

Ki-67 index changes and their association with PR status changes and tumor invasion: a real-world study of patients with non-pCR breast cancer after neoadjuvant chemotherapy

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Abstract

Introduction: Neoadjuvant chemotherapy (NAC) represents a crucial treatment strategy designed to reduce tumor size and facilitate surgical intervention. In patients who do not achieve a pathological complete response (pCR) following NAC, the alteration of receptor status has been observed, but their clinical implications remain unclear. The aim of this study was to investigate the dynamic changes in immunohistochemical (IHC) expression and their association with clinicopathological features in non-pCR breast cancer patients.

Material and methods: A total of 88 non-pCR breast cancer patients diagnosed by core needle biopsy at Baoji Central Hospital between January 1, 2024, and June 1, 2025, were included. Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and the proliferation marker Ki-67 were assessed in biopsy and radical resection tissues after NAC.

Results: A total of 147 breast cancer patients were retrieved, and 88 patients were included in the study. Among them, there were 3 cases of luminal A type (3.42%), 71 cases of luminal B type (80.68%), 7 cases of HER2-enriched type (7.95%) and 7 cases of triple-negative breast cancer (TNBC) type (7.95%). The overall change rate of ER, PR, HER2, Ki-67 was 6.82%, 15.91%, 22.73% and 40.91%, respectively. Changes in the Ki-67 index change were significantly associated with tumor invasion and PR receptor status changes ($p < 0.001$), but not with ER and HER2 changes ($p = 0.267$, $p = 0.620$).

Conclusions: Ki-67 exhibited the highest alteration rate following NAC in non-pCR breast cancer patients, and its change was significantly associated with aggressive tumor features and PR conversion, suggesting a potential association with tumor biological characteristics.

Key words: receptor status changes, neoadjuvant chemotherapy, immunohistochemistry, HER2, biopsy, molecular biology technology.

Introduction

In the United States, breast cancer is the second leading cause of cancer death among women, with approximately 316,000 new cases annually [1]. In China, breast cancer ranked second among the five common newly diagnosed cancers in 2024, with 369,769 cases [2]. Estrogen-relat-

ed breast cancer remains a serious global health issue, with an increasing impact [3]. Based on immunohistochemical (IHC) results, breast cancer is typically classified into four subtypes: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and triple-negative breast cancer (TNBC) [4]. Luminal A and luminal B differ primarily in Ki-67 and progesterone receptor (PR) expression, with luminal B being more aggressive and more frequently requiring chemotherapy. Luminal A is predominantly managed with endocrine therapy and has limited sensitivity to chemotherapy, whereas luminal B is treated with a combination of endocrine therapy and chemotherapy [5, 6]. The HER2 enrichment subtype is driven by HER2 overexpression or gene amplification with the absence of estrogen receptor (ER) and PR expression [7], and is treated with anti-HER2 targeted therapy in conjunction with chemotherapy. TNBC lacks all three key receptors, precluding both endocrine and anti-HER2 targeted therapies, thereby offering limited treatment options [8]. The exact mechanisms of breast cancer development remain unclear. Current evidence points to the involvement of genetic alterations, such as acquired mutations in the ESR1 gene and changes in HER2 status, including amplification or loss [9, 10]. Epigenetic regulation, encompassing DNA methylation and histone modification, also plays a role [11, 12]. Additionally, transcriptional and post-transcriptional regulation affects mRNA and protein expression [13]. Furthermore, multiple signaling pathways, notably the PI3K/AKT/mTOR pathway and the MAPK/ERK pathway are implicated in disease pathogenesis [14, 15].

Neoadjuvant chemotherapy (NAC) is currently indicated for patients with locally advanced breast cancer or early breast cancer requiring systemic treatment. NAC helps slow tumor progression, reduce tumor size, and enable surgical intervention [16]. Breast cancers with distinct IHC profiles demonstrate different survival outcomes after NAC. HER2-positive tumors have the highest likelihood of achieving a pathological complete response (pCR), whereas TNBC is the subtype for which pCR is most clearly associated with improvements in both overall survival (OS) and progression-free survival (PFS) [17].

Although many patients experience substantial therapeutic benefits from NAC, those who do not achieve pCR may not gain additional survival advantages from further treatment. Furthermore, residual cancer lesions in these patients may carry an increased risk of recurrence [18]. Identifying the pathological characteristics of non-pCR patients and predicting the risk of distant recurrence are essential for more precise stratification and biomarker-guided therapy. This approach represents

a key strategy for evaluating post-NAC outcomes in non-pCR patients [19].

In systemic therapy, significant progress has been made through identifying and targeting lineage-specific and cancer protein-specific targets, facilitating a shift from combination chemotherapy to endocrine and HER2-targeted therapies [20]. Current standard treatments include HER2-targeted agents such as trastuzumab and pertuzumab. Emerging small-molecule inhibitors like entrectinib, which can cross the blood-brain barrier and target TRK, ROS1, and ALK, are used for specific molecular subtypes [21, 22]. Lineage-targeting strategies include endocrine-based therapies, such as anti-estrogen therapies and CDK4/6 inhibitors [23, 24].

The use of advanced molecular biology techniques, including gene editing and nanocarriers, may address key challenges in cancer immunotherapy. These innovations may help overcome current therapeutic limitations by enhancing immune cell function, improving tumor targeting, and modulating the immune microenvironment, and may enable the development of diverse new combination treatment strategies [25, 26]. Together, these therapies have contributed to improvements in breast cancer survival and prognosis, expanding treatment options and precision beyond traditional approaches, and aligning with the principles of modern precision medicine and targeted therapy [27].

Receptor status conversion is observed in a notable proportion of breast cancers between biopsy and postoperative resection tissues, with changes in HR and HER2 status not being uncommon [28]. A study of 481 patients with residual cancer nests showed that 15.8% of patients experienced a change in ER status, and 5.2% showed change in HER2 status [29]. Moreover, some initially HER2-negative tumors may convert to HER2-positive after NAC, whereas some initially HER2-positive may lose HER2 expression, becoming HER2-negative or HER2-low [30]. These receptor status conversions are associated with clinical decision-making and prognosis after NAC. Zhang *et al.* found that among 232 patients with pathologically confirmed residual lesions, 85 cases (36.6%) exhibited changes in molecular subtype, and in 45 of these cases (19.3%), treatment modification was required [31]. A meta-analysis of 15 studies demonstrated that loss of HR in recurrent tumors was significantly correlated with shorter OS compared to tumors retaining HR positivity. Furthermore, ER positivity is considered a key predictive factor of prolonged survival after recurrence [32]. In summary, receptor status conversion after NAC is a common and clinically meaningful biological phenomenon, highlighting the potential impor-

tance of dynamic tumor assessment in treatment planning.

This study aimed to comprehensively investigate the evolution of IHC biomarkers in breast cancer patients who did not achieve pCR following NAC. We sought to elucidate the patterns and dynamics of these biomarker changes and to evaluate their associations with clinicopathological characteristics. The ultimate goal is to provide evidence-based insights for optimizing treatment strategies and improving the characterization of the non-pCR patient population. A graphical abstract is shown in Figure 1.

Material and methods

Study design and population

The retrospective cohort study utilized data obtained from the Department of Pathology at Baoji Central Hospital. Consecutive breast cancer patients who underwent surgery following NAC in Baoji Central Hospital between January 1, 2024, and June 1, 2025, were eligible for inclusion.

The inclusion criteria were as follows: (a) histologically confirmed diagnosis of breast cancer by biopsy; (b) unilateral breast disease; (c) availability of ER, PR, HER2 and Ki-67 status in biopsy tissues and radical resection tissues; and (d) no history of other tumors.

The exclusion criteria were as follows: (a) bilateral breast disease; (b) patients with achievement of pCR, defined as the absence of viable residual cancer cells in the surgically resected specimens, upon microscopic examination. These patients were excluded because no post-treatment residual lesions were available for evaluation; (c) insufficient or exhausted tissue blocks for subsequent IHC staining; and (d) history of other tumors.

This research protocol strictly complies with the ethical principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Baoji Central Hospital (Approval Number: BZYL2026-67) [33]. Of 147 patients initially identified, 88 met the prespecified inclusion and exclusion criteria. A study flow diagram is presented in Figure 2.

Clinical and pathological characteristics

Clinical and pathological data were extracted from the hospital electronic medical record system including gender, age, tumor location, tumor size, TNM staging, tumor invasion, and lymph node metastasis (LNM). Determination of the molecular subtypes of breast cancer was performed according to the 2025 edition of the China Society of Clinical Oncology (CSCO) Guidelines for the Diagnosis and Treatment of Breast Cancer [34].

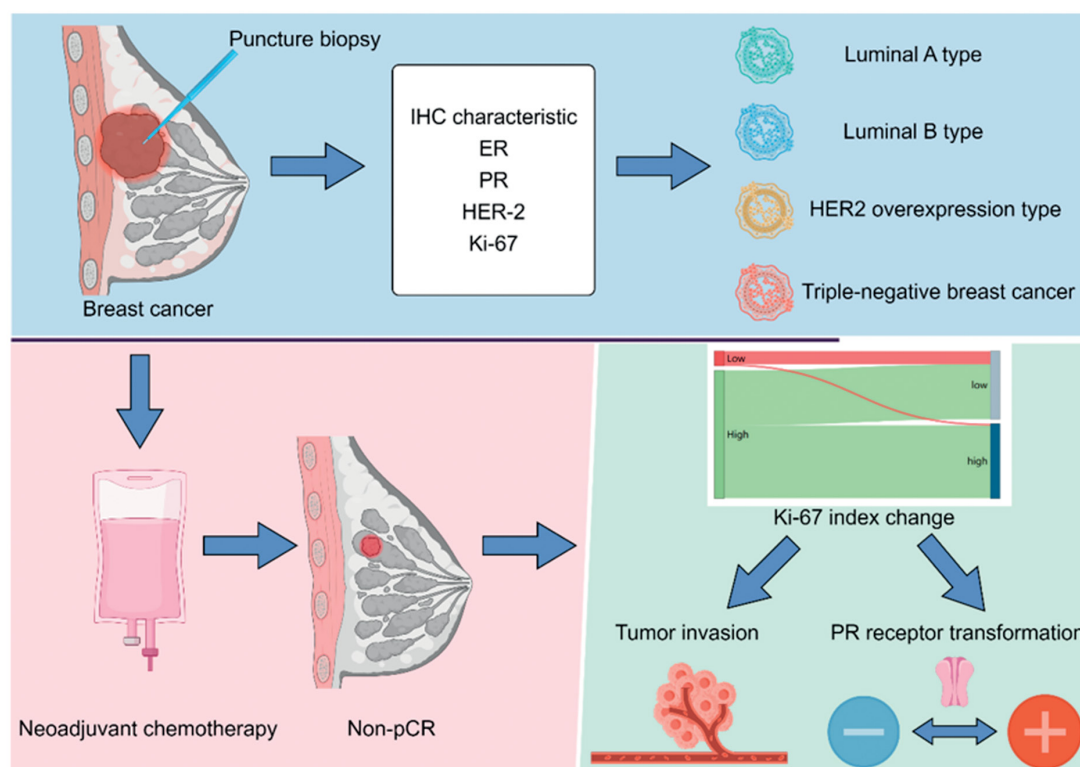


Figure 1. Graphical abstract. Puncture biopsy categorizes breast cancer into four subtypes based on IHC characteristics: luminal A, luminal B, HER2 enriched, and TNBC. In patients who received NAC and exhibited non-pCR, we observed significant associations between changes in the Ki-67 index and tumor invasion, as well as PR status changes

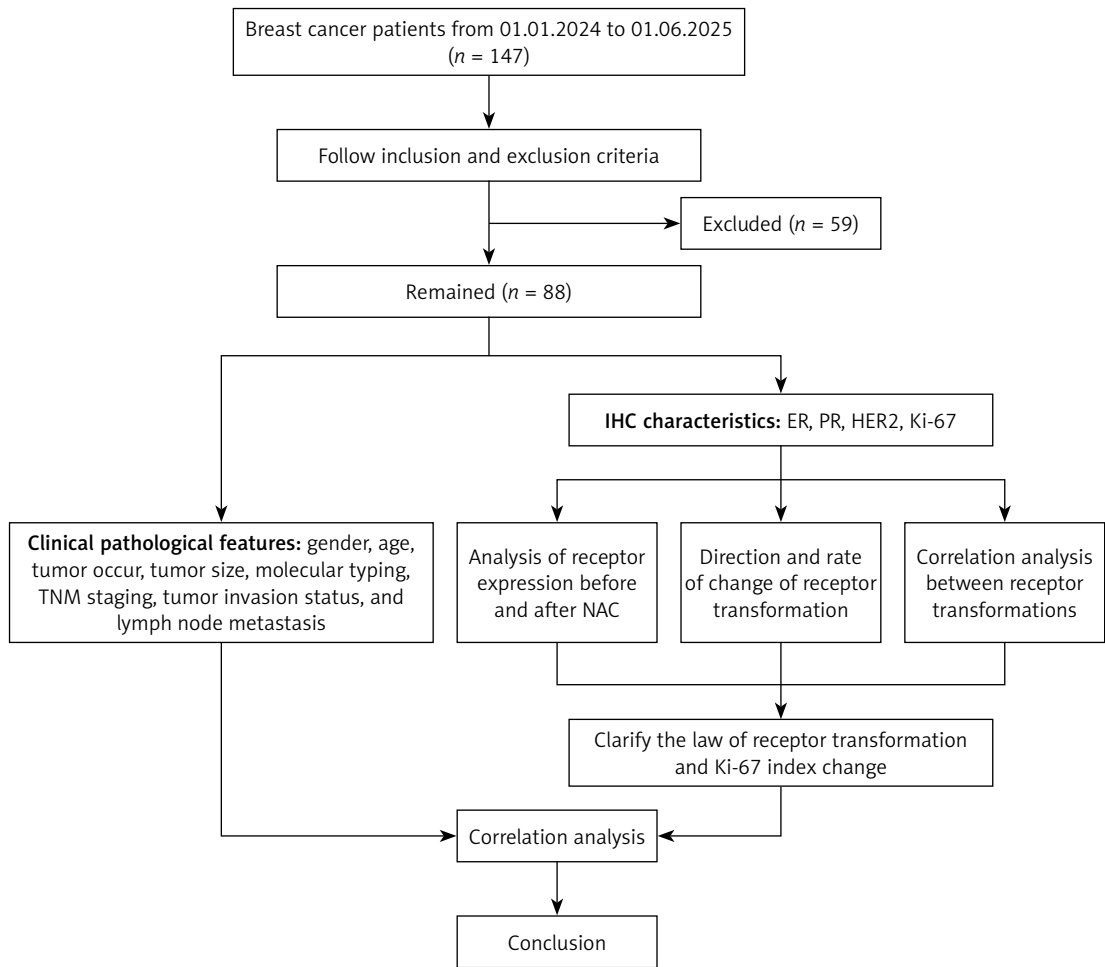


Figure 2. Study design and technical workflow

The subtypes were defined as follows: 1) Luminal A type: ER-positive, PR-positive, HER2-negative, and low Ki-67 (typically < 14%). 2) Luminal B type is subdivided into two categories: 1) ER-positive, PR-positive, HER2-negative, but with high Ki-67 (typically $\geq 14\%$). 2) ER-positive, PR-positive, HER2-positive (regardless of Ki-67 level). 3) HER2 enriched type: ER-negative, PR-negative, and HER2-positive. 4) TNBC: ER-negative, PR-negative, and HER2-negative. The TNM staging system was determined according to the 8th edition guidelines jointly released by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) in 2018 based on histological subtype [35].

Immunohistochemical characteristics

The expression status of ER, PR, HER2, and Ki-67 was collected from preoperative biopsy specimens and compared with corresponding data from postoperative tissue specimens to determine the frequency and type of changes in ER, PR, and HER2. IHC evaluation was performed according to guidelines established by the American Society

of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) in 2023 [36]. ER and PR positivity is defined as the presence of clear positive staining in $\geq 1\%$ of invasive tumor cell nuclei. HER2 evaluation was categorized as follows: 1) IHC 3+: strong, complete membrane staining $\geq 10\%$ of tumor cells; 2) IHC 2+: weak to moderate membrane staining in $\geq 10\%$ of tumor cells; 3) IHC 1+: faint, incomplete membrane staining in any proportion of tumor cells, HER2 negative; 4) IHC 0: no staining or faint staining in $\leq 10\%$ of tumor cells, HER2 negative. According to the China Clinical Oncology Society Guidelines for Breast Cancer Diagnosis and Treatment, a Ki-67 index of 14% serves as the threshold for luminal A and B type breast cancer [37]. All IHC staining results were independently assessed by two pathologists. In cases of disagreement, the final diagnosis was determined by the department director or a senior pathologist.

Tissue processes

All preoperative puncture and postoperative tissue processing and subsequent experiments

were conducted at the Department of Pathology, Baoji Central Hospital. Biopsy tissues and post-operative gross specimens were fixed in paraformaldehyde for 24 hours following acquisition. Gross specimens requiring intraoperative frozen section analysis were promptly submitted for frozen section examination [38]. After completion of the frozen report, the specimens underwent an additional 24 hours of fixation to facilitate subsequent sampling and paraffin embedding. All specimens were thoroughly inspected prior to processing. After confirming the absence of errors, microphotography and detailed documentation were performed. The size, shape, color, texture, and other characteristics of both the tissue and the lesion were meticulously recorded. Samples were collected from the confirmed tumor tissue. In cases where the lesion area was difficult to identify after NAC, extensive sampling was performed based on imaging-guided localization. Puncture tissues were wrapped and folded in embedding paper, and placed in an embedding cassette to prevent loss of the specimen. Gross specimens were sectioned into tissue blocks of approximately 2 × 1.5 × 0.2 cm and stored in embedding cassettes [39]. Tissue dehydration was performed using an automated tissue dehydration instrument (Thermo Fisher Scientific, USA, model: STP420), followed by automatic tissue embedding (Hubei Xiaogan Hongye Medical Instrument Co., Ltd, China, model: BMV III). Dehydrated tissues were removed from the embedding cassettes and embedded in paraffin. All puncture tissues were flattened, neatly arranged, and positioned on the same plane, with gaps of approximately 2–3 mm between tissue blocks to prevent overcrowding. Tissue blocks from the gross specimens were embedded vertically, with the orientation perpendicular to the skin surface. This arrangement enabled simultaneous observation of the full-layer structure, from the surface to the deeper regions and from the tumor center to the periphery in the resulting sections [40]. After embedding, paraffin sections were prepared using a rotary microtome (Leica, Germany, model: RM2235) and dried completely using a constant-temperature slide oven (Hubei Weitai Technology Industrial Co., Ltd., China, model: TK-218V) until complete drying.

Hematoxylin and eosin and immunohistochemical staining

Hematoxylin and eosin (HE) staining was performed using a dyeing and mounting system (Sakura Seiki Co., Ltd., Japan, model: DRS-Prisma-P-JCS). Tissue sections were first fixed to preserve their structural integrity. Hematoxylin staining is then applied to stain the cell nuclei blue. Differ-

entiation was performed to remove hematoxylin from the cytoplasm and extracellular matrix, enhancing nuclear clarity. Finally, sections were counter stained with eosin, rendering cytoplasm and other cellular components red [41]. IHC staining was performed on an automated HIS staining platform (Roche Diagnostics, Germany, model: BenchMark ULTRA PLUS). The following primary antibodies were used: ER (Roche Diagnostics, Germany, product number: M29468), PR (Roche Diagnostics, Germany, product number: M27931), Ki-67 antibody (Roche Diagnostics, Germany, product number: N06786), HER2 (MaiXin Biotechnology Development Co., Ltd., China, product number: 2505280701d). Corresponding secondary antibodies on an automated immunohistochemistry staining platform were applied according to the manufacturer's protocols. Prepared tissue slides were placed into the instrument's slide holder, and the required primary and secondary antibodies were loaded into their designated positions. The instrument automatically performed the following sequential steps: deparaffinization and rehydration, followed by antigen retrieval. Endogenous peroxidase activity was then blocked using hydrogen peroxide solution to reduce background staining. Subsequently, the primary antibodies were automatically dispensed and incubated under temperature-controlled conditions. After completion of the primary incubation, the labeled secondary antibody was automatically added to bind the corresponding primary antibody. Following this, the diaminobenzidine substrate solution was added, and the enzyme catalyzes the substrate to form a brown insoluble precipitate that localizes the target antigen. The cell nuclei were counterstained with hematoxylin. Non-specific nuclear staining was removed using a differentiation solution, and a blue solution enhanced nuclear clarity. After staining, sections were dehydrated through a graded ethanol series, cleared with xylene, and sealed with neutral mounting medium [42]. Tissue sections were scanned using a digital section scanning system (Winmedic, China, model: Science). After conducting a panoramic scan of the slices to determine the target area, a 40× magnified image is taken to clearly display the staining results.

Statistical analysis

Statistical analysis was performed using SPSS version 25.0 (International Business Machines Corporation, USA). Graphical representations were generated using GraphPad Prism 10.0 (American GraphPad Software Company, American) and Statistical Platform for Life Sciences (Yanchang Technology Company, China). Associations between clinicopathological features and receptor status

changes were analyzed. Categorical data were described using frequencies and counts, while quantitative data were presented as means with standard deviations. For categorical variables, χ^2 tests, continuity-corrected χ^2 tests, or Fisher's exact test

were applied based on the type of data, expected frequency, and the total number of cases. The expected frequency for a given cell was calculated as: (the total of the row corresponding to the cell $\times$ the total of the column corresponding to the cell)/total sample size. For a 2×2 contingency table, the standard χ^2 test was used when all expected frequencies were ≥ 5 , and the continuity-corrected χ^2 test was applied when one expected frequency fell within the range of $1 \leq T < 5$ and the total sample size was ≥ 40 . Conversely, Fisher's exact test was employed when any expected frequency was ≤ 1 or the total sample size < 40 . For an $R \times C$ contingency table, the χ^2 test was appropriate when all expected frequencies were ≥ 5 . However, Fisher's exact test was used when more than 20% of the cells had expected frequencies ≤ 5 , or when any expected frequency is < 1 . The significance level for two-tailed tests was set at $\alpha = 0.05$ [43].

Results

Clinical pathological features

A total of 88 patients were included in this study. Among them, 34 cases (38.64%) were younger than 50 years old, and 54 cases (61.36%) were aged 50 years or older. The cohort included 85 women (96.59%). Tumors were located on the left side in 49 cases (55.68%) and on the right side in 39 cases (44.32%). The most frequent tumor size was grade 2, accounting for 61.36% of cases. Luminal B type was predominant, observed in 71 cases (80.68%). Tumor embolus (11.36%) and skin involvement (6.82%) were the most common sites of local extension. LNM was found in 50 cases (56.82%) and absent in 38 cases (43.18%). The most frequent TNM stages were T2 (59.09%), N1 (56.82%), and M1 (96.59%). A summary of clinical pathological features of the 88 patients is present in Table I and Figure 3.

Immunohistochemical characteristics

The expression levels of ER, PR, HER2 and Ki-67 were analyzed in paired biopsy and radical resection tissues from 88 patients. Following NAC treatment, the number of patients with negative ER, PR, and HER2 status increased slightly, while the number of positive patients decreased modestly. Additionally, the Ki-67 index decreased below the established threshold, resulting in a decrease in the number of individuals with high Ki-67 expression. Detailed results are provided in Table II and Figure 4. A representative image of IHC is shown in Figure 5.

Changes of Immunohistochemical characteristics

The changes in individual markers (ER, PR, HER2, and Ki-67) alone do not fully capture the

Table I. Clinical pathological features ($n = 88$)

Characteristic	Cases	Percentage (%)
Age		
< 50 years	34	38.64
≥ 50 years	54	61.36
Gender		
Male	3	3.41
Female	85	96.59
Tumor location		
Left	49	55.68
Right	39	44.32
Tumor size		
Grade 1 (≤ 2 cm)	17	19.32
Grade 2 (2 cm $< x \leq 5$ cm)	54	61.36
Grade 3 (> 5 cm)	17	19.32
Molecular typing		
Luminal A	3	3.42
Luminal B	71	80.68
HER2 enriched	7	7.95
TNBC	7	7.95
Tumor invasion		
None	71	80.68
Nipple	5	5.68
Skin	6	6.82
Tumor embolus	10	11.36
Neuron	2	2.27
LNM		
Yes	50	56.82
None	38	43.18
T stage		
0	1	1.13
1	14	15.91
2	52	59.09
3	12	13.64
4	9	10.23
N stage		
0	22	25.00
1	50	56.82
2	8	9.09
3	8	9.09
M stage		
0	3	3.41
1	85	96.59

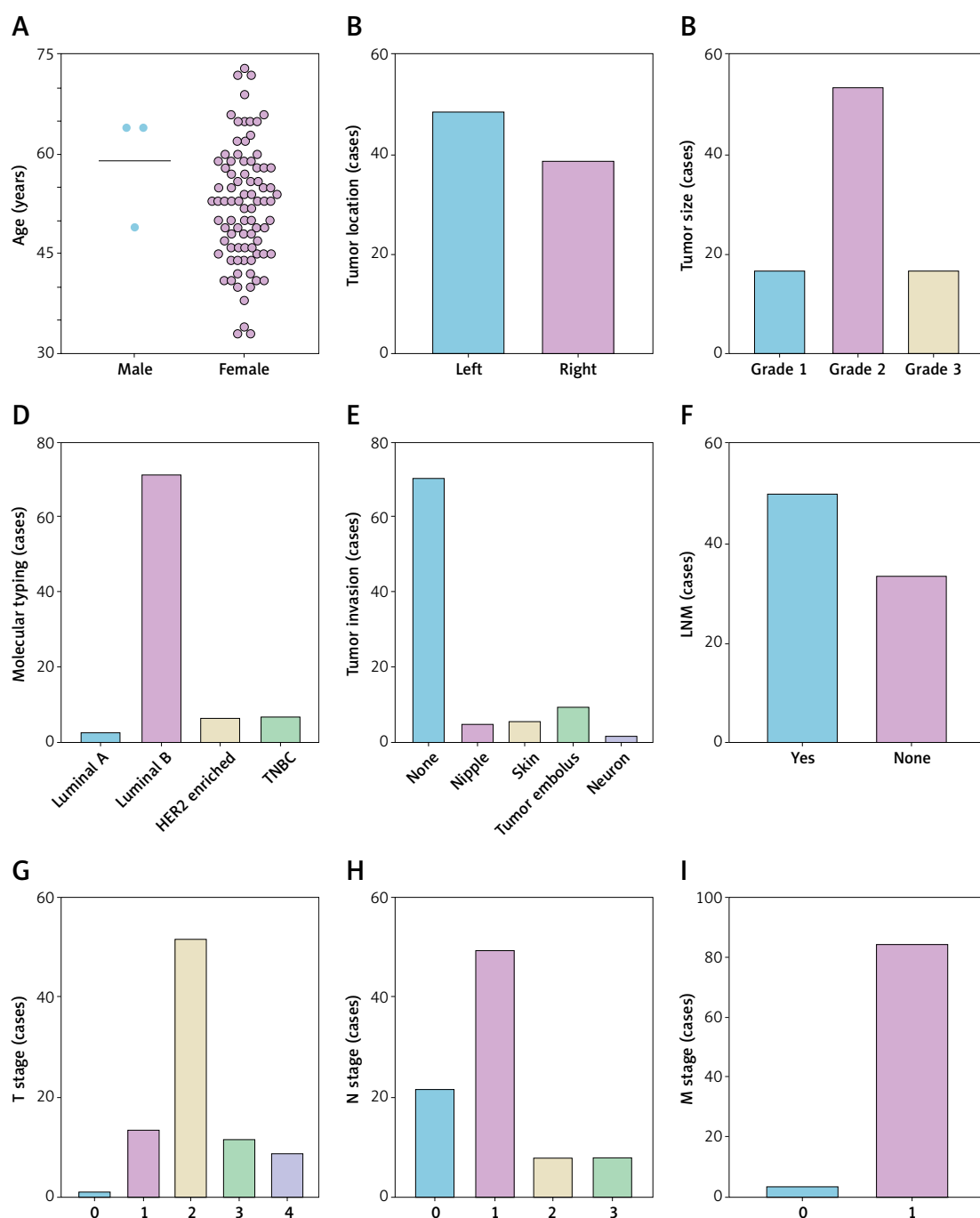


Figure 3. Clinicopathological characteristics of 88 patients with breast cancer. **A** – Age distribution by gender. **B** – Tumor location. **C** – Tumor size. **D** – Molecular typing. **E** – Tumor invasion. **F** – LNM. **G** – T stage. **H** – N stage. **I** – M stage

complexity of receptor status alterations in each patient. Therefore, a more detailed assessment of the specific IHC characteristics was performed. In the ER cohort, 2 cases transitioned from negative to positive, and 4 cases shifted from positive to negative, yielding a total change rate of 6.82%. For PR, 6 cases changed from negative to positive, and 8 cases changed from positive to negative, resulting in an overall change rate of 15.91%. For HER, 8

cases transitioned from negative to positive, and 12 changed from positive to negative, leading to an overall change rate of 22.73%. For Ki-67, 1 case changed from low expression to high expression, whereas 34 cases shifted from high expression to low expression, resulting in the highest overall change rate of 40.91%. Detailed data on the changes of IHC characteristics are shown in Table III and Figure 6.

Association between Ki-67 and clinical pathological features

Based on the variations in the Ki-67 index observed in puncture biopsy and radical resection specimens, the patients were categorized into

Table II. Immunohistochemical characteristics ($n = 88$)

Indicators	Tissue source	
	Puncture biopsy	Radical resection
ER		
Negative	17	20
Positive	71	68
PR		
Negative	20	23
Positive	68	65
HER2		
Negative	29	34
Positive	59	54
Ki-67 index		
Low expression ($\leq 14\%$)	9	42
High expression ($> 14\%$)	79	46

consistent and inconsistent groups. The association between Ki-67 status changes and various clinicopathological characteristics was subsequently analyzed. A significant association was observed between Ki-67 index change and tumor invasiveness ($p < 0.001$, Table IV).

Association between Ki-67 index change and other receptor changes

Given the high conversion rate of Ki-67, its association with other receptor changes was examined. A significant association was found between Ki-67 index change and PR status changes ($p = 0.040$), while no significant associations were observed for ER ($p = 0.267$) and HER2 status changes ($p = 0.620$). Detailed results are provided in Table V.

Discussion

Breast cancer is classified based on its molecular expression profiles into four main subtypes: luminal A, luminal B, HER2 enriched, and TNBC [44]. BRCA1 and BRCA2 are critical tumor suppressor genes located in the nucleus, playing pivotal roles in DNA repair, cell cycle control, protein ubiquitination, and chromatin modification. Loss of function of these genes impairs tumor cell growth, leading

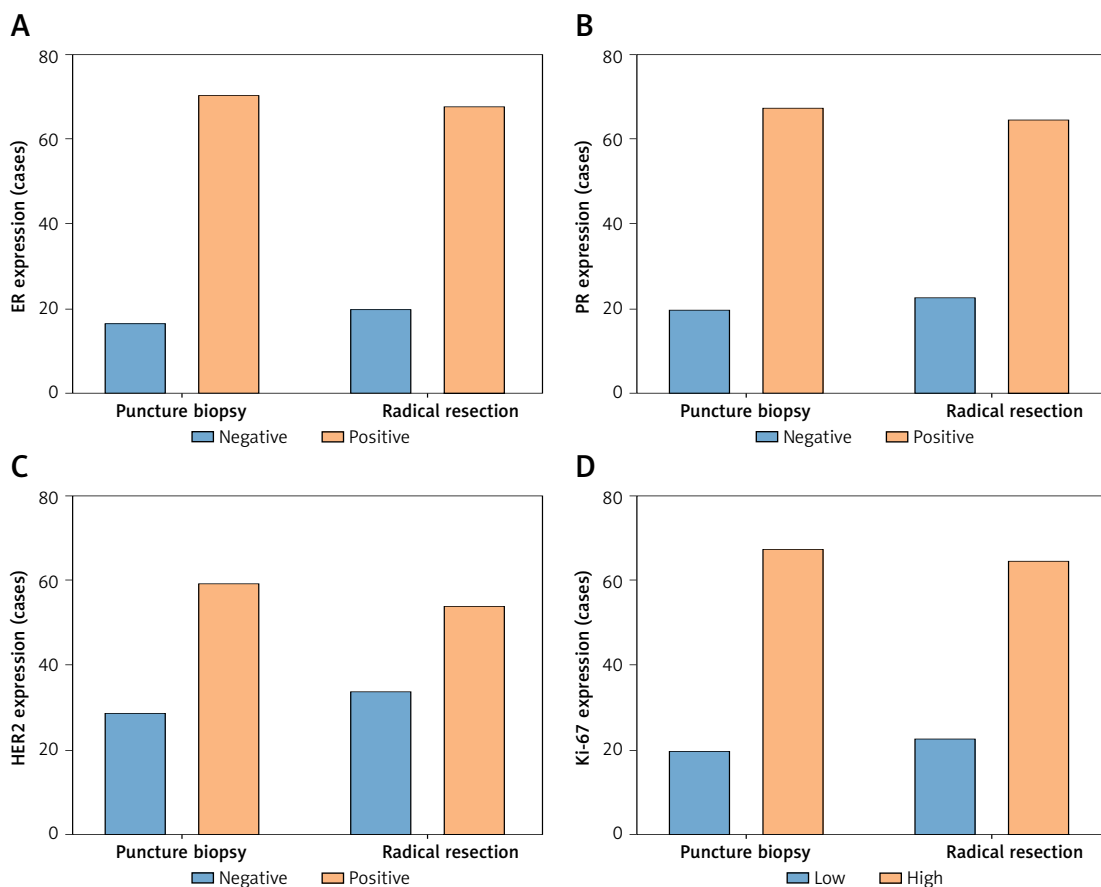


Figure 4. Immunohistochemical characteristics of puncture biopsy and radical resection tissues in breast cancer patients. Bar chart of ER expression (A), PR expression (B), HER2 expression (C), and Ki-67 expression (D)

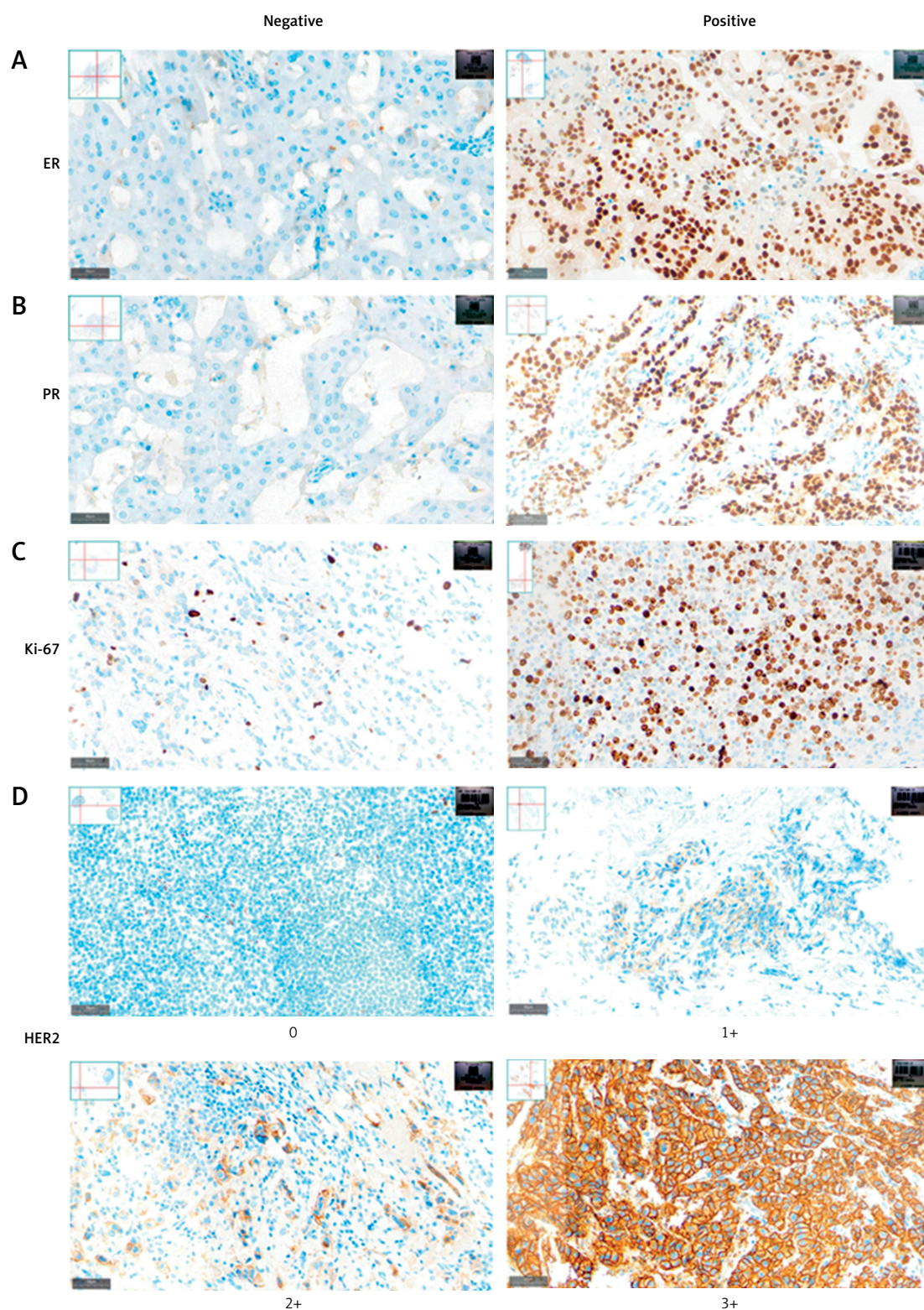


Figure 5. Representative image of IHC characteristics. **A** – ER; **B** – PR; **C** – Ki-67 index; **D** – HER2. After a panoramic scan of the slice, an image with a magnification of 40× was captured, and the scale is shown in the figure

to extensive genomic instability, thereby fostering tumorigenesis and heightening susceptibility to breast cancer [45]. Chronic estrogen exposure can alter the local microenvironment by upregulating growth factors and inflammatory mediators, pro-

moting genomic instability and malignant status changes [46].

Approximately 70% of breast cancer patients have hormone-dependent disease, with their tumor cells expressing ER [47]. Cellular respons-

Table III. Change of IHC characteristics (n = 88)

Indicators	Cases	Change rate (%)
ER		6.82
Negative-negative	16	
Negative-positive	2	
Positive-negative	4	
Positive-positive	66	
PR		15.91
Negative-negative	15	
Negative-positive	6	
Positive-negative	8	
Positive-positive	59	
HER2		22.73
Negative-negative	22	
Negative-positive	8	
Positive-negative	12	
Positive-positive	46	
Ki-67 index		40.91
Low-low expression	8	
Low-high expression	1	
High-low expression	35	
High-high expression	44	

es to ER signaling primarily involve nuclear ER α , ER β , and membrane-bound G protein-coupled ER (GPER, or GPR30) [48]. Among these receptors, ER α plays a pivotal role in breast cancer progression by acting as a transcription factor that modulates the expression of genes associated with cell cycle regulation, proliferation, and apoptosis [49]. As treatment advances, many ER-positive tumor cells may acquire resistance, primarily attributed to hormone evasion, often accompanied with the loss of epithelial characteristics and increased migratory and invasive properties [50]. Hormonal therapy is the primary treatment for ER-positive breast cancer patients, aiming to inhibit estrogen supply or ER α function [51]. Tamoxifen, a well-studied and widely utilized selective ER modulator, stands out as the preferred choice [52]. It may also lower the blood pressure and blood glucose levels in breast cancer patients, exerting a protective role in cardiovascular function [53]. The introduction of aromatase inhibitors such as letrozole, anastrozole, or exemestane effectively hinders the conversion of AR to ER by inhibiting aromatase activity. This action significantly diminishes circulating estrogen levels, thereby impeding the estrogen-dependent proliferation of breast cancer cells [54]. Studies have shown that aromatase inhibitors reduce breast cancer recurrence and improve 10-year survival compared with tamoxifen [55].

PR preparations are primarily used for contraception and hormone therapy. Over time, com-

bined hormone preparations (ER and PR) have gradually been replaced by single-agent PR only drugs [56]. Fitzpatrick *et al.* studied nearly 10,000 women under the age of 50 in the United Kingdom and showed that taking combined contraceptives, or PR contraceptives, increased breast cancer risk, with no substantial difference between the types [57]. Tamoxifen did not confer benefits to patients with ER-/PR+ breast cancer. Gene expression profiles in ER-/PR+ breast cancer patients exhibited a positive association with those of ER-/PR- tumors, resembling TNBC [58]. Notably, the upregulation of inflammation-related genes such as USP1, CDC20, and CASP1 was prominent in TNBC. Conversely, the absence of ER α in HR-positive cell lines rendered them unresponsive to tamoxifen treatment but notably susceptible to the inflammation inhibitor Ac-YVAD-CHO, which specifically impeded tumor proliferation and migration [59].

HER2, a tyrosine kinase receptor encoded by the *erbB2* gene, is a member of the epidermal growth factor receptor (EGFR) family [60]. Its activation occurs through ligand-independent dimerization with other EGFR family members. This activation leads to the initiation of intracellular tyrosine kinase activity, which in turn triggers downstream signaling pathways including PI3K/Akt/mTOR and Ras/Raf/MEK/MAPK [61]. These signaling pathways play crucial roles in regulating cellular processes such as proliferation, migration, invasion, anti-apoptotic activities, and angiogenesis [62]. Trastuzumab, the first monoclonal antibody approved by the FDA for treating HER2-positive breast cancer, functions by impeding the PI3K/Akt/mTOR pathway [63], which is crucial for cell proliferation and survival, and is pivotal in hormone escape mechanisms. Clinical trials have explored antibody-drug conjugation using trastuzumab derivatives, including trastuzumab deruxtecan and trastuzumab emtansine, demonstrating notable extensions in PFS and OS among patients [64].

Ki-67 was developed as a monoclonal antibody from mice in 1983 and recognizes a nuclear antigen present in proliferating cells but absent in quiescent cells. Since its introduction, Ki-67 has been extensively utilized to evaluate the proportion of proliferating cells in tumors [65]. For patients with high-risk luminal B type tumors, characterized by an elevated Ki-67 index ($\geq 30\%$), NAC effectively reduces invasive biological features and tumor burden [66]. This finding is consistent with the population and conclusions of this study. The Ki-67 index can be used as an independent predictor of axillary pCR in patients with HER2-positive breast cancer [67]. In the multivariate model of TNBC patients stratified by the use of capecitabine, Ki-67 remained the only independent prognostic factor slightly associated with the aggrava-

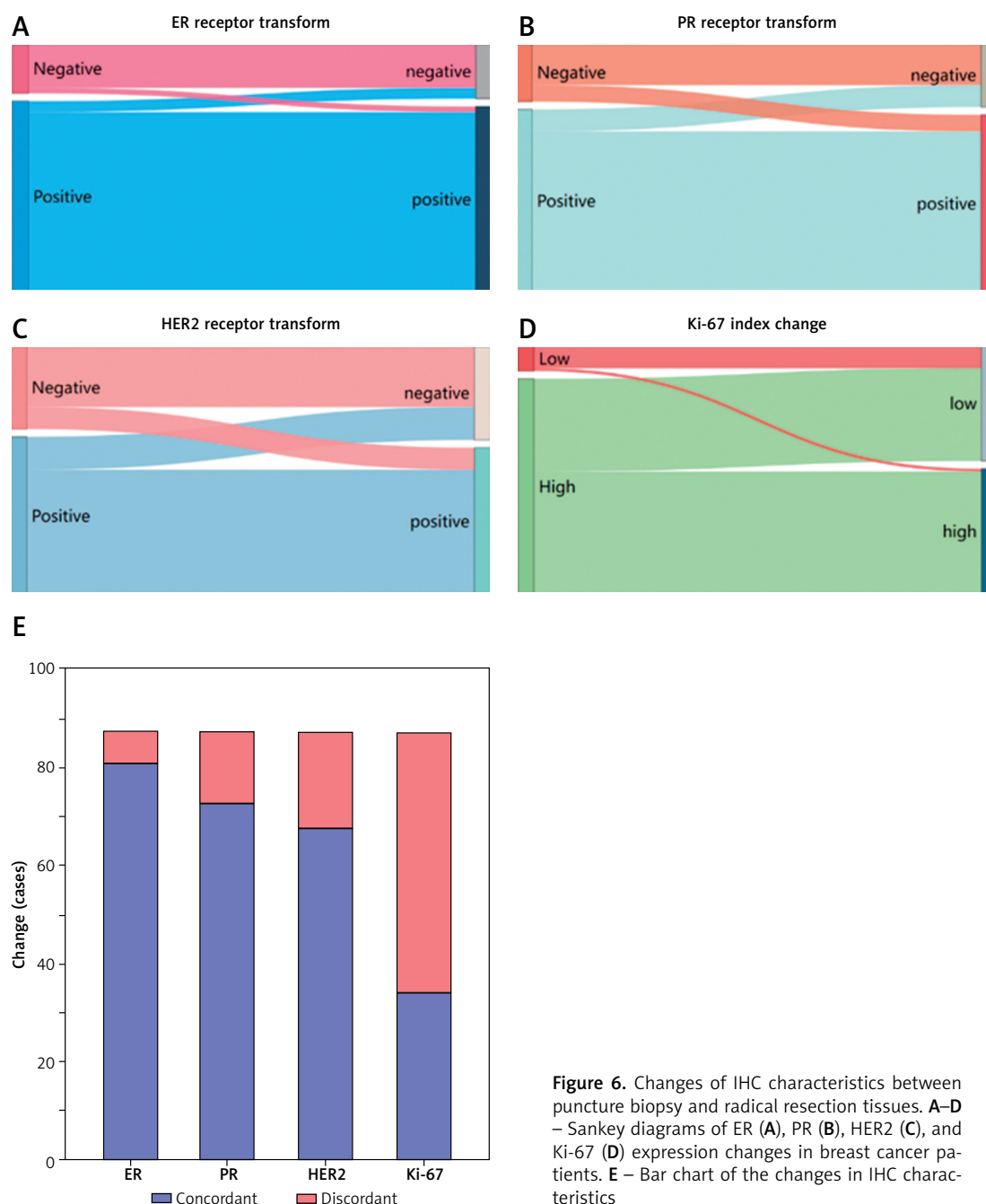


Figure 6. Changes of IHC characteristics between puncture biopsy and radical resection tissues. **A–D** – Sankey diagrams of ER (**A**), PR (**B**), HER2 (**C**), and Ki-67 (**D**) expression changes in breast cancer patients. **E** – Bar chart of the changes in IHC characteristics

tion of DFS [68]. Deep learning has the potential to predict the Ki-67 index from histopathological images of breast cancer tissues. Nevertheless, its clinical application remains constrained by challenges in interpretation, validation, and the integration into pathological workflows, indicating that further development is necessary [69].

The heterogeneity of breast cancer tumors indicates that multiple distinct subtypes may co-exist within a single solid tumor, driven by both intrinsic effects of tumor cells themselves and extrinsic effects from the surrounding TME [70]. The molecular characteristics of breast cancer subtypes are determined by IHC. However, these

subtypes are not static or stationary during NAC, but rather may undergo changes in subtype classification throughout the treatment process [71]. A retrospective cohort study conducted by Antonini M included 369 patients who received NAC treatment but did not achieve pCR. IHC changes were noted in 41.7% of these patients. The DFS and OS of patients exhibiting IHC changes significantly declined, with the poorest outcomes observed in those who converted to HR/HER2-status. These findings underscore the importance of monitoring IHC changes to inform personalized treatment strategies and enhance prognosis assessment [72]. Lu *et al.* conducted a review of

2,417 non-PCR patients who received NAC and found that 14.15% exhibited changes in their biomarker profiles. The change rate was highest among HER2-positive patients following NAC. The prognosis for HR-positive patients who converted

to TNBC or HR-/HER2+ subtypes is significantly poorer. In contrast, TNBC patients with upregulated hormone receptors showed improved outcomes. These findings highlight the influence of subtype conversion on long-term prognoses and

Table IV. Association between Ki-67 index change and clinical pathological features ($n = 88$)

Characteristic	Ki-67		χ^2	p
	Concordant	Discordant		
Age			3.318	0.069
< 50 years	36	18		
≥ 50 years	16	18		
Gender			–	0.266
Male	3	0		
Female	49	36		
Tumor location			0.797	0.372
Left	31	18		
Right	21	18		
Tumor size				
Grade 1 (≤ 2 cm)	11	7	0.171	0.918
Grade 2 (2 cm < $x \leq 5$ cm)	31	23		
Grade 3 (> 5 cm)	10	6		
Molecular typing			–	0.162
Luminal A	3	0		
Luminal B	38	33		
HER2 enriched	6	1		
TNBC	5	2		
Tumor invasion			–	< 0.001
None	36	35		
Nipple	5	0		
Skin	6	0		
Tumor embolus	10	0		
Neuron	1	1		
LNM			1.154	0.283
Yes	32	18		
None	20	18		
T stage			–	0.068
0	0	1		
1	6	8		
2	33	19		
3	5	7		
4	8	1		
N stage			–	0.532
0	15	7		
1	26	24		
2	5	2		
3	6	3		
M stage			–	0.267
0	49	36		
1	3	0		

Table V. Association between Ki-67 index change and other receptor status changes ($n = 88$)

Indicators	Ki-67		χ^2	p
	concordant	discordant		
ER			–	0.267
Concordant	49	36		
Discordant	3	0		
PR			–	0.040
Concordant	51	31		
Discordant	1	5		
HER2			0.179	0.672
Concordant	41	27		
Discordant	11	9		

offer theoretical support for molecularly stratified therapy [73].

The patient study revealed alterations of 6.82% in ER, 15.91% in PR, 22.73% in HER2, and 40.91% in Ki-67 expression levels. Notably, Ki-67 expression exhibited a decreasing trend from high to low levels. Furthermore, a strong association was observed between Ki-67 receptor status changes and both tumor invasion and PR receptor status changes. In a study examining receptor conversion undergoing NAC in 21,755 patients from the Japanese Breast Cancer Registry, the following changes were observed: 4.6% of patients experienced a shift from ER-positive to ER-negative status, 9.3% shifted from ER-negative to ER-positive, 21.4% shifted from HER2-positive to HER2-negative status, and 3.4% shifted from HER2-negative to HER2-positive [74]. Candás *et al.* reported that post-NAC variability was 8.90% for ER, 29.9% for PR, and 13.8% for HER2 [75]. These findings are consistent with the trends observed in the present study, suggesting that this study is representative and reflects the direction of receptor changes in breast cancer patients undergoing NAC.

It has long been established that a high Ki-67 score is associated with tumor invasion-related parameters, such as large tumor size, high histological grade, and lack of steroid hormone receptor [76]. A more favorable response to NAC has been observed in tumors with a Ki-67 index above 19% [77]. A lower Ki-67 index after NAC is associated with better specific survival. Furthermore, the survival rate of low-risk patients with residual lesions and at least 48% reduction of Ki-67 after NAC is equivalent to that of pCR patients [78]. A study by Ono *et al.* reported that when the Ki-67 cutoff value is set at 20%, the expression of PR is negatively associated with the Ki-67 index regardless of the cutoff level [79]. This is also consistent with our findings.

The clinical significance of IHC characteristics is mainly reflected in two aspects. First, it guides subsequent treatment of NAC based on the dy-

namic changes in receptor status undergoing NAC. Second, it enables the prediction of patients' survival outcomes by assessing the IHC characteristics. Gong first observed changes in HER2 receptor status during NAC in breast cancer in 2005 [80]. With advances in oncology and pathology, it is now recognized that receptor conversion during NAC is not coincidental but rather associated with baseline receptor status. Long *et al.* documented that NAC reduced the expression of ER, PR, and Ki-67 in ER+/HER2– patients. Moreover, the loss of PR was notably greater than that of ER, which was significantly associated with lower baseline PR expression [81]. Rey-Vargas *et al.* also found that PR and Ki-67 were altered after NAC, but not in patients who did not receive NAC [82]. A recent retrospective multicenter study showed that in ER-positive patients, PR and HER2 status significantly changed from positive to negative after NAC, supporting the need for repeat testing before and after treatment [83]. Vemuru *et al.* conducted a follow-up receptor expression analysis of 240 patients with residual lesions after NAC, of whom 126 (52.5%) underwent repeat receptor testing. After NAC, 37 cases (29%) exhibited receptor status changes. These changes prompted modifications in adjuvant therapy for 8 patients (6%), and necessitated further screening for 16 cases [84]. Sung *et al.* proposed reassessing the HER2 status in surgically excised specimens after NAC to evaluate the prognostic implications of HER2 status changes and the potential benefits of antibody-drug conjugates for patients [85].

Numerous studies have examined the impact of IHC characteristics on survival and prognosis. It is widely accepted that gaining HR expression is associated with a more favorable prognosis. HR positivity may improve prognosis by increasing sensitivity to endocrine therapy and promoting tumor differentiation. Conversely, the acquisition or loss of HER2 expression does not show a clear association with prognosis, possibly due to the genomic instability of tumors. Li *et al.* reported that

DFS and OS were shorter in patients with negative HR status than in patients with unchanged HR. Patients who transitioned from HR negative to positive or remained HR negative demonstrated improved outcomes with DFS and OS. Additionally, patients who converted to HER2 positive status exhibited better DFS compared to those who remained HER2 positive [86]. Monisha's research demonstrated that patients with receptor status changes after NAC had better OS and DFS compared to those without changes in receptor status, with cases where ER and HER2 status changed from positive to negative showing longer DFS and OS [87]. Dowling's meta-analysis incorporating 22 studies suggested that HR significantly improved DFS, while HR loss was associated with significant deterioration of DFS and OS [88].

This study focused solely on patients who underwent repeat marker testing both before and after surgery, under the condition that they did not achieve a pCR, indicating the presence of residual lesions. The cohort includes a relatively small number of luminal A type breast cancer patients, which may indicate that luminal A type tumors exhibit reduced sensitivity to NAC. Consequently, these tumors are more frequently managed through pre-endocrine therapy or surgery instead of NAC. As a result, fewer patients with luminal A tumors participated in the non-PCR pathway of NAC examined in this study.

In addition, this study ensured that patients received comparable treatment in the same period. For example, National Comprehensive Cancer Network (NCCN) 2024 guidelines revised the preferred regimen for preoperative/adjuvant treatment of HER2 to TDM-1. This study did not include systematic follow-up data for survival analysis, which limited the evaluation of long-term treatment outcomes. Future research should involve prospective, multi-center studies and the establishment of an active, standardized long-term follow-up mechanism.

Tumor heterogeneity is a core biological characteristic of breast cancer, which is characterized by differential receptor expression profiles and molecular typing of different cell subsets within the same tumor. With the in-depth study of mechanisms underlying receptor status changes, it has been widely recognized that the expression status of ER, PR, and HER2 in breast cancer during NAC is not constant, but follows a dynamic evolutionary pattern. For patients exhibiting receptor status changes after NAC, clinical practice requires accurate identification and evaluation of such changes based on receptor re-expression in metastatic or residual lesions, with dynamic adjustment of the treatment strategy accordingly. The core goal of this strategy is to optimize follow-up treatment options and identify potential benefi-

ciaries of different drugs (such as endocrine therapy, targeted therapy or chemotherapy), thereby effectively avoiding overtreatment or undertreatment and advancing the diagnosis and treatment paradigm towards individualization and precision medicine. Currently, some scholars have called for the inclusion of receptor status changes after NAC in clinical pathway management, as well as its incorporation as an important basis for revising and updating breast cancer diagnosis and treatment guidelines. Emerging technologies, including deep learning and case-omics, facilitate the multi-center construction and validation of large-scale models to analyze receptor status changes and survival prognosis in breast cancer patients. These advances can effectively inform early treatment decisions and enhance the accessibility of precision oncology [89].

Looking to the future, guiding targeted drug selection and precise treatment decisions based on receptor status changes characteristics not only represents a focus of clinical research but is also expected to become a key pathway for improving patient efficacy and quality of life.

This study revealed that following NAC, the expression levels of ER, PR, HER2, and the Ki-67 index in breast cancer patients showed varying degrees of changes, with which Ki-67 demonstrating the highest change rate. Further analysis indicated that changes in the Ki-67 index were significantly associated with increased tumor invasion and changes in PR status. Future research should further investigate the biological mechanisms underlying changes in Ki-67 and other IHC characteristics in breast cancer patients with non-pCR following NAC. A deeper understanding of these mechanisms may provide a more robust theoretical foundation for optimizing treatment strategies and improving clinical outcomes.

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Ethical approval

This study was approved by the Ethics Committee of Baoji Central Hospital (Approval Number: BZYL2026-67).

Conflict of interest

The authors declare no conflict of interest.

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