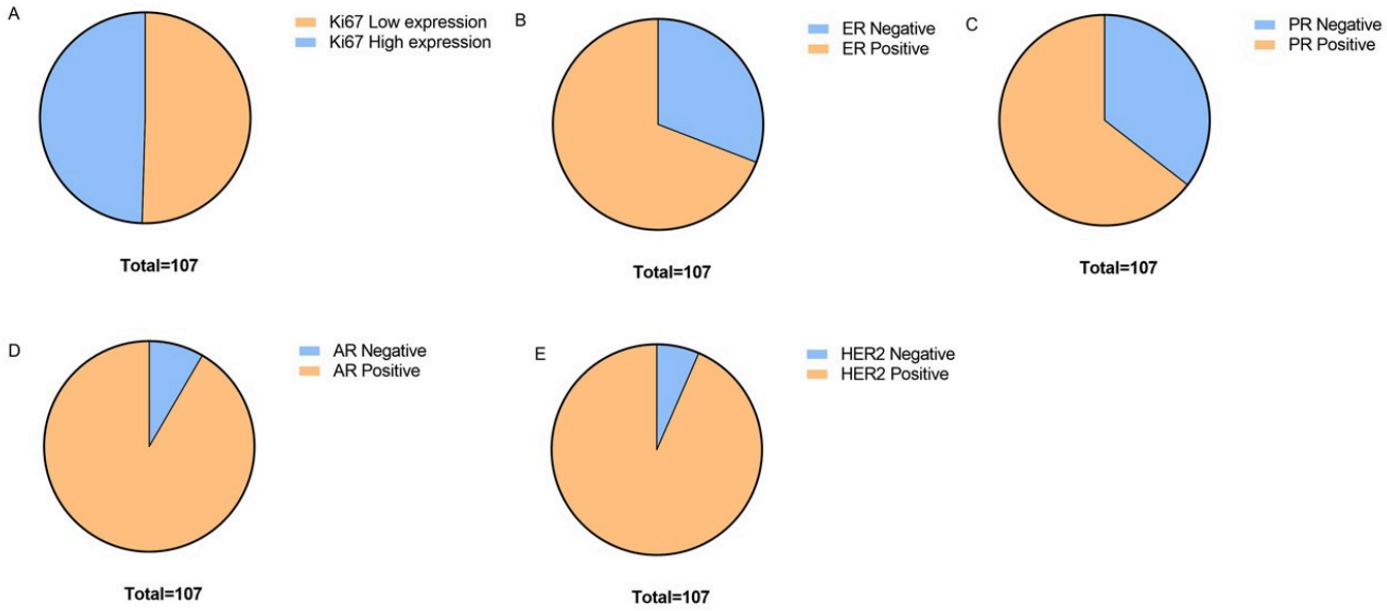


Figure 2

Pie chart of the Ki-67 index (A), ER (B), PR (C), AR (D), and HER-2 (E).

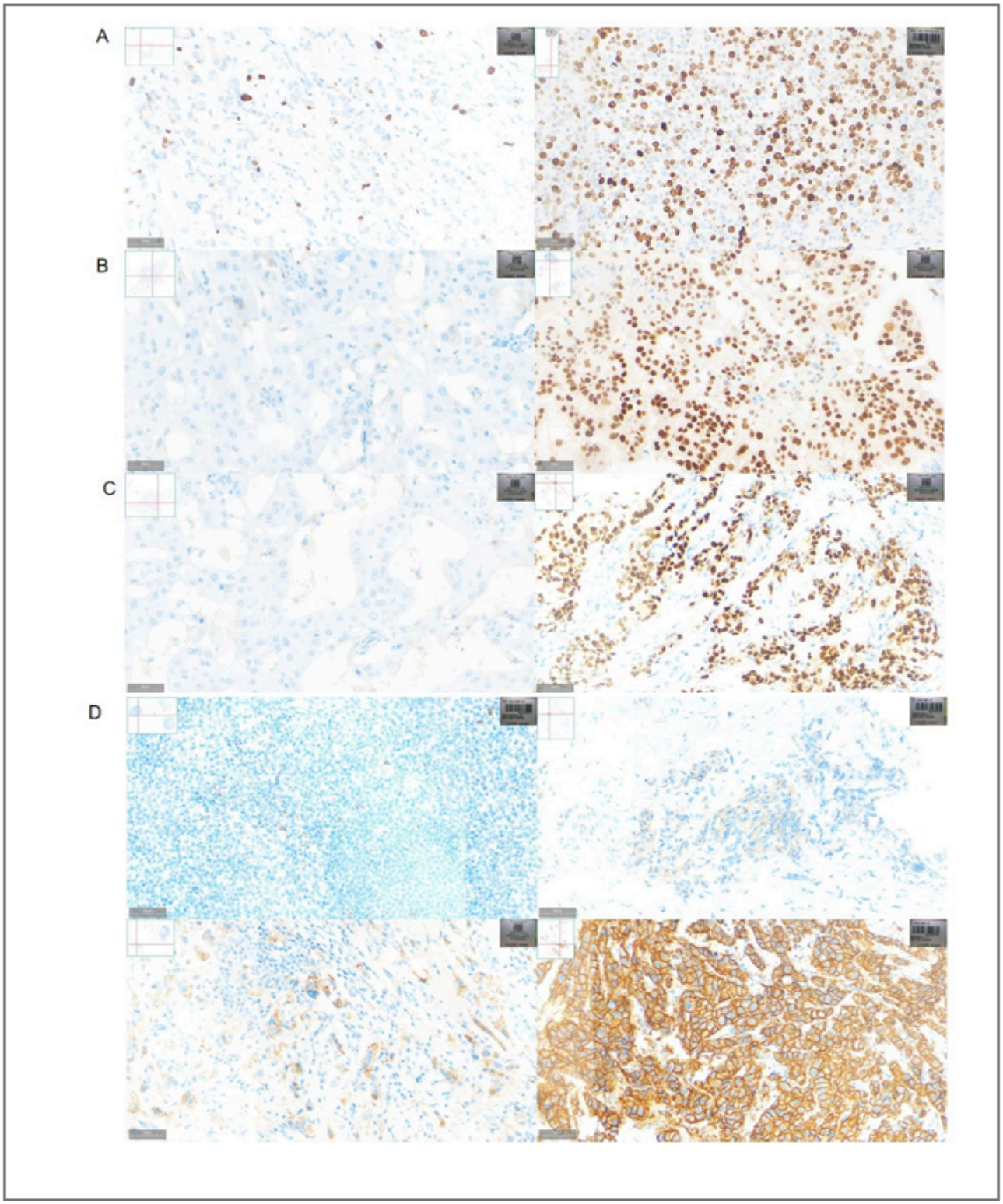


Figure 3

Negative results are shown on the left, and positive results are shown on the right, including the Ki-67 index (A), ER (B), PR (C). (E) From left to right and top to bottom, they are HER-2 from 0, 1+, 2+ to 3+ in sequence.

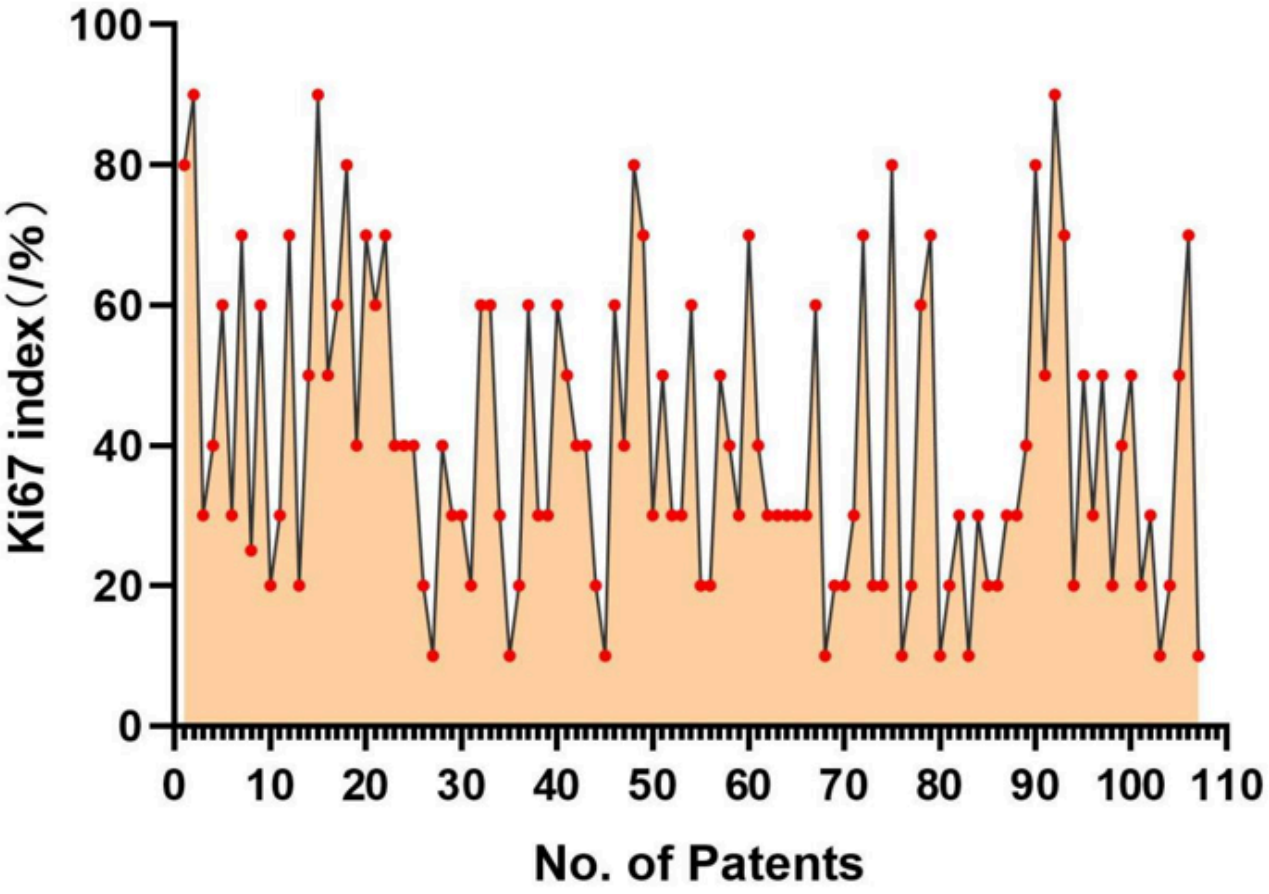


Figure 4

Scatter plot of the Ki-67 index distribution.

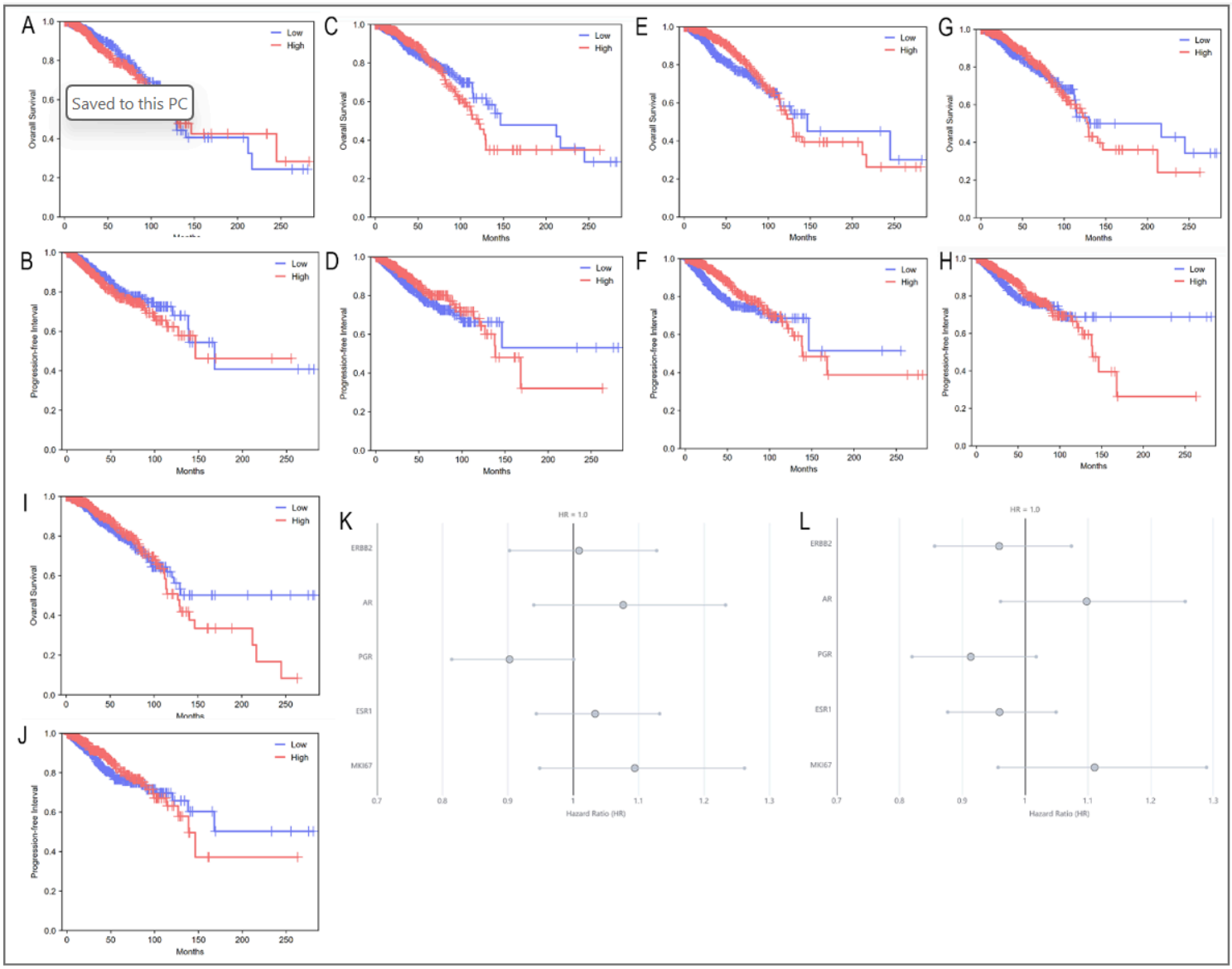


Figure 5

Single-gene and polygene survival analysis. Survival curves of OS and PFS for MKI67 (A, B), ESR1 (C, D), PGR (E, F), AR (G, H) and ERBB2 (I, J). Hazard ratio plots for OS and PFS derived from multigene survival analysis (K, L).