# WOMEN IN SURGICAL ONCOLOGY: 2021

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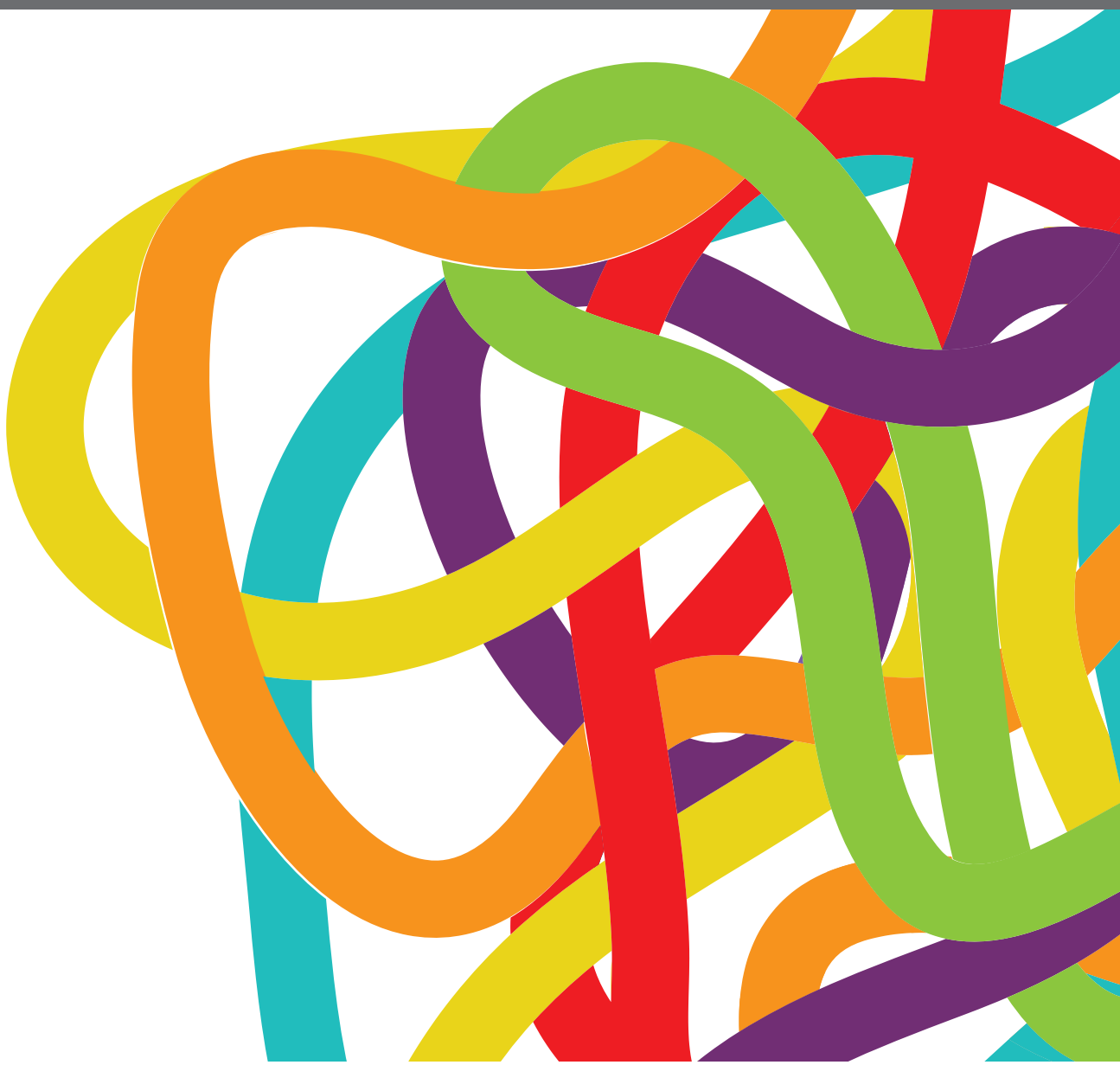

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# WOMEN IN SURGICAL ONCOLOGY: 2021

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# Editorial: Women in surgical oncology: 2021

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Editorial on the Research Topic:
Women in surgical oncology: 2021

Currently, female researchers represent merely a minority, accounting for an estimated 29.3% who end up covering this position worldwide, with a great variability according to each country (1). Specifically, Central Asia exhibits the greatest proportion of female researchers with an estimated 48.2% as opposed to South and West Asia with the lowest count globally (i.e. 18.5%) (1).

In response to such a large gender gap in the scientific research community, the UNESCO Institute for Statistics (UIS) is in the midst of developing new indicators in order to better comprehend the reasons behind women's decisions to pursue one career over another. Several could be the reasons implicated in limiting and discouraging women's access to the scientific community, including ancient biases and gender stereotypes. By further understanding such issues, the UIS project concurrently aims at reducing the gender inequality in science, technology, engineering and mathematics (STEM) fields, by possibly promoting reforms in policies and implementing changes in favor of gender equality in all countries with the ultimate goal of empowering women (2).

The present Research Topic spans through various fields of surgical oncology, including breast, ovarian, colorectal, gastric, and esophageal cancer and includes research papers by women involved in oncological surgery.

Breast cancer represents, at present, the most common malignancy among women of all ages. Li et al. focused on retrospectively analyzing the outcomes of three surgical options on disease-free and overall survival rates of young women with breast cancer below the age of 35 years. They found that both survival rates were significantly improved in those patients who underwent breast-conserving surgery compared to mastectomy. On the other hand, Wang et al. used the Surveillance, Epidemiology, and End-results (SEER) database of the US National Cancer Institute registry for the determination of differences in patient survival of each different treatment modality for invasive micropapillary carcinoma (IMPC) of the breast. Authors show how in women with early-stage IMPC, breast-conserving therapy is equivalent to mastectomy in terms of survival outcomes. Xu et al. also assessed breast cancer from a molecular point of view and found that Ferroptosis-related prognostic signature could be proposed

as novel biomarkers for the prediction of breast cancer prognosis as they seem connected to the immune microenvironment. Molecular and cellular mechanisms of pyroptosis in patients with breast cancer were looked into also by Ren et al.. The inflammation-dependent programmed cell death mediated by inflammasomes, known as pyroptosis, plays a substantial role in the progression of breast cancer and authors suggest how pyroptosis-related genes could be used as new prognostic biomarkers or even targets for breast cancer treatment.

Epithelial ovarian cancer is one of the most aggressive gynecologic cancers. Gao et al. retrospectively evaluated the prognostic impact of retroperitoneal lymphadenectomy in patients with ovarian clear cell cancer. Authors found no survival benefit in patients undergoing retroperitoneal lymphadenectomy and was not an independent predictor of tumor recurrence. In their study, Jiang et al. introduce a new, minimally invasive surgical approach for the treatment of giant ovarian cysts > 20 cm in diameter. All patients successfully underwent single-port laparoscopic surgery for the removal of serous or mucinous cystadenomas.

Wong et al. determined predictors of morbidity and mortality after palliative surgery in patients with peritoneal carcinomatosis due to various primary malignancies. Authors found elevated preoperative albumin levels and a good Eastern Cooperative Oncology Group (ECOG) performance status were independently associated with better short term outcomes following palliative gastrointestinal surgery, supplying a simplified model to predict superior responders to surgical treatment.

Colorectal cancer is another one of the most common cancers, ranking third as the leading cause of death in both men and women. Hashimoto et al. analyzed immunohistochemical data in order to identify protein expression patterns in stages II-III colorectal cancer that could somehow predict patient outcomes. A high expression of Tenascin-C was identified as a single prognostic marker and was correlated with a worst prognosis in both stages of colorectal cancer.

Although radical resection for gastric cancer is currently the only curative treatment option, recurrence after surgery is most commonly peritoneal. Xiang et al. aimed at establishing a reference value for the creation of treatment strategies by identifying current methods for predicting and preventing peritoneal recurrence following surgical resection. Authors highlight how an early gastric cancer diagnosis and a limited loco-regional extension of the primary tumor reduce the risk of peritoneal recurrence, together with intraperitoneal chemotherapy and adjuvant chemotherapy.

Finally, this Research Topic also includes a Study Protocol of a prospective multicenter study on patient participation for clinical trials (Weis et al.). This study aims to develop patient-centered trial information material for this randomized controlled trial and to increase patient acceptance and compliance with randomized treatment strategies and trials.

Promoting gender equality, dismantling stereotypes, and encouraging women to pursue STEM jobs are all necessary to shift entrenched beliefs. Therefore, Frontiers in Oncology is pleased to provide this Research Topic to highlight the contributions of female researchers in all areas of oncology. The work provided here demonstrates the range of oncology research across the board and shows new developments in theory, experimentation, and methodology with applications to interesting and current issues.

## Author contributions

All authors contributed to manuscript revision, read, and approved the submitted version.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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ORIGINAL RESEARCH
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# Expression Patterns of Microenvironmental Factors and Tenascin-C at the Invasive Front of Stage II and III Colorectal Cancer: Novel Tumor Prognostic Markers

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**Background:** Biological markers expressed in cancer cells and the surrounding cancer-associated fibroblasts (CAF) can be used for prediction of patient prognosis in colorectal cancer (CRC). Here, we used immunohistochemical techniques to evaluate cancer cells' expression of specific biomarkers that are closely associated with neoplastic progression.

**Methods:** Immunohistochemical markers included Ki-67, p53, β-catenin, MMP7, E-cadherin and HIF1-α. We also characterized microenvironmental markers expressed by CAF, including expression of α-smooth muscle actin, CD10, podoplanin, fibroblast specific protein 1, platelet derived growth factor β, fibroblast association protein, tenascin-C (TNC), ZEB1 and TWIST1. The study population consisted of 286 CRC patients with stage II and III disease. Stage II and III CRC were divided into a first and a second cohort (for validation). The CRCs were stratified using cluster analysis. To identify the utility of prognostic markers in stage II and III CRC, univariate and multivariate analyses were performed in both cohorts.

**Results:** Stage II and III CRCs were stratified into 3 subgroups. Specific subgroups were significantly correlated to disease-free survival using univariate and multivariate analyses in the first cohort. High expression of TNC was identified as a single prognostic marker in both cohorts by univariate and multivariate analyses.

**Conclusions:** We suggest that the presence of a specific subgroup defined by multiple markers can be used for prediction of CRC outcome in stages II and III. In addition, we showed that high expression of TNC was correlated with a poorer prognosis in stages II and III of CRC.

**Keywords: cancer-associated fibroblast, colorectal cancer, cluster analysis, prognostic marker, tenascin-C**

## INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the third leading cause of cancer death in both men and women in the United States (1). These trends for incidence and mortality are common worldwide (1). Remarkable progress has been made in the diagnosis and treatment of CRC. In spite of such advances, CRC is often discovered at an advanced stage at which point achieving a cure is very difficult (2). Therefore, the development of effective markers to predict patient prognosis of CRC is greatly needed.

The outcome of patients with CRC can be predicted by prognostic factors, such as the TNM staging system proposed by the UICC and AJCC (3, 4). Additionally, novel and promising prognostic biomarkers are listed in the WHO classification 2019 (5). There are 2 histological processes that are present within the tumor microenvironment at the invasive front of CRC: tumor budding and the desmoplastic reaction (DR) (6–9). Tumor budding is defined as single cells or clusters of up to four tumor cells at the invasion front of CRC (6–8). It is closely associated with both local and distant metastases and is therefore a histological biomarker of tumor progression and a poor prognosis (6–8). The classification of the DR was recently proposed by Ueno et al. as a prognostic histological marker (9). A pronounced desmoplastic stromal reaction in the microenvironment involves complex cellular interactions at the invasive front (10). This theory posits that cooperation between cancer cells and cancer associated fibroblasts (CAFs) present within the tumor microenvironment is necessary to support tumor growth and progression (10, 11). In addition, the microenvironment itself plays an important role in neoplastic progression and metastasis in CRC (10, 11). Whereas such histological findings are widely used as markers for establishing a patient's prognosis, they do not explain the underlying cellular processes that promote tumor growth and metastasis (12, 13). Therefore, the discovery of additional markers would be very beneficial. We propose that identification of protein expression patterns in cancer cells and CAFs could provide new biological insights and guide the development of new therapies for CRC (12, 13).

In this study, we analyzed immunohistochemical data to identify possible protein expression patterns in stages II and III of CRC that predict patient outcome. We focused on markers that are closely associated with tumor growth and progression within the microenvironment.

## MATERIALS AND METHODS

### Patients

CRC patients who underwent curative surgery at stages II or III at Iwate Medical University Hospital from January 2009 to December 2015 were included in the present study. In total, 286 patients were included the first cohort (148 cases) and in a second cohort for validation (138 cases), which were evaluated through a retrospective analysis. We used a block randomization method in the research design to select and divide participants into different groups or conditions in order to avoid bias in the selection of two cohorts. Paraffin embedded tissues were well preserved, medical records were complete and patient status had been followed up, including overall survival and disease-free survival data that were confirmed through telephone interviews and by the mail. In addition, cases with invasion beyond the proper muscular layer were included for determination of the desmoplastic reaction (9). Finally, patients who underwent preoperative chemoradiotherapy and emergency surgery were excluded. In addition, patients who had evidence of hereditary non-polyposis colorectal cancer or familial adenomatous polyposis were not enrolled. The clinicopathological variables characterizing the patients included tumor location, stage and t stage, histological type, lymphatic/venous invasion and tumor budding. The variables were recorded according to the General Rules for Management of the Japanese Colorectal Cancer Association (**Table 1**) (14). In addition, DR classification was determined based on Ueno's classification (9).

This study was approved by the local ethics committee of Iwate Medical University (approval number MH2020-070), and all patients provided informed consent.

#### Determination of Disease-Free Survival

We determined the duration of disease-free survival at which metastasis was discovered during the follow-up period (2 times/year to 3 times/year) using computed tomography.

### Chemotherapeutic Treatment After Surgery for Stage II or III CRC

Following surgery, Capecitabine or UFT/UZEL (Tegafur Uracil + Calcium Folinate) were administered in stage II CRC (20/140 cases), whereas FOLFOX, including the drugs leucovorin calcium (folinic acid), fluorouracil and oxaliplatin were used in stage III CRC (85/146 cases). The other 181 patients, including 120 cases in stage II and 61 cases in stage III did not receive additional chemotherapy following surgery.

#### Determination of Sample Size

The sample size required to identify differences in overall and disease-free survival between cohorts was determined using JMP Pro 13.0 software (SAS, Tokyo, Japan). From the calculation, at least 120 cases were required. The statistical power (detection power) was set to 0.8, which is commonly used in medical studies.

### Tissue Microarray Construction (TMA)

The TMAs were assembled using a manual tissue array (Azumaya Co, Tokyo, Japan). Five mm tissue cores were taken from each targeted lesion and placed into a recipient block containing 12 cores including 10 cancer tissues and 2 cores for control tissues (normal colon; CRC). After construction, 3-micron sections were cut and stained with hematoxylin and eosin on the initial slides to

**Abbreviations:** CAF, Cancer associated fibroblast; CRC, colorectal cancer; TMA, tissue microarray; MMP7, matrix metalloproteinase-7; FSP1, fibroblast specific protein 1; PDGFR-b, platelet derived growth factor receptor beta; FAP, fibroblast associated protein; ZEB1, zinc finger E-box binding homeobox 1; TWIST1, twistrelated protein 1.

**TABLE 1** | Clinicopathological findings in stage II and III colorectal cancer.

| | | Cohort 1 (%) | Cohort 2 (%) | |
|---|---|---|---|---|
| Total | | 148 | 138 | *p* value |
| Age, median (range) (y) | | 67.5 (34–94) | 70.0 (41–88) | 0.1583 |
| Sex | Man | 90 (60.8) | 81 (58.7) | 0.7193 |
| | Woman | 58 (39.2) | 57 (41.3) | |
| Location | Right colon | 30 (20.3) | 32 (23.2) | 0.4271 |
| | Left colon | 64 (43.2) | 49 (35.5) | |
| | Rectum | 54 (36.5) | 57 (41.3) | |
| pT | pT3 | 129 (86.5) | 111 (80.4) | 0.1474 |
| | pT4 | 19 (13.5) | 27 (19.6) | |
| Stage | II | 71 (48.0) | 69 (50.0) | 0.8129 |
| | III | 77 (52.0) | 69 (50.0) | |
| Histological type | WDA | 17 (11.5) | 26 (18.8) | 0.0681 |
| | MDA | 121 (81.8) | 109 (79.9) | |
| | PDA | 2 (1.4) | 1 (0.7) | |
| | PAP | 6 (4.1) | 1 (0.7) | |
| | MUC | 2 (1.4) | 1 (0.7) | |
| Lymphatic invasion | Positive | 130 (87.8) | 129 (93.5) | 0.4404 |
| | Negative | 18 (12.2) | 9 (6.5) | |
| Venous invasion | Positive | 129 (87.2) | 128 (92.8) | 0.4534 |
| | Negative | 19 (12.8) | 10 (7.2) | |
| Tumor budding | Low | 117 (79.1) | 108 (78.3) | 0.8861 |
| | High | 31 (20.9) | 30 (21.7) | |
| Desmoplastic reaction | Mature | 65 (43.9) | 61 (44.2) | 0.9877 |
| | Intermediate | 53 (35.8) | 49 (35.5) | |
| | Immature | 30 (20.3) | 28 (20.3) | |
| Disease-free survival, median (range) (d) | | 1857 (33–3196) | 1835 (93–3308) | |
| Overall survival, median (range) (d) | | 3077 (52–3196) | 2195 (93–3308) | |

*WDA, well-differentiated adenocarcinoma; MDA, moderately differentiated adenocarcinoma; PDA, poorly differenced adenocarcinoma; PAP, papillary carcinoma; MUC, mucinous carcinoma.*

verify the histologic diagnosis. Serial sections were cut from the TMA block for immunohistochemical staining.

## Immunohistochemistry

Tumors were routinely fixed in 20% neutral-buffered formalin and embedded in paraffin wax. Three-micron-thick paraffin sections were cut, dewaxed, and rehydrated. Microarray slides were incubated in 3% hydrogen peroxide to block endogenous peroxidase. Antigen retrieval was performed using an autoclave-based method, followed by incubation with the primary antibody overnight at 4°C in a high humidity cabinet. Slides were processed using the Dako Autostainer Universal Staining System (Dako, Glostrup, Denmark) (12). The specimens were treated with citrate buffer (pH 6.0) using a microwave [three times for 5 min, 750 W; cat. no. H2500; Microwave Processor (Bio-Rad Laboratories, CA, USA)] and then reacted with antibodies, as previously described. Antibodies used in this study were classified into 2 subgroups: epithelial (cancer cells) and interstitial (cancer associated fibroblasts, CAF) markers. Antibodies targeting CAFs included the following: α-smooth muscle actin (α-SMA, Dako 1A4), CD10 (Dako, 56C6), podoplanin (Dako, D2-40), fibroblast specific protein 1 (FSP1; S100A4, Dako, polyclonal), platelet derived growth factor receptor (PDGFR-β; 28E1, Cell Signaling Technology), fibroblast association protein (FAP, Abcam, EPR20021) and tenascin-C (IBL, 4F10TT). For EMT, we utilized zinc finger E-box binding homeobox 1 (ZEB1, Sigma-Aldrich, polyclonal) and Twist-related protein 1 (TWIST1, Abcam, Twist2C1a). CAFs were recognized as "spindle-shaped cells" by experienced pathologists (T.S. and N.U.). Cytoplasmic staining of tumor cells was conducted with antibodies against α-SMA, CD10, podoplanin, FSP1, PDGFR-β, FAP and tenascin-C. Nuclear staining of fibroblasts was based on positivity for ZEB1 and TWIST1 expression. Furthermore, antibodies targeting cancer cells in this study included Ki-67 (Dako, MIB1) for proliferative activity, p53 (Dako, Do7) for p53 mutation, β-catenin (Dako, β-catenin-1) for activation of Wnt signaling, a central signal transducer in CRC, MMP7 (Daiichi Fine Chemical, 141-7B2) for cancer progression, E-cadherin (Dako, NCH-38) for cellular adhesion and HIF1-α (Novus Biologicals, polyclonal) for cancer-specific metabolic marker which may be associated with tumor progression. Detailed information of antibodies is summarized in **Supplementary Table 1**.

## Assessment of Scoring of Immunohistochemical Expression

The expression of the markers was scored for both the intensity and extent of immunopositivity, as described in a previous report with slight modification (15). The immunostaining intensity of the cancer cells and CAFs in the CRCs was classified into 4 categories as follows: negative, weak, moderate and strong. The immunostaining extent was semi-quantified as follows: 0%, 1-25%, 26-50%, 51-100%. The combination of intensity and extent was scored. Scores 2–3 were defined as a positive staining pattern, as shown in **Supplementary Table 2**. In addition, the score was also sub-classified into low (score 0-1) and high expression (score 2-3). Assessment of scoring was performed

by two pathologists. If agreement was not obtained between the pathologists, we asked an additional pathologist regarding the assessment. Finally, the score was determined by agreement of more than two pathologists.

In the present study, a wide range of expression levels was observed for all the markers. Thus, we selected the deepest invasive region as a target area to measure the expression levels of markers.

### Hierarchical Analysis of the Expression of CAF and EMT Markers

Hierarchical cluster analysis was performed for clustering of the samples according to the expression level in order to achieve maximal homogeneity for each group and the greatest differences between the groups using open-access clustering software (Cluster 3.0 software; bonsai.hgc.jp/~mdehoon/software/cluster/software.htm). The clustering algorithm was set to centroid linkage clustering, which is the standard hierarchical clustering method used in biological studies.

### Statistical Analysis

Data were analyzed using JMP Pro 13.0 software (SAS, Tokyo, Japan). Data obtained for clinicopathological features (sex, location, pT, stage, histological type, lymphatic invasion, venous invasion, tumor budding, desmoplastic reaction, overall survival, disease-free survival) and subgroup (subgroups 1, 2 and 3) were analyzed using Fisher's exact test. In addition, the comparison of the age distributions within each subgroup was performed using the Kruskal-Wallis test. If multigroup comparisons were needed for statistical analysis, we used Bonferroni corrections.

Kaplan-Meier analyses were performed using a log-rank test for survival analyses. Univariate and multivariate analyses were conducted with Cox proportional hazards model to identify statistical differences for prediction of overall and disease-free survival. The level of significance was $p < 0.05$, and the confidence interval (CI) was determined at the 95% level.

## RESULTS

A representative figure is shown in **Figure 1**. In addition, the cancer invasive front is depicted in **Supplementary Figure 1**.

## ANALYSES OF CLINICOPATHOLOGICAL VARIABLES AND BIOLOGICAL MARKERS IN THE FIRST COHORT

### Hierarchical Clustering Based on Marker Scores in First Cohort

We performed hierarchical clustering based on marker scores to evaluate differences in expression patterns of cancer cell-, CAF- and EMT-related markers in stage II and III CRC. Three distinct subgroups were stratified, as shown in **Figure 2**. The vertical line shows the expression of each marker in cancer cells and fibroblasts and the horizontal lines denote "relatedness" between samples. There was no statistical difference in the frequency of clinicopathological variables among subgroups 1, 2 and 3. Although immature desmoplastic reaction present in subgroup 1 showed a high frequency among the 3 subgroups, such association between the 3 subgroups did not quite reach a statistically significant level ($p = 0.0508$). However, the frequency of disease-free survival was significantly higher in subgroup 1 than in subgroup 2 ($p<0.0001$). Detailed data are shown in **Table 2**.

### Survival Analyses of Each Subgroup in the First Cohort

Kaplan–Meier analyses were performed to determine the association between the disease-free survival frequencies and the subgroups. Subgroup 1 had a poorer disease-free survival, compared to subgroup 2 ($p < 0.0001$). However, overall survival did not differ among the subgroups (**Supplementary Figure 2**).

### The Association of Clinicopathological Variables and Subgroups With Survival of Stage II and III CRC Patients: Univariate and Multivariate Analyses of the First Cohort Using a Cox Proportional Hazards Model

The univariate analysis of stage II and III CRC patients (**Table 3a**) identified 5 factors: histologic type (mucinous carcinoma vs. well differentiated adenocarcinoma), stage (II *vs* III), desmoplastic reaction (mature *vs* immature) and subgroup (1 *vs* 2; 1 *vs* 3). **Table 3b** reveals that 3 factors (mucinous carcinoma *vs* well differentiated adenocarcinoma, mature DR *versus* immature DR, subgroup 1 *versus* 2) were retained in the multivariate analysis using a Cox proportional hazards model

Using a similar method, we performed univariate analysis for screening of overall survival of stage II and III CRC patients. As a result, 3 factors, including stage (II *vs* III), desmoplastic reaction (mature *vs* immature), and subgroup (1 *vs* 2) were identified in univariate analysis (**Table 3c**). However, no factors were retained in multivariate analysis (**Table 3d**).

### Association of Individual Markers With Individual Subgroups in the First Cohort

The frequency of positive scores (score 2 or 3) of SMA was higher in subgroup 2 than in subgroup 1. There were statistically significant differences in the frequencies of positive scores among subgroups 1, 2 and 3 (subgroup 1, 2 > 3). In addition, significant differences in the frequencies of positive scores for tenascin-C between subgroups 1 and 2, and 3 were found (subgroup 1 > 2, 3). The frequency of the positive score for ZEB1 was statistically higher in subgroup 2 than in subgroup 3. Next, there was a statistically significant difference in the

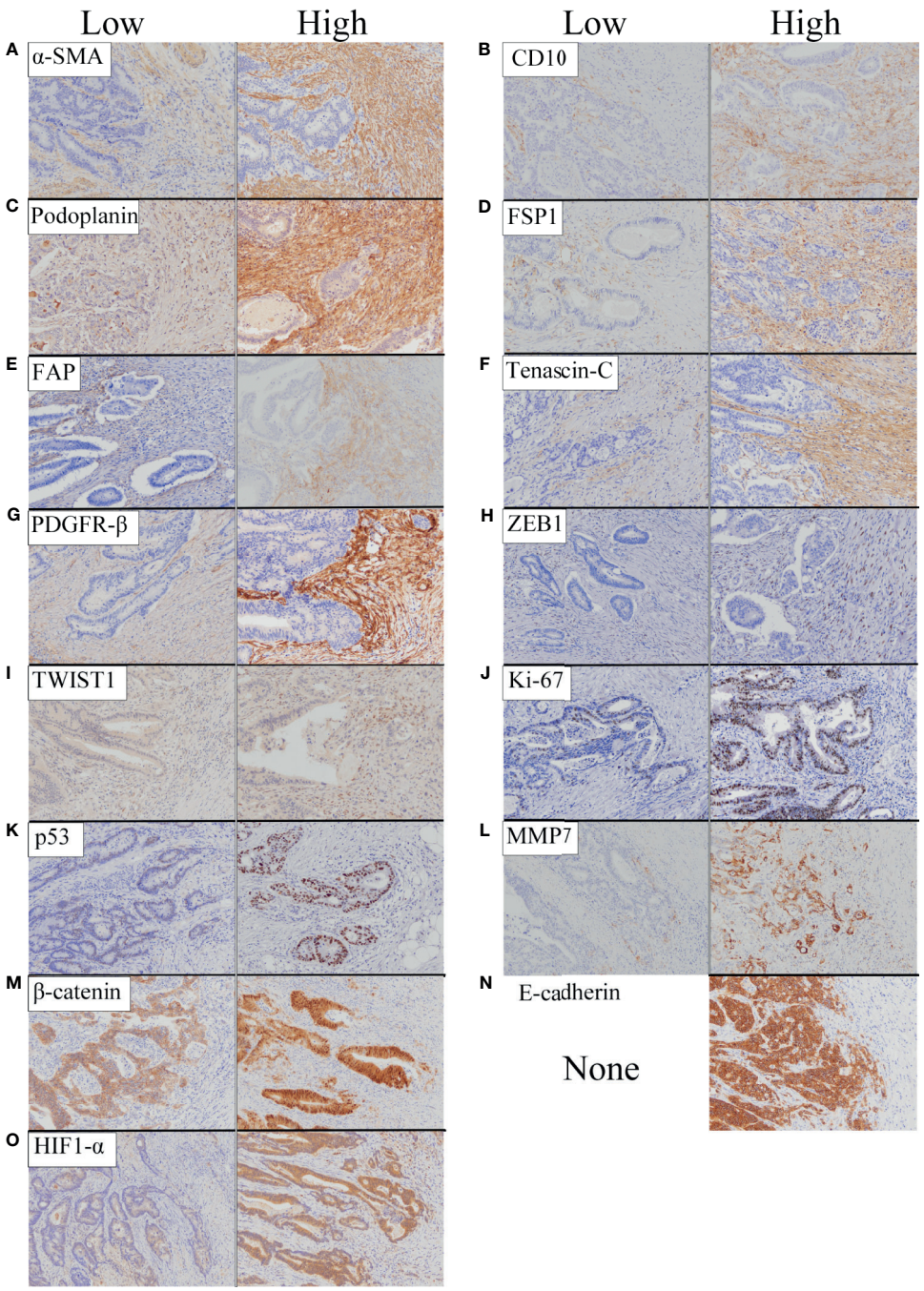


**FIGURE 1** | Representative features of immunohistochemical staining of biological markers we examined based on expression level (low and high). **(A)** α-SMA. **(B)** CD10. **(C)** Podoplanin. **(D)** FSP1. **(E)** FAP. **(F)** Tenascin-C. **(G)** PDGFR-β. **(H)** ZEB1. **(I)** TWIST1. **(J)** Ki-67. **(K)** p53. **(L)** MMP7. **(M)** β-catenin. **(N)** E-cadherin. **(O)** HIF1-α.

frequencies of positive scores for TWIST1 between subgroup 3 and subgroup 1 (subgroup 1 > 3). The positive score for p53 was significantly greater in subgroup 2 than in subgroups 1 and 3. Furthermore, there was a significant difference in the frequencies of positive scores for p53 between subgroups 1 and 3. Finally, we observed statistically significant differences in the frequencies of positive MMP7 scores among subgroups 1 and 2, and 3 (subgroup 1, 2 > 3). Detailed data are shown in **Figure 3**.

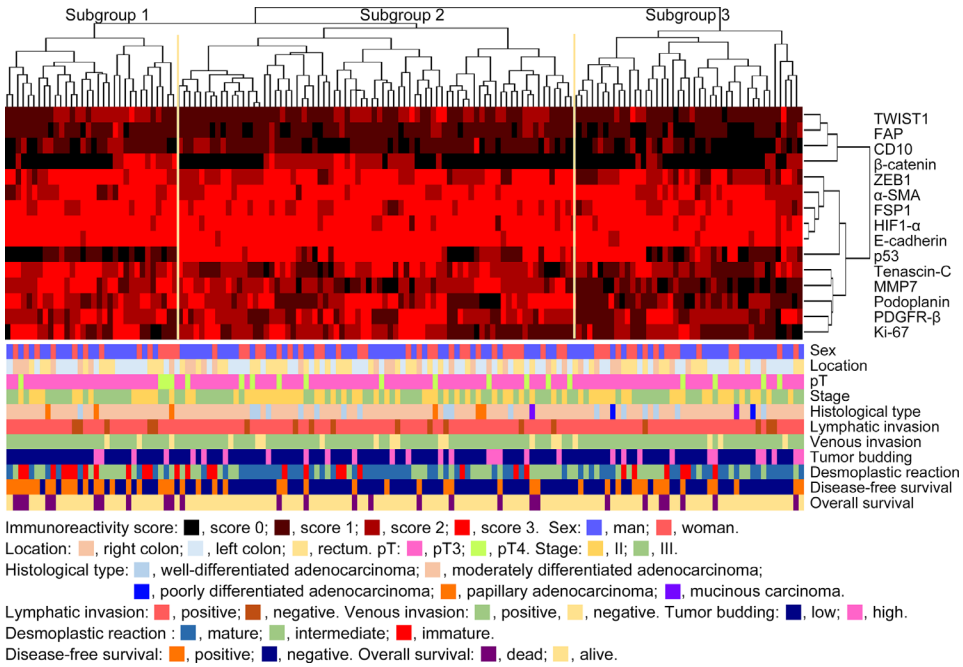


**FIGURE 2** | Hierarchical cluster analysis of colorectal cancer patients with stage II or III disease based on the expression patterns of cancer cells and cancer-associated fibroblast (CAF) proteins in the first cohort. The examined CRCs were subclassified into 3 subgroups.

**TABLE 2** | Clinicopathological variables according to each subgroup in the first cohort.

| | | Subgroup 1 (%) | Subgroup 2 (%) | Subgroup 3 (%) | *p* value |
|---|---|---|---|---|---|
| Total | | 32 | 74 | 42 | |
| Age median (range) (y) | | 69.0 (43–94) | 67.0 (34–92) | 67.5 (42–88) | 0.1971 |
| Sex | Man | 19 (59.4) | 45 (60.8) | 26 (61.9) | 1.0000 |
| | Woman | 13 (41.6) | 29 (39.2) | 16 (38.1) | |
| Location | Right colon | 7 (21.9) | 15 (20.3) | 8 (19.0) | 0.9516 |
| | Left colon | 15 (46.9) | 30 (40.5) | 19 (45.2) | |
| | Rectum | 10 (31.2) | 29 (39.2) | 15 (35.7) | |
| pT | pT3 | 28 (87.5) | 63 (85.1) | 38 (90.5) | 0.7678 |
| | pT4 | 4 (12.5) | 11 (14.9) | 4 (9.5) | |
| Stage | II | 13 (40.6) | 37 (50.0) | 21 (50.0) | 0.6456 |
| | III | 19 (59.4) | 37 (50.0) | 21 (50.0) | |
| Histological type | WDA | 1 (3.1) | 10 (13.5) | 6 (14.3) | 0.1125 |
| | MDA | 28 (87.5) | 60 (81.1) | 33 (78.6) | |
| | PDA | 0 (0.0) | 0 (0.0) | 2 (4.8) | |
| | PAP | 3 (9.4) | 3 (4.1) | 0 (0.0) | |
| | MUC | 0 (0.0) | 1 (1.4) | 1 (2.4) | |
| Lymphatic invasion | Positive | 27 (84.4) | 65 (87.8) | 38 (90.5) | 0.7149 |
| | Negative | 5 (15.6) | 9 (12.2) | 4 (9.5) | |
| Venous invasion | Positive | 29 (90.6) | 61 (82.4) | 39 (92.9) | 0.2694 |
| | Negative | 3 (9.4) | 13 (17.6) | 3 (7.1) | |
| Tumor budding | Low | 28 (87.5) | 58 (78.4) | 31 (73.8) | 0.3738 |
| | High | 4 (12.5) | 16 (21.6) | 11 (26.2) | |
| Desmoplastic reaction | Mature | 8 (25.0) | 36 (48.6) | 21 (50.0) | 0.0508 |
| | Intermediate | 11 (34.4) | 27 (36.5) | 15 (35.7) | |
| | Immature | 13 (40.6) | 11 (14.9) | 6 (14.3) | |
| Disease-free survival | Positive | 20 (62.5)* | 15 (20.3)* | 15 (35.7) | 0.0002 |
| | Negative | 12 (37.5) | 59 (79.7) | 27 (64.3) | |
| Overall survival | Dead | 10 (31.6) | 10 (13.5) | 7 (16.7) | 0.0999 |
| | Alive | 20 (62.5) | 64 (86.5) | 35 (83.3) | |

*WDA, well-differentiated adenocarcinoma; MDA, moderately differentiated adenocarcinoma; PDA, poorly differenced adenocarcinoma; PAP, papillary carcinoma; MUC, mucinous carcinoma; *p < 0.0001.*

**TABLE 3** | Association of clinicopathological variables and subgroups with disease-free survival and overall survival in the first cohort in univariate and multivariate analyses.

| Variables | | a. Univariate analysis | | | b. Multivariate analysis | | | c. Univariate analysis | | | d. Multivariate analysis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HR | 95%CI | *p* value | HR | 95%CI | *p* value | HR | 95%CI | *p* value | HR | 95%CI | *p* value |
| | | Disease-free survival | | | | | | Overall survival | | | | | |
| Sex | Woman *vs* man | 1.420 | 0.807-2.477 | 0.2207 | | | | 1.456 | 0.674-3.126 | 0.3335 | | | |
| Location | Rectum *vs* Left colon | 1.488 | 0.784-2.864 | 0.2238 | | | | 1.336 | 0.549-3.429 | 0.5257 | | | |
| | Right colon *vs* Left colon | 1.671 | 0.778-3.476 | 0.1821 | | | | 1.411 | 0.492-3.950 | 0.5104 | | | |
| | Right colon *vs* Rectum | 1.123 | 0.534-2.260 | 0.7508 | | | | 1.056 | 0.393-2.627 | 0.9084 | | | |
| pT | pT4 *vs* pT3 | 1.488 | 0.646-3.007 | 0.3261 | | | | 2.477 | 0.967-5.630 | 0.0581 | | | |
| Stage | III *vs* II | 2.319 | 1.299-4.322 | 0.0041 | 1.816 | 0.981-3.520 | 0.0577 | 2.575 | 1.139-6.570 | 0.0222 | 2.264 | 0.972-5.923 | 0.0586 |
| Histological type | MUC *vs* WDA | 10.443 | 1.857-58.726 | 0.0253 | 9.556 | 1.537-59.396 | 0.0350 | 4.994 | 0.448-55.676 | 0.2455 | | | |
| Lymphatic invasion | Positive *vs* Negative | 2.444 | 0.895-10.069 | 0.0865 | | | | 3.596 | 0.762-64.238 | 0.1226 | | | |
| Venous invasion | Positive *vs* Negative | 1.473 | 0.643-4.255 | 0.3878 | | | | 1.677 | 0.499-10.431 | 0.4493 | | | |
| Tumor budding | High *vs* Low | 1.386 | 0.707-2.548 | 0.3275 | | | | 1.502 | 0.636-3.299 | 0.3383 | | | |
| Desmoplastic reaction | Immature *vs* Intermediate | 1.766 | 0.894-3.446 | 0.1003 | | | | 1.788 | 0.725-4.257 | 0.2004 | | | |
| | Immature *vs* Mature | 3.023 | 1.486-6.183 | 0.0025 | 2.190 | 1.012-4.793 | 0.0467 | 3.660 | 1.316-10.947 | 0.0132 | 2.473 | 0.821-7.870 | 0.107 |
| | Intermediate *vs* Mature | 1.711 | 0.869-3.435 | 0.1200 | | | | 2.048 | 0.793-5.893 | 0.1407 | | | |
| Subgroup | Subgroup 1 *vs* 2 | 3.626 | 1.860-7.224 | 0.0002 | 3.012 | 1.512-6.127 | 0.0018 | 2.643 | 1.082-6.459 | 0.0335 | 2.082 | 0.825-5.261 | 0.1185 |
| | Subgroup 1 *vs* 3 | 2.024 | 1.036-4.049 | 0.0392 | 1.710 | 0.819-3.672 | 0.1538 | 2.058 | 0.789-5.676 | 0.1393 | | | |
| | Subgroup 3 *vs* 2 | 1.791 | 0.868-3.697 | 0.1135 | | | | 1.284 | 0.466-3.347 | 0.6154 | | | |

*WDA, well-differentiated adenocarcinoma; MUC, mucinous carcinoma; HR, hazard ratio; 95%CI, 95% confidence interval.*

### The Association of Clinicopathological Variables and Individual Markers With the Survival of Stage II and III CRC Patients: Univariate and Multivariate Analyses of the First Cohort

With regard to disease-free survival, 3 variables (stage II *vs* III; mature *vs* immature; mucinous carcinoma *vs* well differentiated adenocarcinoma) and one marker (tenascin-C) were identified in univariate analysis (**Table 4a**). Among those 4 parameters, 2 variables, including desmoplastic reaction and histological type and one marker, tenascin-C, were retained in multivariate analysis (**Table 4b**). In overall survival, stages (II *vs* III) and desmoplastic reaction (mature *vs* immature) were identified in univariate analysis (**Table 4c**). Desmoplastic reaction (mature *vs* immature) was retained in multivariate analysis (**Table 4d**).

## ANALYSES OF CLINICOPATHOLOGICAL VARIABLES AND INDIVIDUAL MARKERS IN THE SECOND COHORT (VALIDATION)

### The Association of Clinicopathological Variables and Individual Markers With the Survival of Stage II and III CRC Patients: Univariate and Multivariate Analyses of the Second Cohort

With regard to disease-free survival, 5 variables (pT3 *vs*. pT4; stage II *vs*. III; positive venous invasion *vs*. negative venous invasion; low grade budding *vs*. high grade budding; mature *vs*. immature) and 2 markers (tenascin-C and β-catenin) were identified in univariate analysis (**Table 5a**). However, only 1 factor (tenascin-C) was retained in multivariate analysis (**Table 5b**). In overall survival, 4 variables (pT3 *vs*. pT4; stage II vs. III and desmoplastic reactions (mature *vs*. immature; and intermediate *vs*. mature) and 2 individual markers (tenascin-C and Ki-67) were detected in univariate analysis (**Table 5c**). Only the positive expression of tenascin-C was retained in multivariate analysis (**Table 5d**).

## DISCUSSION

Certain proteins expressed by microenvironmental cells play crucial roles in neoplastic progression of CRC. Those proteins may be derived from cancer cells or from stromal cells (sometimes termed "cancer-associated fibroblasts" (CAFs) (12, 13). Proteins expressed by cancer cells and CAFs interact with one another and this interaction is likely important at the invasive front (12, 13). According to that theory, the combination of proteins from cancer cells and CAFs mediate tumor growth and progression (12, 13). In the present study, specific expression patterns could be correlated with the prognosis of stage II and III CRC patients. Therefore, the current results suggest that a specific subgroup (identified here by stratification) can be used to evaluate the role and significance of various proteins produced by microenvironmental cells. Finally, in the present study, subgroup 1 was correlated with disease-free survival. However, the presence in subgroup 1 did not correlate with overall survival. The reason remains unknown.

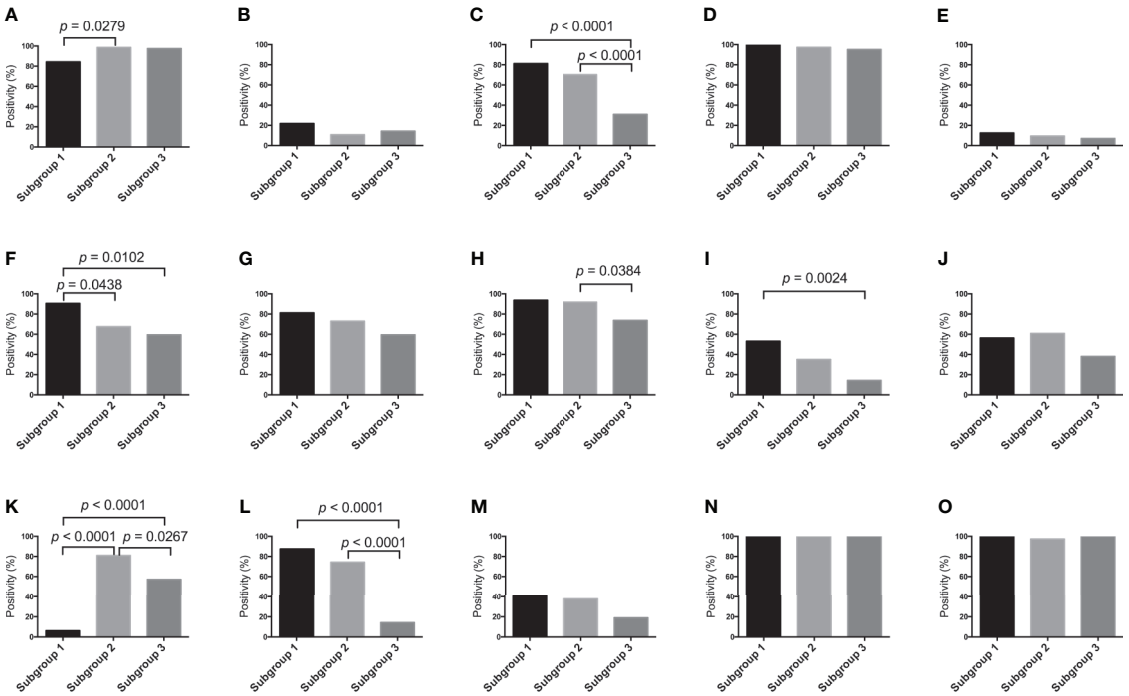


**FIGURE 3** | Marker expression levels. **(A)** Expression level of each marker in the first cohort. **(A)** α-SMA, **(B)** CD10, **(C)** Podoplanin, **(D)** FSP1, **(E)** FAP, **(F)** Tenascin- C, **(G)** PDGFR-β, **(H)** ZEB1, **(I)** TWIST, **(J)** Ki-67, **(K)** p53, **(L)** MMP7, **(M)** β-catenin, **(N)** E-cadherin and **(O)** HIF1-α.

In the current study, we used 15 microenvironment-related markers (cancer cell markers and CAF markers) to identify associations of expression patterns with patient outcomes. Among the cancer cell-related markers, a high Ki-67-positive rate and overexpression of p53 were considered to reflect the characteristics of tumors. Intranuclear expression of β-catenin and high expression of MMP7, E-cadherin, and HIF1-α are closely associated with tumor budding, which is a key histological feature occurring in the cancer microenvironment (16–18). By contrast, stromal markers, including α-SMA, CD10, podoplanin, FSP1, PDGFR β, FAP, and TNC, were used as CAF markers. These markers are thought to be associated with enhanced progression of CAFs. Based on these findings, we suggest that the microenvironment-related markers used in the current study may be suitable for identification of the molecular mechanisms of neoplastic progression and cancer metastasis in the tumor microenvironment.

Tenascin-C (TNC) is an extracellular matrix molecule that drives the progression of many types of human cancer. The basis for its actions remains unclear (19). TNC is associated with organogenesis accompanying cell proliferation and migration, resulting in the epithelial-mesenchymal transition (EMT) that might result from interactions between cancer cells and stromal cells (20). EMT is the process by which polarized epithelial cells are converted into mesenchymal cells during cancer progression. As a result, carcinoma cells lose their epithelial polarity and intercellular connections, allowing them to escape the surrounding epithelium (20, 21). The expression of TNC facilitates such phenotypic changes, alterations that are enhanced by TGF-β, a promoter of EMT (19–21). Murakami et al. revealed that TNC in primary CRC stroma might be a novel biomarker that is predictive of postoperative prognosis (21). Finally, TNC may promote EMT-like change and proliferation, alterations that lead to poor prognosis in CRC patients (20).

TNC may be involved in cancer growth and metastatic processes *via* the Hedgehog (HH) signaling pathway, caused either by mutations in the pathway (ligand independent) or through HH overexpression (ligand dependent) (22). HH signaling starts with secretion of the HH ligand, followed by secretion of Patched (PTC), the transmembrane protein Smoothened (SMO) and three GLI (Glioma-associated oncogene) zinc finger transcription factors (23). The HH/GLI1 pathway promotes cancer growth, stem cell self-renewal and metastatic behavior in advanced CRC (24). Human CRC stem cells require active HH/GLI1 signaling for survival and self-renewal (25). Our finding suggests that activation of CAF at the invasive front is caused by high expression of TNC facilitated *via* HH signaling (26). In addition, accumulating evidence suggests that activated HH signaling plays an important role in neoplastic transformation as well as the development of drug resistance of human cancers (27). Thus, HH signaling during tumorigenesis and the development of chemo-resistance are closely associated. Those findings suggest that therapeutic strategies might target such signals in human cancers and their relapse (26, 27). For example, cyclopamine is an HH signal pathway antagonist and consequently is expected to improve the survival of patients with CRC by inhibiting the proliferation of colon cancer cells (28). Previous study showed that cyclopamine treatment results in decreased levels of mRNA coding for HH, SMO and PTCH, all of

**TABLE 4** | Association of clinicopathological variables and individual marker with disease-free survival and overall survival in the first cohort in univariate and multivariate analyses.

| Variables | | a. Univariate analysis | | | b. Multivariate analysis | | | c. Univariate analysis | | | d. Multivariate analysis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HR | 95%CI | *p* value | HR | 95%CI | *p* value | HR | 95%CI | *p* value | HR | 95%CI | *p* value |
| | | Disease-free survival | | | | | | Overall survival | | | | | |
| Stage | III *vs* II | 2.319 | 1.299-4.322 | 0.0041 | 1.760 | 0.943-3.430 | 0.0762 | 2.575 | 1.139-6.570 | 0.0222 | 2.237 | 0.959-5.856 | 0.0629 |
| Histological type | MUC *vs* WDA | 10.443 | 1.857-58.726 | 0.0253 | 15.097 | 2.505-90.973 | 0.0132 | 5.001 | 0.929-33.667 | 0.2451 | | | |
| Desmoplastic reaction | Immature *vs* Intermediate | 1.766 | 0.894-3.446 | 0.1003 | | | | 1.788 | 0.725-4.257 | 0.2004 | | | |
| | Immature *vs* Mature | 3.023 | 1.486-6.183 | 0.0025 | 2.358 | 1.115-5.033 | 0.0250 | 3.660 | 1.316-10.947 | 0.0132 | 3.076 | 1.085-9.352 | 0.0347 |
| | Intermediate *vs* Mature | 1.711 | 0.869-3.435 | 0.1200 | | | | 2.048 | 0.793-5.893 | 0.1407 | | | |
| α-SMA | Positive *vs* Negative | 0.684 | 0.250-2.821 | 0.5468 | | | | 0.628 | 0.187-3.905 | 0.5532 | | | |
| CD10 | Positive *vs* Negative | 1.690 | 0.798-3.254 | 0.1606 | | | | 1.412 | 0.472-3.453 | 0.5025 | | | |
| Podoplanin | Positive *vs* Negative | 1.019 | 0.583-1.821 | 0.9477 | | | | 1.039 | 0.486-2.307 | 0.9215 | | | |
| FSP1 | Positive *vs* Negative | 0.677 | 0.209-4.150 | 0.6111 | | | | 0.357 | 0.072-6.461 | 0.3876 | | | |
| FAP | Positive *vs* Negative | 0.501 | 0.120-1.392 | 0.2067 | | | | 0.278 | 0.154-1.334 | 0.1270 | | | |
| Tenascin-C | Positive *vs* Negative | 2.694 | 1.293-6.557 | 0.0065 | 2.317 | 1.068-5.870 | 0.0324 | 2.052 | 0.840-6.130 | 0.1201 | | | |
| PDGFR-β | Positive *vs* Negative | 0.988 | 0.550-1.863 | 0.9680 | | | | 0.764 | 0.351-1.786 | 0.5176 | | | |
| ZEB1 | Positive *vs* Negative | 1.269 | 0.584-3.325 | 0.5730 | | | | 2.094 | 0.623-13.018 | 0.2638 | | | |
| TWIST1 | Positive *vs* Negative | 1.512 | 0.851-2.639 | 0.1554 | | | | 1.185 | 0.533-2.540 | 0.6673 | | | |
| Ki-67 | Positive *vs* Negative | 1.139 | 0.653-2.011 | 0.6477 | | | | 1.245 | 0.579-2.736 | 0.5754 | | | |
| p53 | Positive *vs* Negative | 0.629 | 0.359-1.098 | 0.1023 | | | | 0.504 | 0.231-1.077 | 0.0767 | | | |
| MMP7 | Positive *vs* Negative | 1.621 | 0.890-3.069 | 0.1096 | | | | 1.205 | 0.560-2.734 | 0.6383 | | | |
| β-catenin | Positive *vs* Negative | 0.770 | 0.401-1.395 | 0.3974 | | | | 0.979 | 0.419-2.127 | 0.9578 | | | |
| E-cadherin* | | | | | | | | | | | | | |
| HIF1-α | Positive *vs* Negative | 0.461 | 0.101-8.172 | 0.4961 | | | | 0.297 | 0.062-5.344 | 0.3187 | | | |

*WDA, Well-differentiated adenocarcinoma; MUC, mucinous carcinoma; HR, Hazard ratio; 95%CI, 95%confidence interval. *could not analyze, why all cases were positive expression of the marker.*

which were highly expressed in colon cancer cell lines (28). These findings may influence potential therapeutic strategies because TNC expression by CAF may be targeted in future molecular therapies.

High expression of TNC was reported to be a prognostic marker for CRC through induction of EMT and cell proliferative activity (20). According to that study, TNC may facilitate EMT-like changes and could be associated with a poor prognosis of CRC patients. This finding is consistent with other data showing that cancer cell-derived TNC promotes cancer cell invasion *via* EMT regulation. Thus, it is a novel indicator of poor prognosis (29). In the present study, we found that even in stages II and III, intermediate stages that account for the majority of surgically resected CRC, TNC was an independent prognostic marker. This result was validated by analysis of a second cohort. The present results showed that TNC in primary CRC stroma has the potential to be a novel biomarker that predicts postoperative prognosis.

There are some limitations to this study. First, the immuno histochemical markers we used in the present study may not yield consistent results. For clinical application, immunohistochemical reagents must be reliable and reproducible. In that regard, many immunohistochemical markers that are closely associated with the formation of the microenvironment have been analyzed (12, 13). In the current study, 15 microenvironment-related markers, including Ki-67, p53, β-catenin, MMP7, E-cadherin, and HIF1-α (for cancer cells) and CD10, podoplanin, FSP 1, PDGFR β, FAP, TNC, ZEB1, and TWIST1 (for CAFs) were used. Briefly, Ki-67 positivity and p53 overexpression have been widely used as characteristics of tumors. The remaining factors, including β-catenin, MMP7, E-cadherin, and HIF1-α, are closely associated with the formation of the cancer microenvironment. In addition, stromal factors could be classified as CAF or EMT markers. The two stromal markers used in this study were considered CAF markers given that all markers we used were expressed in CAFs. These CAF markers are suitable for identifying the functions of CAFs. Therefore, we concluded that the immunohistochemical markers examined in this study were all involved in generation of the tumor microenvironment at the invasive front. Finally, analysis of these immunohistochemical markers should yield reliable and reproducible results, as demonstrated in the current study. Second, the heterogeneous expression of the markers examined in this study may be problematic when determining marker expression levels (30). Although it may be difficult to avoid this problem, we suggest that the invasive front of cancer cells, which is critical for tumor progression, may be the best region for measuring the immunohistochemical expression levels of the chosen markers (10, 11). Finally, although there are many different reports regarding prognostic factors in CRC (31, 32), the different results may reflect the choice of markers, patient stage, heterogeneity of expression, staining platform, judging methods and cut-off value. In the present study, we suggest that the current results are reliable and reproducible under the conditions we employed.

## CONCLUSIONS

Cancer cells and CAFs express many proteins that modulate neoplastic progression and metastasis. In the present study, we

**TABLE 5 |** Association of clinicopathological variables and individual markers with disease-free survival and overall survival in the second cohort in univariate and multivariate analyses.

| Variables | | a. Univariate analysis | | | b. Multivariate analysis | | | c. Univariate analysis | | | d. Multivariate analysis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HR | 95%CI | *p* value | HR | 95%CI | *p* value | HR | 95%CI | *p* value | HR | 95%CI | *p* value |
| | | Disease-free survival | | | | | | Overall survival | | | | | |
| Sex | Woman *vs* Man | 0.819 | 0.430-1.512 | 0.5283 | | | | 0.919 | 0.395-2.048 | 0.8320 | | | |
| Location | Rectum *vs* Left colon | 1.982 | 0.976-4.345 | 0.0588 | | | | 1.617 | 0.639-4.603 | 0.3180 | | | |
| | Right colon *vs* Left colon | 1.094 | 0.417-2.774 | 0.8505 | | | | 1.088 | 0.313-3.613 | 0.8894 | | | |
| | Right colon *vs* Rectum | 1.813 | 0.850-4.310 | 0.1278 | | | | 1.486 | 0.560-4.632 | 0.4393 | | | |
| pT | pT4 *vs* pT3 | 2.279 | 1.142-4.307 | 0.0208 | 1.527 | 0.714-3.112 | 0.2662 | 2.630 | 1.065-5.986 | 0.0369 | 1.936 | 0.746-4.670 | 0.1667 |
| Stage | III *vs* II | 3.114 | 1.633-6.334 | 0.0004 | 1.991 | 0.977-4.301 | 0.0581 | 4.599 | 1.847-13.885 | 0.0007 | 2.619 | 0.980-8.321 | 0.0552 |
| Histological type | WDA *vs* MDA | 0.627 | 0.264-1.492 | 0.2657 | | | | 0.787 | 0.269-2.303 | 0.6533 | | | |
| Lymphatic invasion | Positive *vs* Negative | 4.334 | 0.944-76.833 | 0.0620 | | | | 2.447 | 0.516-43.777 | 0.3125 | | | |
| Venous invasion | Positive *vs* Negative | 4.855 | 1.057-86.076 | 0.0401 | 4.073 | 0.838-73.423 | 0.0904 | 2.722 | 0.574-48.690 | 0.2501 | | | |
| Tumor budding | High *vs* Low | 2.291 | 1.170-4.285 | 0.0169 | 1.667 | 0.825-3.237 | 0.1497 | 2.090 | 0.847-4.752 | 0.1052 | | | |
| Desmoplastic reaction | Immature *vs* Intermediate | 1.520 | 0.712-3.204 | 0.2736 | | | | 1.227 | 0.451-3.118 | 0.6754 | | | |
| | Immature *vs* Mature | 2.322 | 1.076-4.976 | 0.0324 | 0.993 | 0.415-2.340 | 0.9867 | 3.293 | 1.092-10.245 | 0.0348 | 1.596 | 0.493-5.305 | 0.4312 |
| | Intermediate *vs* Mature | 1.527 | 0.733-3.202 | 0.2558 | | | | 2.684 | 1.021-7.793 | 0.0452 | 1.895 | 0.696-5.686 | 0.2143 |
| α-SMA | Positive *vs* Negative | 1.423 | 0.437-8.736 | 0.6078 | | | | 1.580 | 0.333-28.264 | 0.6303 | | | |
| CD10 | Positive *vs* Negative | 0.657 | 0.267-1.391 | 0.2883 | | | | 0.704 | 0.295-1.941 | 0.4698 | | | |
| Podoplamin | Positive *vs* Negative | 0.752 | 0.399-1.496 | 0.4020 | | | | 0.564 | 0.251-1.343 | 0.1872 | | | |
| FSP1 | Positive *vs* Negative | 0.355 | 0.108-2.185 | 0.2181 | | | | 0.296 | 0.061-5.139 | 0.3172 | | | |
| FAP | Positive *vs* Negative | 0.819 | 0.425-1.520 | 0.5323 | | | | 4.045 | 0.853-72.358 | 0.0866 | | | |
| Tenascin-C | Positive *vs* Negative | 5.025 | 2.164-14.621 | 0.0001 | 3.973 | 1.656-11.795 | 0.0012 | 4.527 | 1.559-19.172 | 0.0036 | 3.188 | 1.038-13.882 | 0.0421 |
| PDGFR-β | Positive *vs* Negative | 1.290 | 0.685-2.566 | 0.4395 | | | | 1.416 | 0.611-3.661 | 0.4288 | | | |
| ZEB1 | Positive *vs* Negative | 0.683 | 0.248-2.825 | 0.5463 | | | | 0.562 | 0.166-3.510 | 0.4713 | | | |
| TWIST1 | Positive *vs* Negative | 1.067 | 0.581-1.971 | 0.8331 | | | | 1.237 | 0.553-2.818 | 0.6028 | | | |
| Ki-67 | Positive *vs* Negative | 1.667 | 0.885-3.316 | 0.1157 | | | | 2.952 | 1.187-8.906 | 0.0185 | 2.114 | 0.834-6.465 | 0.1191 |
| p53 | Positive *vs* Negative | 1.362 | 0.730-2.663 | 0.3378 | | | | 1.255 | 0.552-3.096 | 0.5955 | | | |
| MMP7 | Positive *vs* Negative | 0.651 | 0.354-1.199 | 0.1671 | | | | 0.823 | 0.368-1.875 | 0.6358 | | | |
| β-catenin | Positive *vs* Negative | 2.119 | 1.143-4.084 | 0.0169 | 1.502 | 0.796-2.940 | 0.2121 | 1.865 | 0.830-4.440 | 0.1323 | | | |
| E-cadherin* | | | | | | | | | | | | | |
| HIF1-α* | | | | | | | | | | | | | |

*WDA, Well-differentiated adenocarcinoma; MUC, mucinous carcinoma; HR, Hazard ratio; 95%CI, 95%confidence interval. *Could not analyze, why all cases were positive expression of the marker.*

found that specific expression patterns may allow the prediction of patient outcome in CRC. In addition, the expression of TNC by CAFs might be a potential prognostic biomarker in stage II and III CRC patients. These results highlight a potential role for TNC in CRC tumor progression and provide novel mechanistic insights into the roles of HH, as it is associated with high expression of TNC in driving CRC progression. Our findings also suggest that TNC could be a critical target gene for the treatment of CRC. However, further study will be needed in the near future to confirm these results.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of Iwate Medical University Hospital (approval number MH2020-070). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## AUTHOR CONTRIBUTIONS

MH, who is the first author, constructed the figures and tables and performed the statistical analyses. MO assisted statistical analyses. YK and HU supported pathological interpretation of desmoplastic reactions. NY and NU helped in the interpretation of pathological findings. KO and AS provided clinical support during the preparation of the manuscript. TS, who is the corresponding author, contributed to the preparation of the manuscript, including all aspects of the data collection and analysis. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.690816/full#supplementary-material

**Supplementary Figure 1 |** Histological features of the invasive front. **(A)** Low-power view of the invasive area of CRC. **(B)** High-power view of the invasive area of CRC (mature type desmoplastic reaction). **(C)** Low-power view of the invasive area of CRC. **(D)** High-power view of the invasive area of CRC (intermediate type desmoplastic reaction). **(E)** Low-power view of the invasive area of CRC. **(F)** High-power view of the invasive area of CRC (immature type desmoplastic reaction).

**Supplementary Figure 2 |** Kaplan-Meier analyses of the disease-free survival **(A)** and overall survival **(B)** based on each subgroup of the first cohort.

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# Comprehensive Analysis of Ferroptosis-Related LncRNAs in Breast Cancer Patients Reveals Prognostic Value and Relationship With Tumor Immune Microenvironment

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**Background:** Breast cancer (BC) is a heterogeneous malignant tumor, leading to the second major cause of female mortality. This study aimed to establish an in-depth relationship between ferroptosis-related LncRNA (FRlncRNA) and the prognosis as well as immune microenvironment of the patients with BC.

**Methods:** We downloaded and integrated the gene expression data and the clinical information of the patients with BC from The Cancer Genome Atlas (TCGA) database. The co-expression network analysis and univariate Cox regression analysis were performed to screen out the FRlncRNAs related to prognosis. A cluster analysis was adopted to explore the difference of immune microenvironment between the clusters. Furthermore, we determined the optimal survival-related FRLncRNAs for final signature by LASSO Cox regression analysis. Afterward, we constructed and validated the prediction models, which were further tested in different subgroups.

**Results:** A total of 31 FRLncRNAs were filtrated as prognostic biomarkers. Two clusters were determined, and C1 showed better prognosis and higher infiltration level of immune cells, such as B cells naive, plasma cells, T cells CD8, and T cells CD4 memory activated. However, there were no significantly different clinical characters between the clusters. Gene Set Enrichment Analysis (GSEA) revealed that some metabolism-related pathways and immune-associated pathways were exposed. In addition, 12 FRLncRNAs were determined by LASSO analysis and used to construct a prognostic signature. In both the training and testing sets, patients in the high-risk group had a worse survival than the low-risk patients. The area under the curves (AUCs) of receiver operator characteristic (ROC) curves were about 0.700, showing positive prognostic capacity. More notably, through the comprehensive analysis of heatmap, we regarded LINC01871, LINC02384, LIPE-AS1, and HSD11B1-AS1 as protective LncRNAs, while LINC00393, AC121247.2, AC010655.2, LINC01419, PTPRD-AS1, AC099329.2, OTUD6B-AS1, and LINC02266

were classified as risk LncRNAs. At the same time, the patients in the low-risk groups were more likely to be assigned to C1 and had a higher immune score, which were consistent with a better prognosis.

**Conclusion:** Our research indicated that the ferroptosis-related prognostic signature could be used as novel biomarkers for predicting the prognosis of BC. The differences in the immune microenvironment exhibited by BC patients with different risks and clusters suggested that there may be a complementary synergistic effect between ferroptosis and immunotherapy.

**Keywords: breast cancer, ferroptosis, lncRNA, prognosis, immune microenvironment**

## INTRODUCTION

Breast cancer (BC) is the most common cancer among women in the world, leading to the second largest cause of female mortality, and its morbidity and mortality are increasing year by year (1). Due to the large population base in China, the number of cases and deaths of female BC in China ranks first in the world (2). Although with the development of people's awareness of physical examination and prevention and the all-out support of diversified treatment methods, such as surgery, radiotherapy, the prognosis of BC has been significantly improved. However, the prognosis of advanced BC is still disappointing (3, 4). According to the previous research, BC is a malignant solid tumor formed by the long-term action of multiple genes and factors, accompanied by obvious heterogeneity, resulting in a diversified tumor microenvironment and different responses (5, 6). Increasing evidence showed that lncRNA plays a unignorable role in regulating the occurrence and development of various cancers, such as BC (7), endometrial cancer (8), and liver cancer (9). Therefore, we need to explore the potential molecular mechanisms to maximize the benefits of existing methods to promote the progress in the diagnosis and treatment of BC.

Ferroptosis is an iron-dependent, novel programmed cell death pattern distinct from apoptosis, cell necrosis, and autophagy that can be triggered by acute and chronic cellular stress caused by abnormal lipid metabolism and biochemical processes (10–12). The previous studies have proved that the activation of ferroptosis can promote the killing effect of body on tumors, especially for tumors that have developed resistance to the traditional treatments, such as BC, which has become a very promising anti-tumor direction (13–15). Glutathione peroxidase 4 (GPX4) can be used by BC to gain the ability to endure drug resistance, conversely, the loss of GPX4 function can reverse the formation of BC drug resistance, which leads to the persistent ferroptosis process of cells and prevents tumor recurrence, suggesting that targeting GPX4 is a therapeutic strategy for acquired drug resistance (13). In addition, siramesine combined with lapatinib promotes the death of BC cells by increasing reactive oxygen species (ROS) through iron transport disruption, independent of downstream targets (members of the EGFR family) and cathepsin B, which suggests that the other targets of siramesine and lapatinib are associated with ferroptosis and provides hope for overcoming apoptotic resistance in BC (16). In addition to GPX4, a previous study reported that other ferroptosis-related genes, such as iron, ACSL4, SLC7A11, and SLC3A, could be promising targets for BC treatment (17, 18). However, there are few studies on ferroptosis and immune microenvironment of BC, and unified insights are still lacking but urgently needed.

In the present study, we downloaded and integrated the gene expression data and the clinical information of patients with BC from the TCGA dataset. The cluster analysis was then adopted to explore the difference of immune microenvironment. Afterward, the prognostic signature associated with ferroptosis was determined to construct the predicting models and further validated these models. Collectively, not only did our results demonstrate that the prognostic models accurately predicted the prognosis of patients with BC, but also preliminarily revealed the differences in the immune microenvironment in the process of ferroptosis, which provided some thoughts and insights for the combination of immunotherapy and ferroptosis in clinical diagnosis and treatment of BC.

## MATERIALS AND METHODS

### Data Collection and Process

The gene expression data of the transcriptome (such as, mRNA and LncRNA) and the clinical information of BC patients were downloaded from the TCGA dataset (19), of which the clinical information included the survival time, survival status, age, gender, grade, stage, T stage, N stage, and M stage. Specifically, we preliminarily screened 1,178 cases of transcriptome data, such as 112 cases of the normal and 1,066 cases of the tumor, and 1,053 cases of clinical data that include 911 cases of survival and 142 cases of death. A list of ferroptosis-related genes was extracted from FerrDb (http://www.zhounan.org/ferrdb/operations/download.html), and the expression of ferroptosis-related genes was extracted.

### Screening of the Prognostic FRLncRNAs

For picking out the target ferroptosis-related LncRNAs (FRlncRNAs), a co-expression network analysis was adopted to show the relationship between lncRNAs and ferroptosis-related genes. The LncRNAs with $|r| \geq 0.5$ and $P < 0.001$ were confirmed as FRlncRNAs. Then, the univariate Cox regression analysis was used to screen the prognosis-related FRlncRNAs, and the

results were presented in the form of forest plot. Further, we drew a heatmap and compared the differential expression of these FRlncRNAs in normal tissues and tumor tissues using the rank sum test.

## Hierarchical Consensus Clustering Based on the Prognostic FRlncRNAs

According to the FRlncRNAs related to prognosis, the hierarchical consensus clustering was used to perform the classification of TCGA cohort (20). To obtain robust classification, we adopted an unsupervised consensus approach implemented in the R package "Consensus Cluster Plus" (21). Moreover, the relative change in area under the cumulative distribution function (CDF) curve was employed to determine the optimal number of clusters, k, which was further verified by the total within sum of squares (WSS) and the gap statistics. The difference of survival probability and clinical information (age, stage, T stage, N stage, and M stage) between clusters were investigated.

## Evaluation of the Correlation With Immune Features

Inspired by the success of immunotherapy in the patients with BC in recent years (22), we further explored whether there was an immunological explanation for the survival differences between the clusters. Set PD-L1 gene as the strongest example, we carried out Pearson's correlation coefficient to test the co-expression and correlation between the hub gene and the prognostic FRlncRNAs in tumor tissues. Besides, ESTIMATE algorithm was performed to calculate the immune and stromal scores to quantify the presence of stromal cells and the infiltration of immune cells in tumor samples. To observe the differences of immune cells among clusters in the tumor microenvironment in a more detailed way, CIBERSORT, a gene expression-based deconvolution algorithm to describe the cell constitution of tissues (23), was performed to intuitively display the distribution of immune cells, which was showed in the violin plot generated by the vioplot package.

## Gene Set Enrichment Analysis (GSEA) Between Clusters

Gene Set Enrichment Analysis determines whether the predetermined gene sets have statistically significant differences between the two biological states in a computational method (24). In view of the consensus clustering, we conducted GSEA analysis in clusters with the aim of mining survival differences. By adjusting the *p*-value, the enrichment pathway for each phenotype was classified *via* the normalized enrichment score (NES). An NES >1 and false discovery rate (FDR) < 0.05 denoted statistical significance.

## Construction and Validation of the Prediction Models

Based on the results of univariate Cox regression analysis, the least absolute shrinkage and selection operator (LASSO) regression was applied to select the optimal survival-related FRlncRNAs, which were involved in the final modeling. According to the coefficients derived from LASSO regression and expression levels of FRlncRNAs included in final models, the prognostic risk score formula was constructed as follows:

$$risk\ score = \sum_{n=1}^{i} \left(\beta_n * expression\ of\ gene_n\right)$$

where β is the regression coefficient.

We randomly divided the patients with BC from TCGA into two groups according to the ratio of 1:1, one group as the training set and the other group as the validation set. In the training set, we calculated the risk scores of the patients with BC and classified these patients into the high-risk group and the low-risk group on the basis of the median risk score as the threshold. The Kaplan–Meier (K–M) survival analysis was used to prove whether there was a survival difference between the two groups. The receiver operator characteristic (ROC) curve was built by using the survival ROC package to assess the efficiency of the prognostic model. In the test group, the same processes were performed to validate the prognostic model of this group. Independent cohort validation is important for prognostic signatures. In the current study, the GSE69031 cohort was used to validate our OS signature (25). The expression data of the genes included in the final signature were obtained and substituted into the equation for risk score calculation. All patients in this cohort were stratified into low- or high-risk groups. The prediction accuracy of signature in the independent validation cohort was evaluated by ROC curve and K–M survival analyses.

## Identification of the Independence of Risk Score Prognostic Model

The clinical information, such as age and stage, was integrated to testify the independence of the prognostic model with the combination of risk score. For that, the univariate and multivariate Cox regression analyses were performed to verify the independence of the prognostic model in the training cohort and test cohort, respectively.

## Validation of the Risk Score Prognostic Model Between Different Subgroups

Through stratification of clinical data, K–M curves were drawn, respectively to study whether the genetic risk scores were applicable to patients in different groups. Specifically, we divided patients into the two categories based on age >65 and age <65, T1-2 and T3-4, N0 and N1–3, M0 and M1, Stage I–II and Stage III–IV, respectively, and calculate the difference in survival curves between the high-risk and low-risk patients in each category, so as to expand the applicability of risk scores.

## Comprehensive Analysis of the Differences Between High- and Low-Risk Groups

We enrolled all eligible patients and divided them into the high-risk and low-risk groups according to the calculation above. The clinical information (age, stage, T stage, N stage, and M stage), immune score, and clusters were integrated to exhibit the differences between high-risk and low-risk groups. More importantly, we also considered the expression differences of

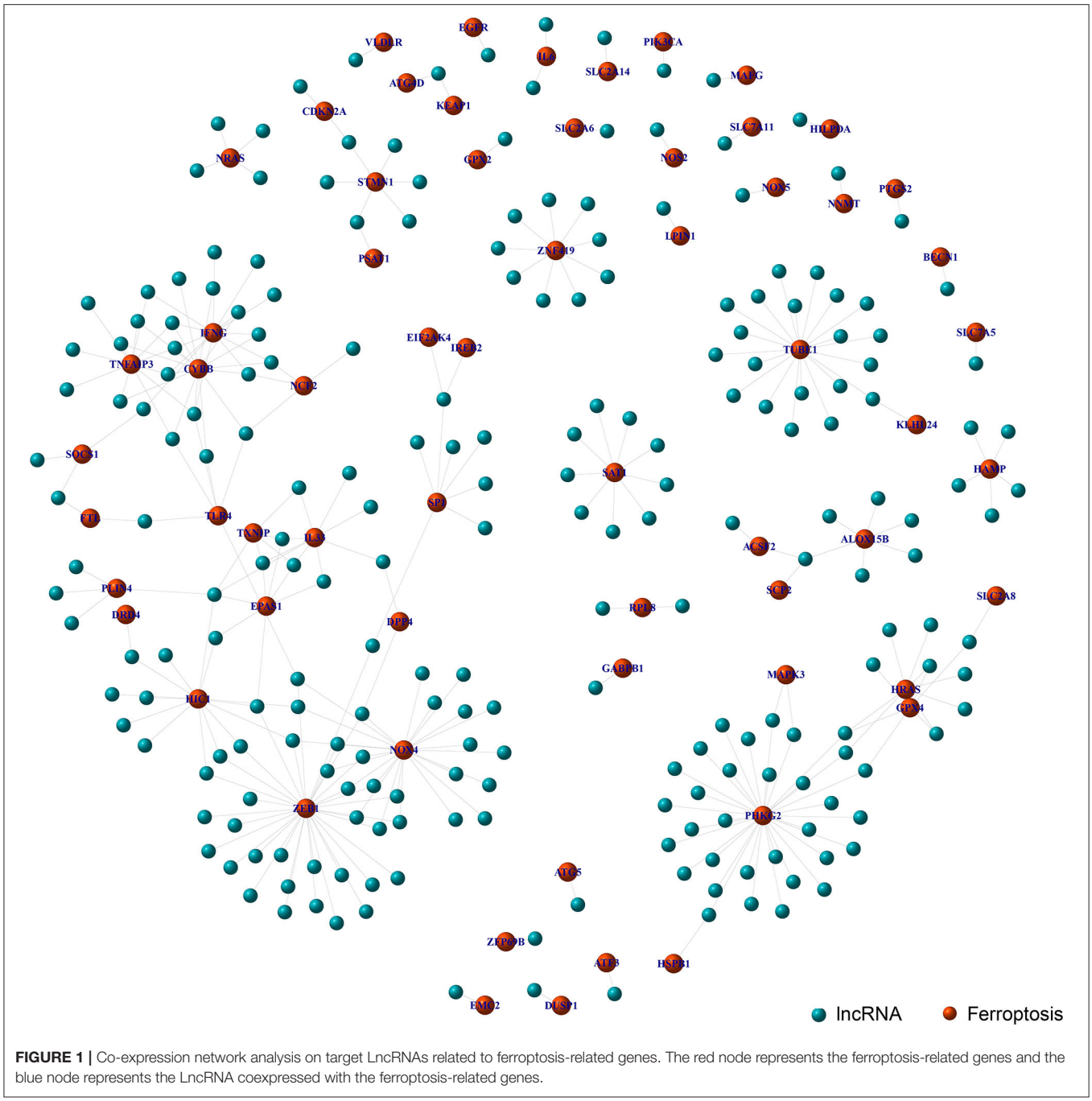


**FIGURE 1 |** Co-expression network analysis on target LncRNAs related to ferroptosis-related genes. The red node represents the ferroptosis-related genes and the blue node represents the LncRNA coexpressed with the ferroptosis-related genes.

the survival-related FRlncRNAs involved in the final modeling, hoping to provide a theoretical basis for finding new therapeutic targets for BC.

# RESULTS

## The LncRNAs Associated With Ferroptosis Genes

Through the co-expression analysis, we sorted out the 63 FRLncRNAs, and visualized the relationship by co-expression network (**Figure 1**). In the **Figure 1**, the red nodes represent the ferroptosis-related genes, and the blue nodes represent LncRNAs co-expressed with ferroptosis-related genes. These LncRNAs were used as FRlncRNAs for the subsequent analyses.

## Identification of the FRlncRNAs Related to Prognosis

To explore the influence of FRlncRNAs on the prognosis of patients with BC, the univariate Cox regression analysis was used to preliminarily determine 31 FRlncRNAs related to the prognosis, which were visualized by forest map (**Figure 2**). If

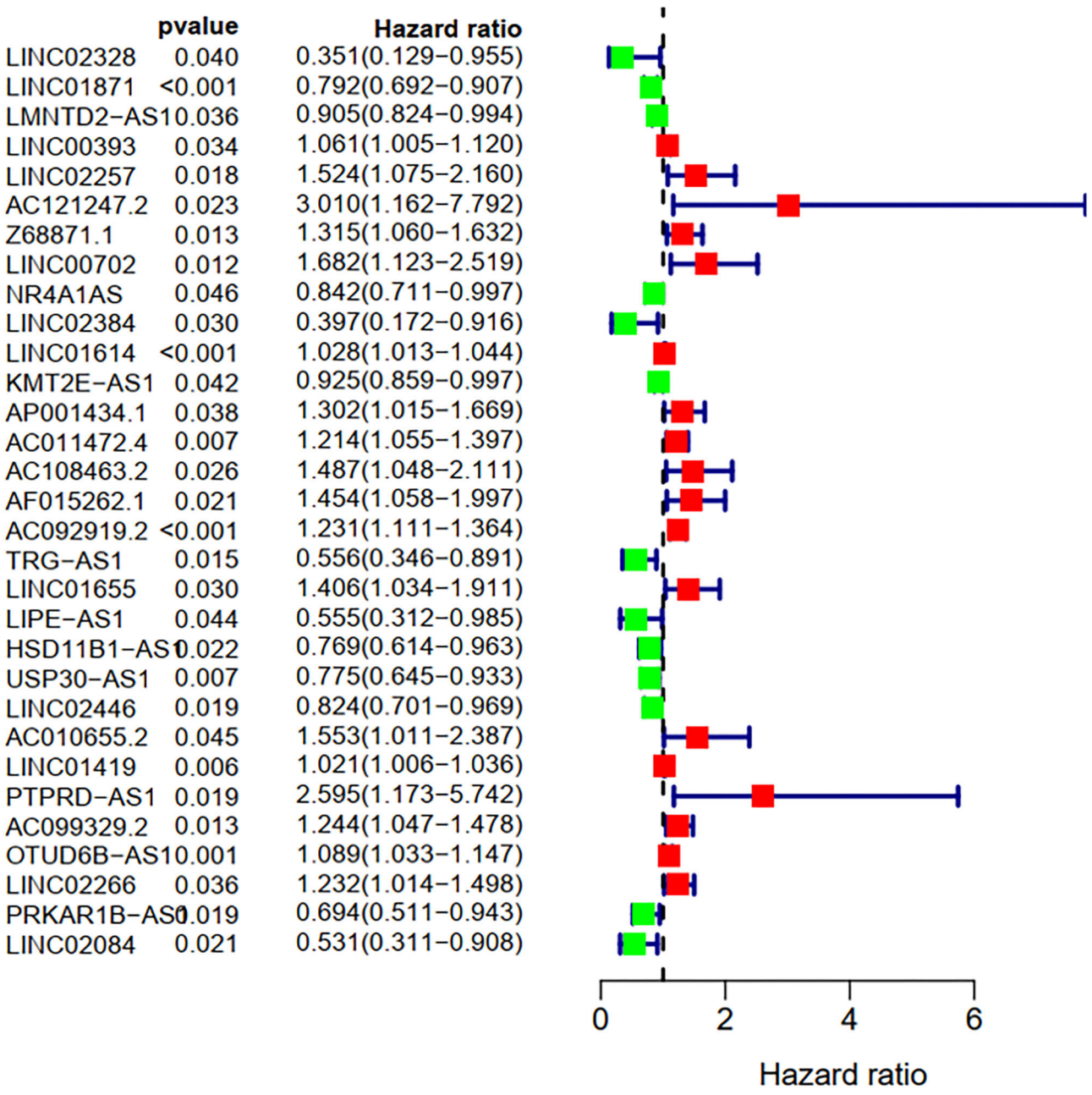


**FIGURE 2 |** Univariate Cox regression analysis of the FRLncRNAs related to prognosis. The red and green boxes represent risk factors or protective factors, respectively.

the hazard ratio (HR) >1, the higher the expression level, the higher the risk of patients. On the contrary, HR < 1 means that the higher the expression level, the lower the risk of patients. Further, the differential analysis was employed to exhibit the expression level of 31 prognosis-related FRlncRNAs between the normal tissues and tumor tissues. LINC00702, AC121247.2, HSD11B1-AS1, NR4A1AS, AC011472.4, LIPE-AS1, etc., were lower expressed in tumor tissues, conversely, AP001434.1, LINC02257, LINC01655, LINC01614, AF015262.1, LMNTD2-AS1, etc., were highly expressed in tumor tissues (**Figure 3**).

## FRlncRNAs-Based Clusters Associated With Prognosis

Our study confirmed that some FRlncRNAs were related to the prognosis of patients, and were expressed in a heterogeneous manner among tumor patients. In order to better understand the intertumoral heterogeneity of BC, we conducted an unsupervised

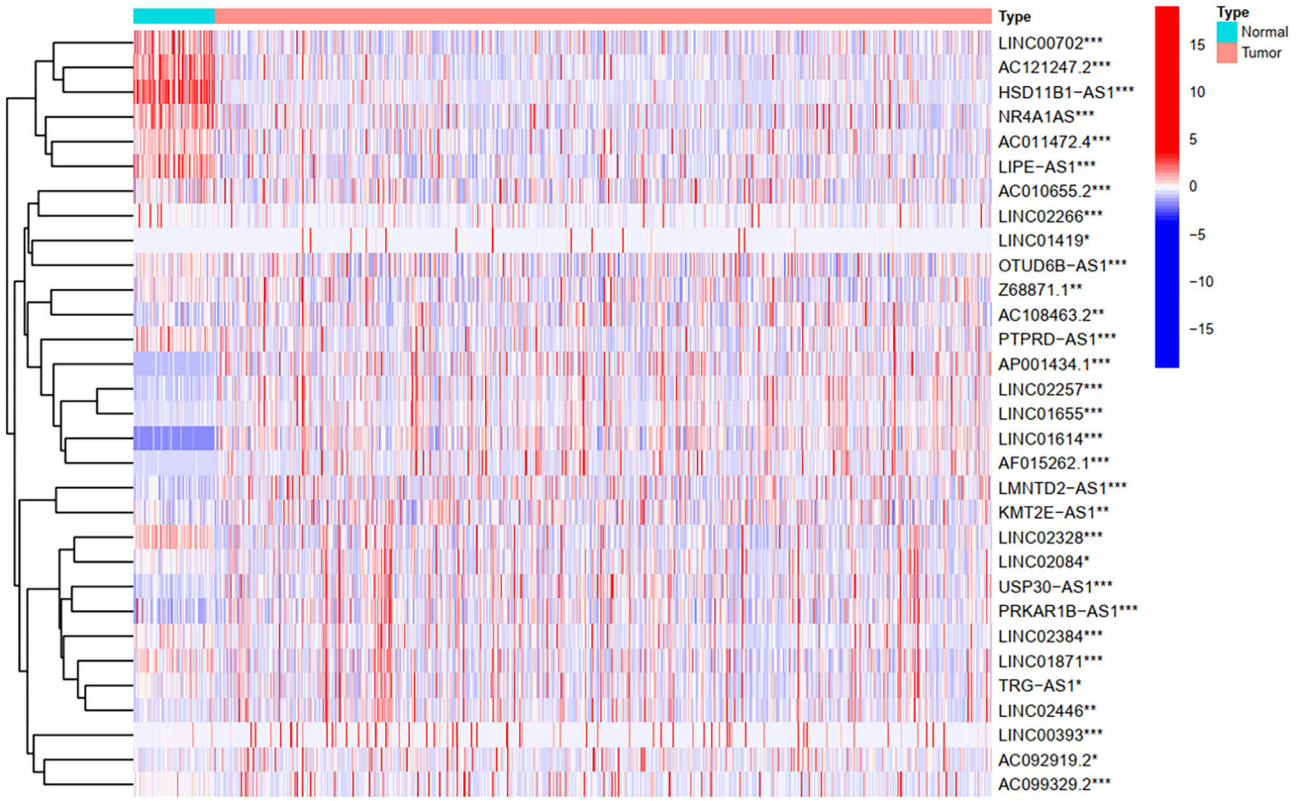


**FIGURE 3 |** The heatmap shows the differential expression of the prognostic between normal and tumor tissues. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

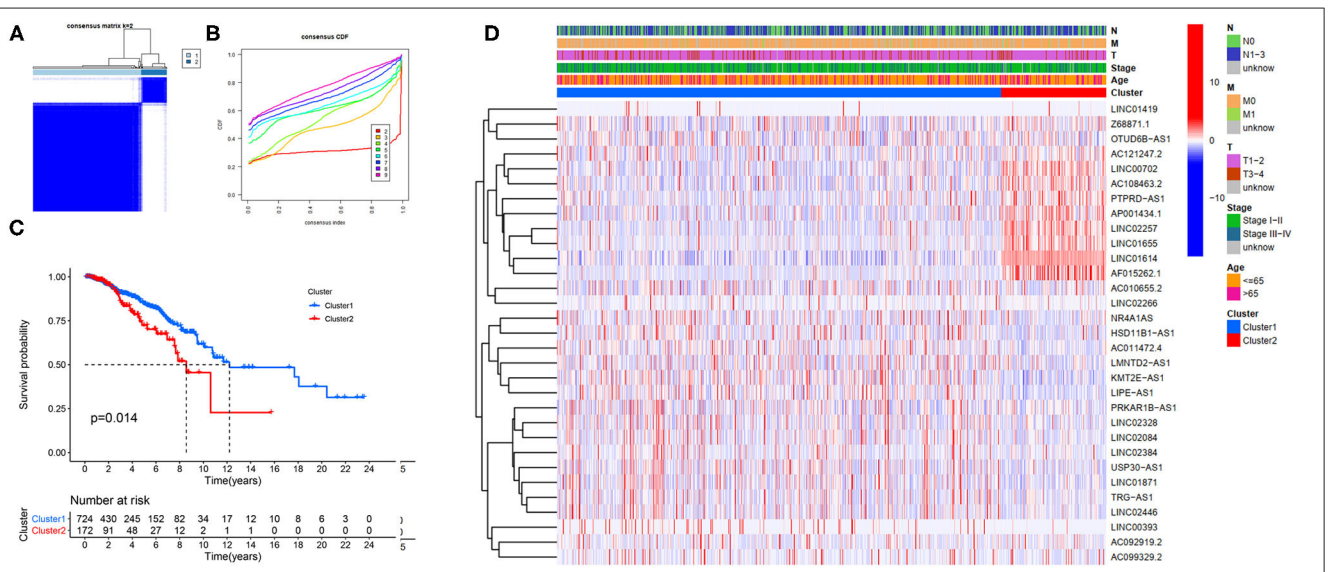


**FIGURE 4 |** Hierarchical consensus clustering based on the prognostic FRLncRNAs. **(A)** Consensus clustering analysis identification of two clusters ($n = 896$); **(B)** Cumulative distribution function (CDF) for $k = 2$–9; **(C)** Kaplan–Meier (K–M) curves for the 896 patients breast cancer (BC) stratified by cluster; **(D)** Heatmap on the prognostic FRLncRNAs ordered by clusters. The association with clusters, survival probability, and clinical information (age, stage, T stage, N stage, and M stage) were investigated.

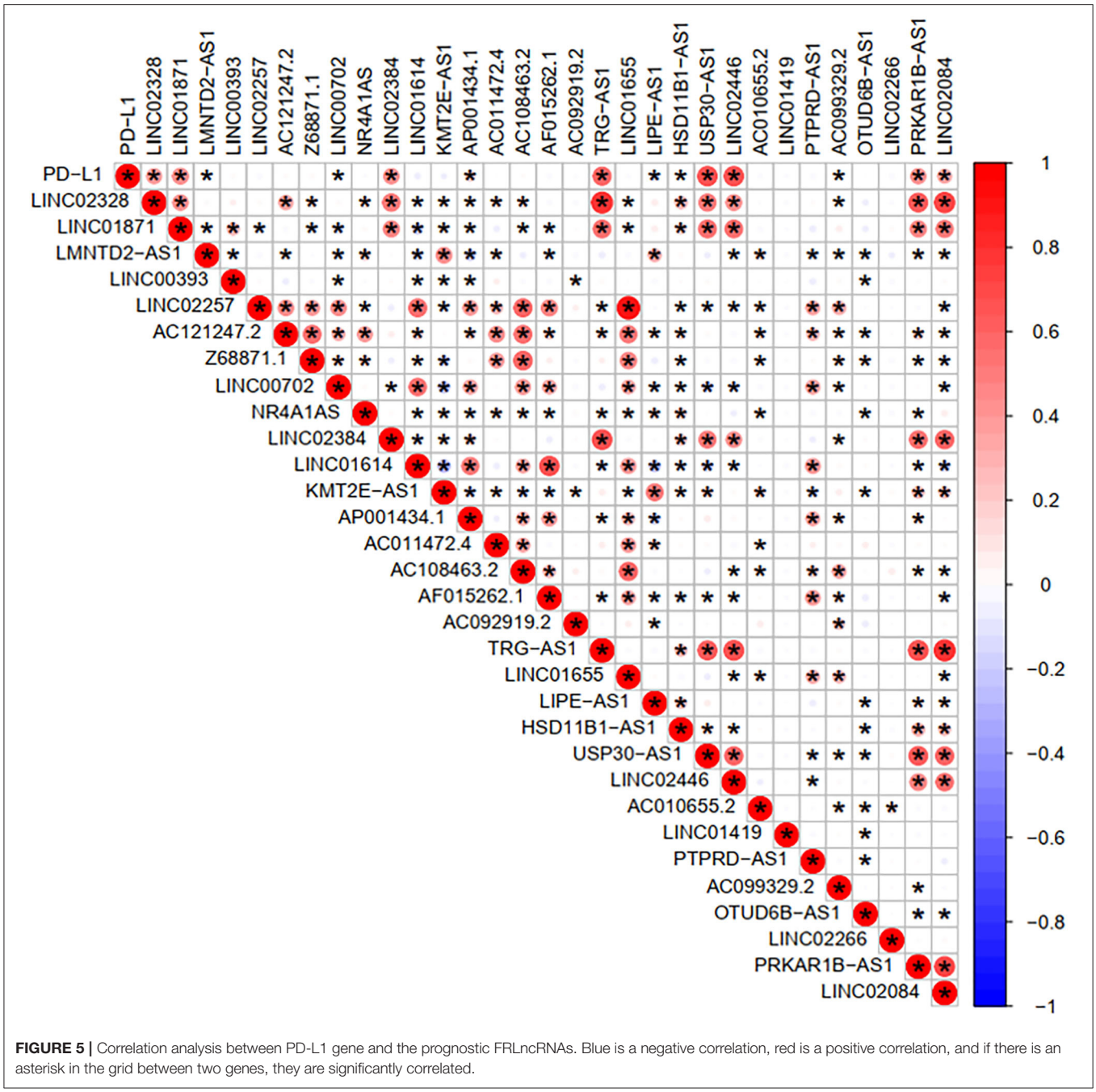


**FIGURE 5 |** Correlation analysis between PD-L1 gene and the prognostic FRLncRNAs. Blue is a negative correlation, red is a positive correlation, and if there is an asterisk in the grid between two genes, they are significantly correlated.

consensus analysis to explore the influence of FRlncRNAs on the occurrence and development of BC from multiple perspectives. Using the similarities in the expression of prognosis-related FRlncRNAs, we choose the value of $k = 2$ as optimal selection (**Figures 4A,B**). Consequently, the two clusters of samples were determined as follow: C1 ($n = 726$, 80.8%) and C2 ($n = 172$, 19.2%). We then applied K–M curves to compare the differences in survival between different clusters and found that C1 tended to carry a good prognosis (**Figure 4C**). Next, we researched whether there was a correlation between the clinical data and clusters (**Figure 4D**). It was obvious that the expressions of FRlncRNAs in the upper right corner of heatmap were upregulated in C2, with a more red color, such as AP001434.1, LINC02257, LINC01655, LINC01614, and AF015262.1. More interestingly, we noticed that most of these FRlncRNAs upregulated in C2 with poor prognosis were also upregulated in tumor tissue, suggesting that these FRlncRNAs may act as tumor promoters to accelerate tumor migration and progression. At the same time, we could also compare whether there were differences in clinical traits in different clusters. **Figure 4D** shows no significance in

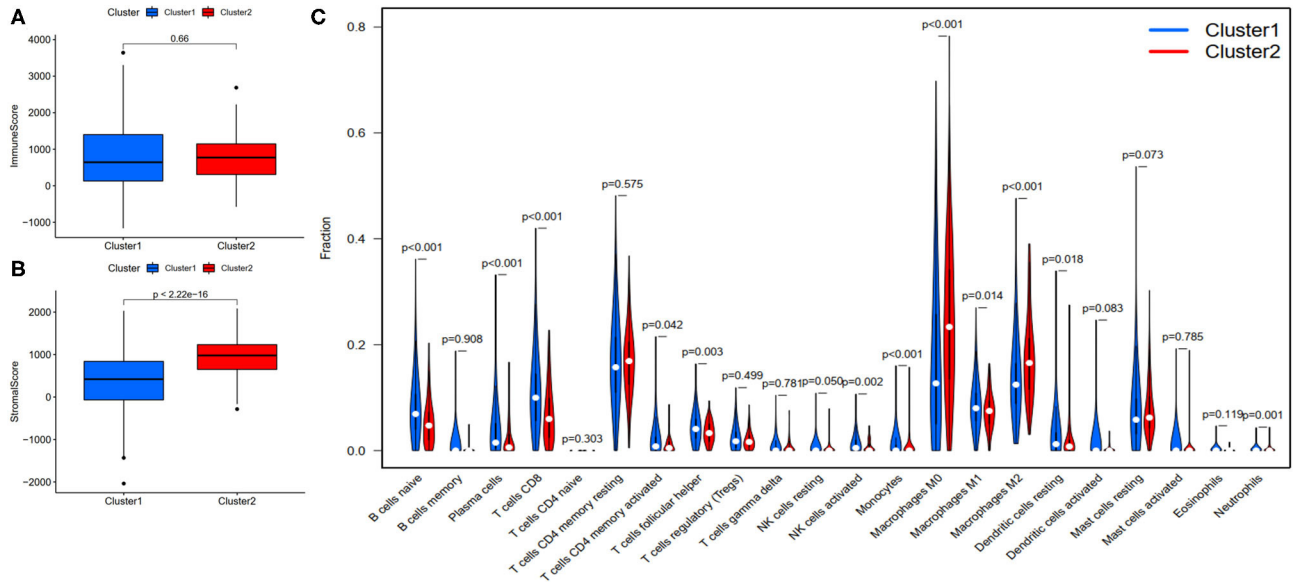


**FIGURE 6 |** Evaluation of the correlation with immune features between clusters. **(A)** Immune score between clusters; **(B)** Stromal score between clusters; **(C)** The violin plot of comparison of 22 types of immune cells between clusters.

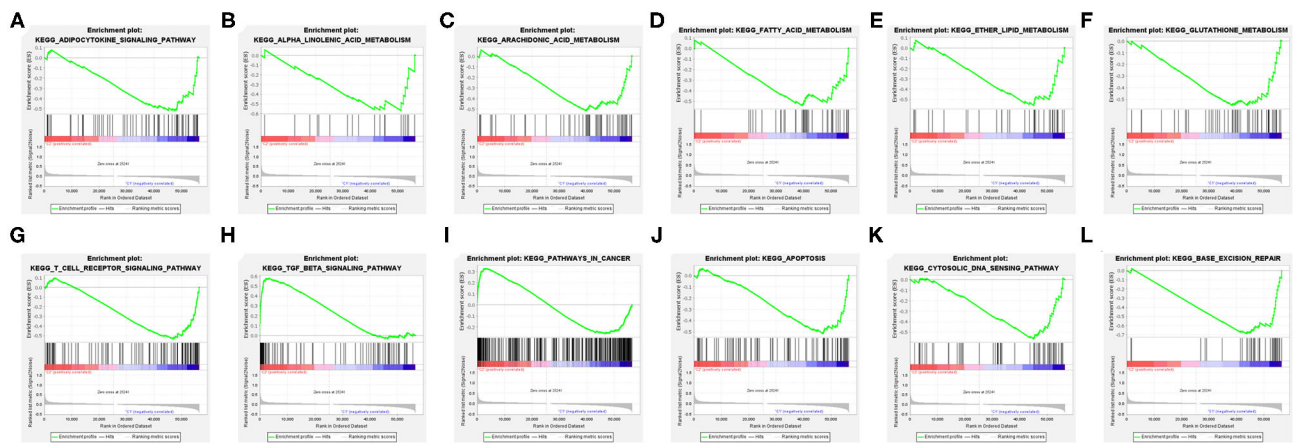


**FIGURE 7 |** Gene Set Enrichment Analysis (GSEA) between the clusters **(A–L)**.

the clinical parameters indicating no differences between these characteristics in the various clusters.

## FRlncRNAs-Based Prognostic Differences Significantly Associated With Immune Features

To investigate the correlation of FRlncRNAs with immune features, we conducted further analysis from the perspective of immunology. The correlation analysis was performed to explore the relationship between prognosis-related FRlncRNAs and PD-L1 gene (**Figure 5**). The result implies that PD-L1 was significantly associated with some FRlncRNAs, such as LINC02328, LINC01871, LMNTD2-AS1, LINC00702, LINC02384, AP001434.1, TRG-AS1, LIPE-AS1, HSD11B1-AS1, USP30-AS1, LINC02446, AC099329.2, PRKAR1B-AS1, and LINC02084. Furthermore, we investigated the differences of immune microenvironment between the clusters. Intriguingly, we found that there was no difference in immune cells between C1 and C2 (**Figure 6A**), whereas C2 was associated with higher stromal scores compared with C1 (**Figure 6B**). Furthermore, violin plot analysis showed that the levels of cell infiltration (**Figure 6C**), such as B cells naïve, plasma cells, T cells CD8, T cells CD4 memory activated, T cells follicular helper, natural killer (NK) cells activated, monocytes, macrophages M1, dendritic cells resting, and neutrophils were higher in C1 than in C2, whereas the levels of macrophages M0 and macrophages M2 were lower in C1 than in C2. Collectively, the turbulent changes of

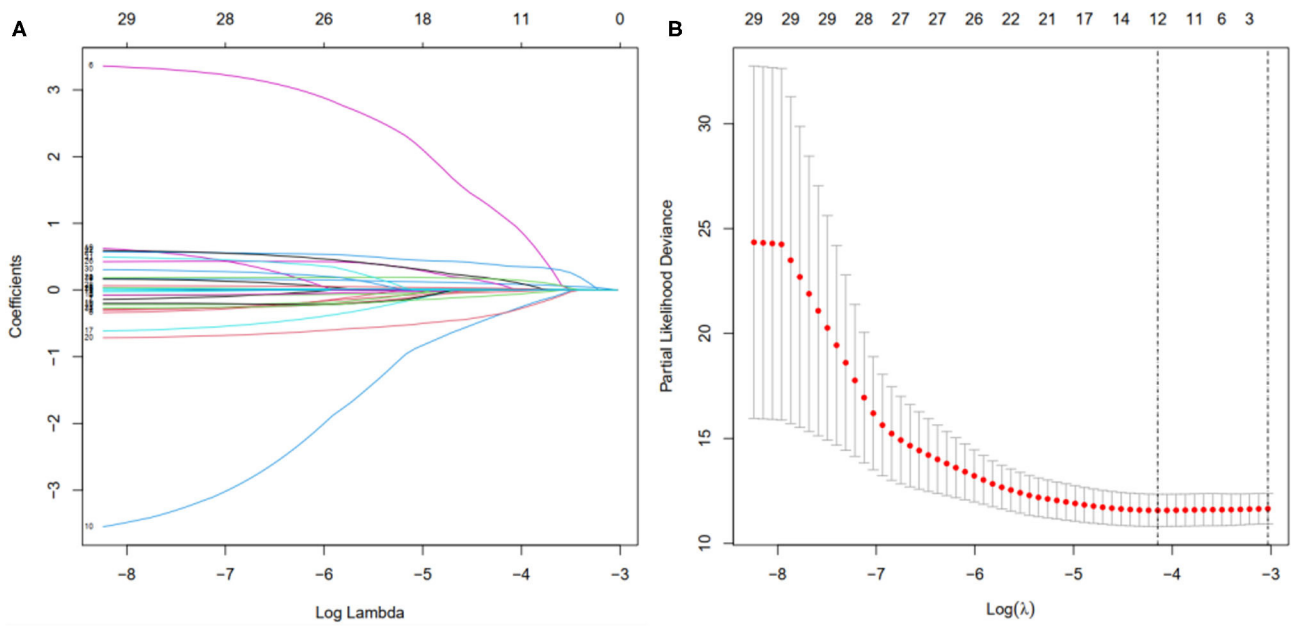


**FIGURE 8 |** Selection of the optimal survival-related LncRNAs by LASSO Cox regression. **(A)** LASSO coefficient profiles of the candidate survival-related LncRNAs. A coefficient profile plot was produced against the log λ sequence. **(B)** Dotted vertical lines were drawn at the optimal values using the minimum criteria.

immune cells in the tumor immune microenvironment may support the conclusion that C2 had a poor prognosis to some extent.

## The Differential Biological States in Clusters Identified by GSEA Analysis

The explanation from the immune point of view made us understand the impact of target FRlncRNAs on tumor microenvironment more thoroughly. We further conducted GSEA analysis to explore the biological signal pathways with obvious differences between the clusters. Some metabolism-related pathways, in relation to "adipocytokine signaling pathway," "linolenic acid metabolism," "arachidonic acid metabolism," "fatty acid metabolism," "ether lipid metabolism," and "glutathione metabolism" were significantly enriched in C1, among which the pathways related to lipid metabolism accounted for the majority (**Figures 7A–F**). In addition, we found that immune-associated pathway was enriched, such as "T cell receptor signaling pathway" and "TGF-beta signaling pathway" (**Figures 7G,H**). Besides, the enrichment of some carcinogenic pathways was also exposed, such as "pathway in cancer," "apoptosis," "cytosolic DNA sensing pathway," and "base excision repair" (**Figures 7I–L**).

## Establishment and Validation of the Prognostic Signature in the Patients With BC

First, according to the ratio of 1:1, the patients with BC were randomly divided into a training set and a validation set, both of which included 448 patients with complete information, as described in the previous literature (26). Second, according to 31 prognostic FRlncRNAs calculated by univariate Cox regression analysis, we then performed LASSO regression analysis to pick out the optimal prognosis-related FRlncRNAs with nonzero coefficients (**Figures 8A,B**). Consequently, 12-FRLncRNA signature was determined. The formula of the final model was listed as follows: risk score = −0.250*expression of LINC01871+0.091*expression of LINC00393+1.058* expression of AC121247.2-0.330* expression of LINC02384-0.336* expression of LIPE-AS1-0.072* expression of HSD11B1-AS1+0.361* expression of AC010655.2+0.018* expression of LINC01419+0.051* expression of PTPRD-AS1+0.137* expression of AC099329.2+0.024* expression of OTUD6B-AS1+0.157* expression of LINC02266. We then calculated the risk scores and divided patients into high- and low-risk groups by the median risk score in the training set (**Figure 9A**). The relationships between the survival status and survival times of patients with BC ranked by risk scores were depicted in **Figure 9B**. In addition, a heatmap was plotted to show the expression profiles of 12 FRLncRNAs (**Figure 9C**). To study the relationship between the risk score and survival probability, K–M curves were carried out in **Figure 9D**. Patients in the high-risk group had a worse survival probability, whereas those in the low-risk group had a better survival probability. Furthermore, the ROC curve was plotted to verify the predictive ability of the models, whose AUC of the ROC curves was 0.693, revealing a positive prognostic ability (**Figure 9E**).

To verify the results of the training set, we employed the same models on the patients in the testing set (**Figure 10**). The results showed that the high-risk patients showed significantly worse survival probability than the low-risk groups (**Figure 10D**),

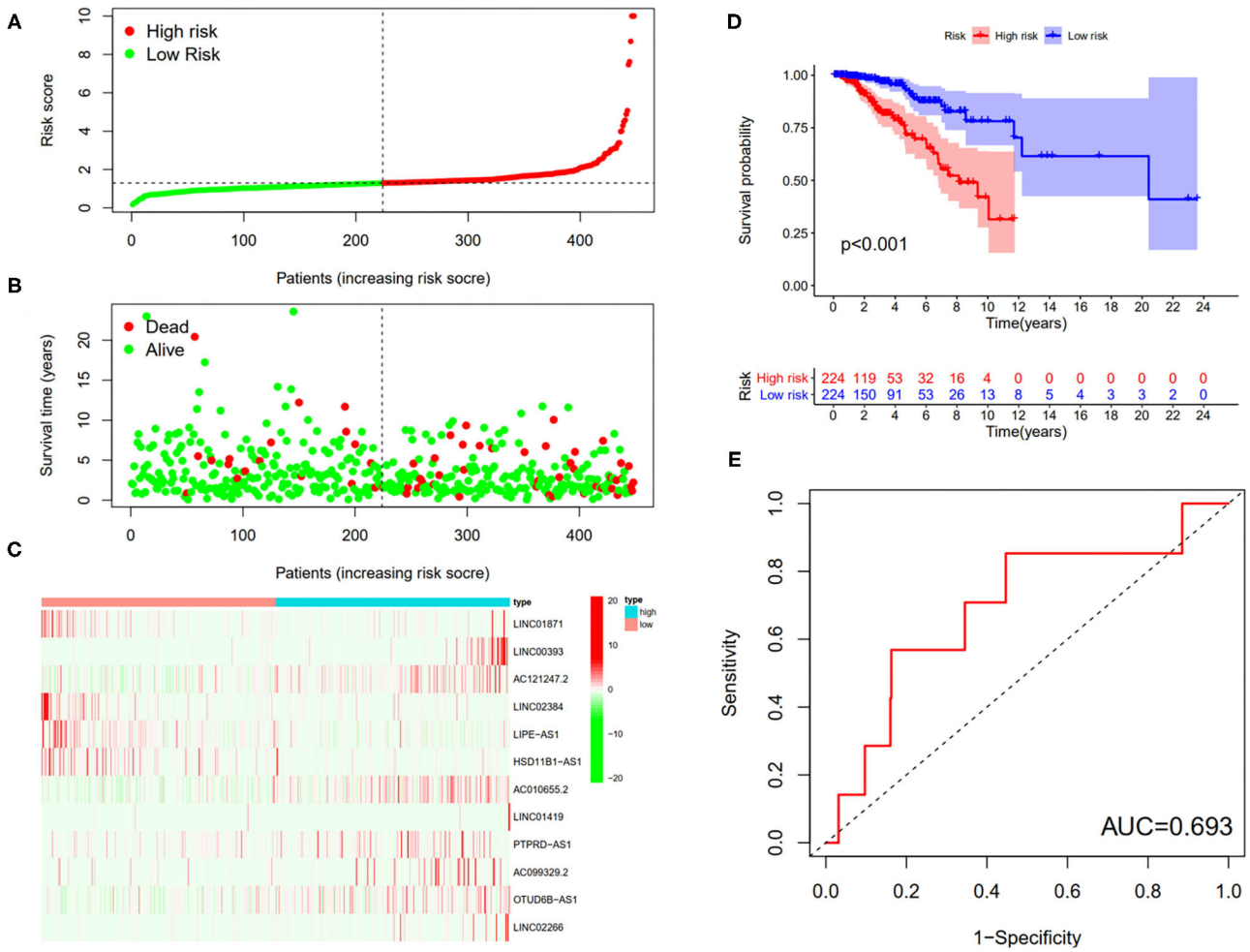


**FIGURE 9 |** The 12-LncRNA signature in the training set. **(A)** The distribution of risk score; **(B)** the survival time and status of patients; **(C)** the bottom shows the heatmap of 12-LncRNA expression profile. Colors from red to green indicate decreasing expression level from high to low; **(D)** the K–M curves for high- and low-risk groups. Purple color represents the low-risk group, whereas red color represents the high-risk group; **(E)** receiver operator characteristic (ROC) curves for patients with BC in testing set. AUC, area under the curve.

whose AUC of ROC was 0.655 (**Figure 10E**), which suggested that the prognostic model could satisfactorily predict the prognosis of patients with BC. In addition, the prognostic values of the risk score of patients with BC in the GSE69031 cohort were calculated. The results indicated that patients in the high-risk group showed a worse prognosis than the low-risk patients (**Figure 10F**). The AUC values of signature to predict the OS was 0.681 (**Figure 10G**). Generally, our signature showed satisfactory performance in the independent cohorts, which indicated that these signatures are robust prognostic biomarkers.

## Exploration of Risk Score as an Independent Prognostic Factor

In view of the complexity of a variety of clinical factors, further univariate and multivariate Cox regression analyses were performed to explore the independence of risk scores (**Figures 11A,B**). Collectively, the risk score was an independent prognostic factor, independent of other clinical factors, in both the training and validation sets ($P < 0.05$). Generally, our research indicated that the patients with higher risk scores were a worse prognosis.

## Model Validation of Clinical Grouping

First of all, we divided patients into the two categories based on age >65 and age <65, T1-2 and T3-4, N0 and N1-3, M0 and M1, Stage I-II, and Stage III-IV. By employing the same models, we plotted K–M survival curves for each subgroup (**Figure 12**). For example, in **Figures 12C,D**, patients with T stage at T1 and T2 showed statistically significant differences in their survival curves, and patients with T3 and T4 also showed statistically significant differences in their survival curves, indicating that the risk score was applicable to the patients with different T stages ($P < 0.05$). Similarly, in other grouping variables, such as age<65 and age> 65 (**Figures 12A,B**), in N0 and N1–3 patients (**Figures 12E,F**), in early and late-stage patients (**Figures 12G,H**), value of $P < 0.05$, indicating that the risk score of this gene was applicable

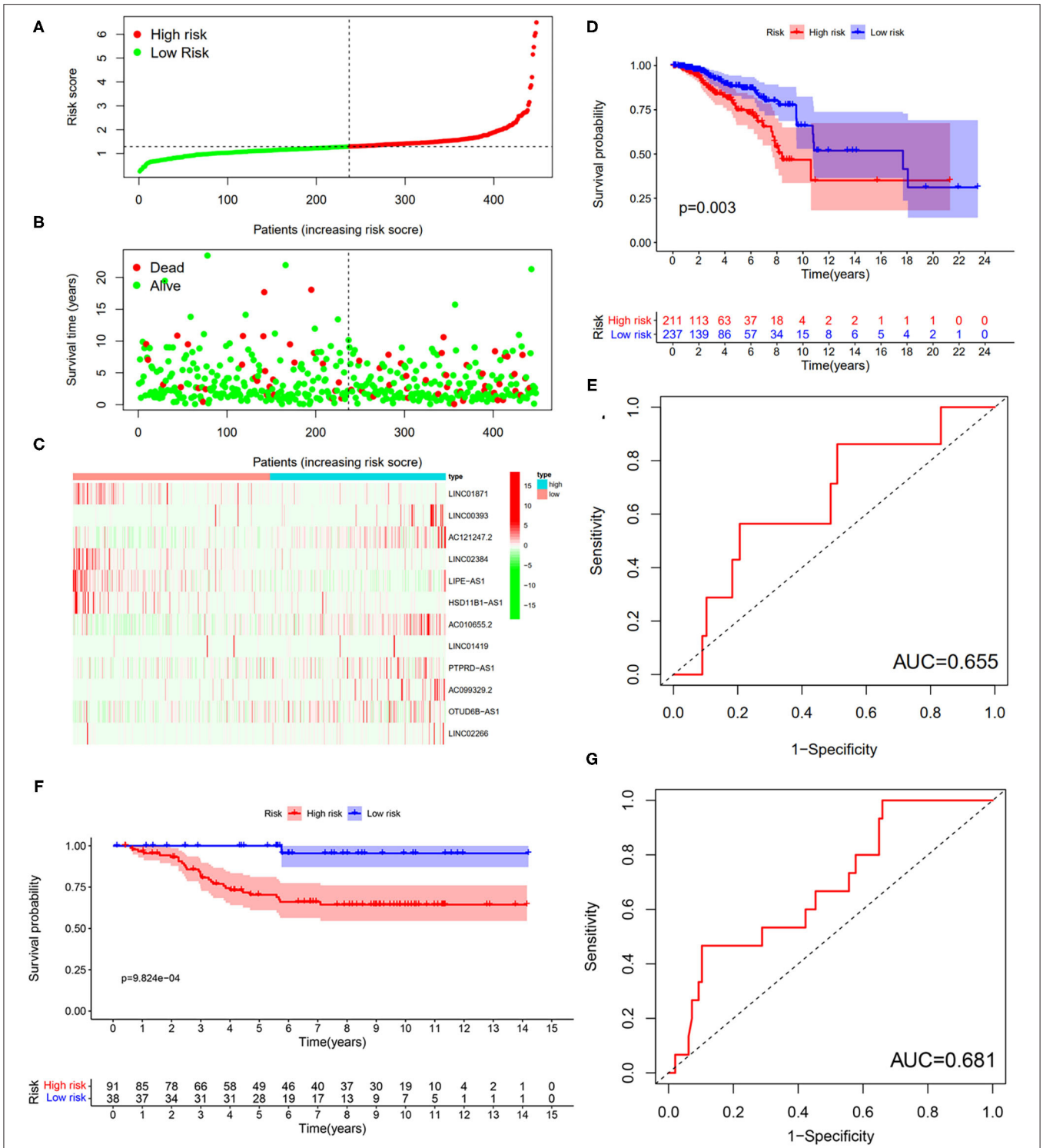


**FIGURE 10 |** Testing for the 12-LncRNA signature. **(A)** The distribution of risk score; **(B)** the survival time and status of patients; **(C)** the bottom shows the heatmap of 12-LncRNA expression profile. Colors from red to green indicate decreasing the expression level from high to low; **(D)** the K–M curves for high- and low-risk groups. Purple color represents the low-risk group, whereas red color represents the high-risk group; **(E)** ROC curves for patients with BC in the testing set. **(F)** The K–M curves for the high- and low-risk groups in GSE69031 cohort. Purple color represents the low-risk group, whereas red color represents the high-risk group; **(G)** ROC curves for patients with BC in GSE69031 cohort. AUC, area under the curve.

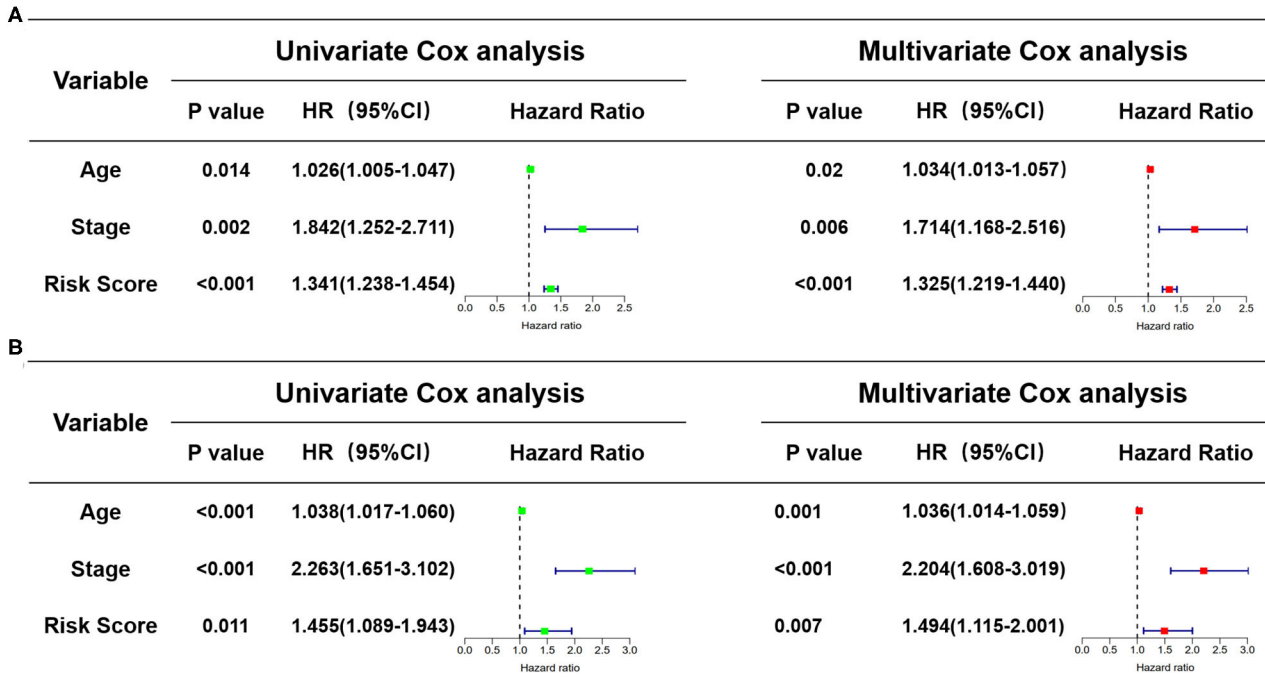


**FIGURE 11 |** Identification of the independence of risk score prognostic model by the Cox regression analyses. **(A)** The univariate and multivariate Cox regression analyses of the risk score in the training cohort. **(B)** The univariate and multivariate Cox regression analyses of the risk score in the test cohort. The green boxes represent risk factors of the univariate Cox regression analysis and the red boxes represent risk factors of the multivariate Cox regression analysis.

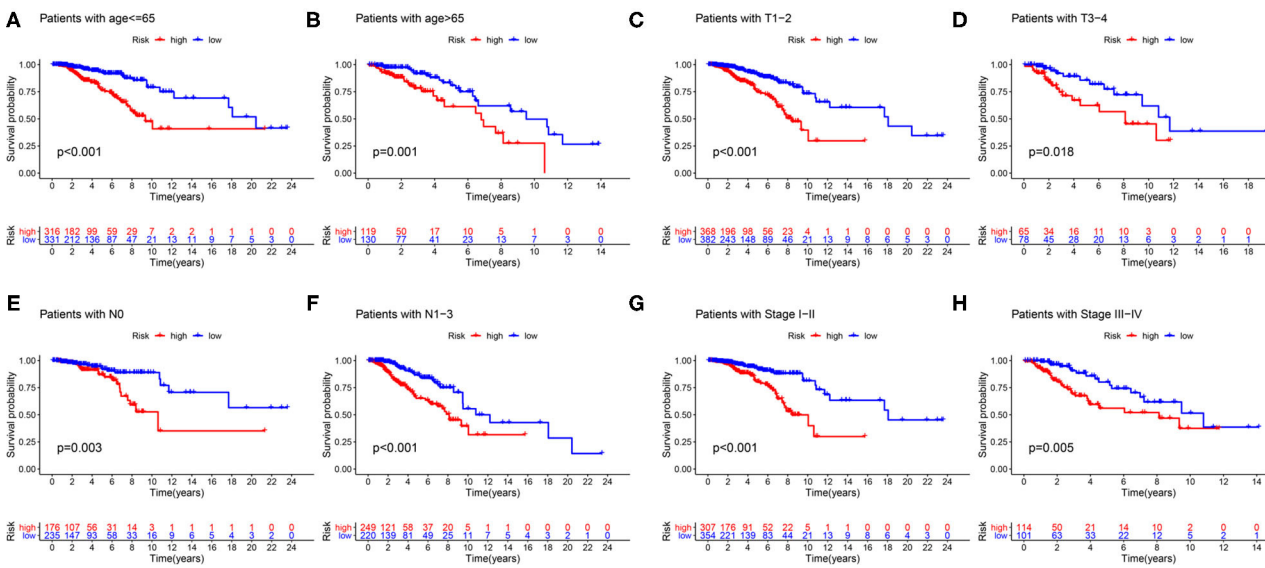


**FIGURE 12 |** Validation of the risk score prognostic model among the different clinical groups. **(A)** Age ≤65; **(B)** age >65; **(C)** T1-2; **(D)** T3-4; **(E)** N0; **(F)** N1–3; **(G)** stage I–II; **(H)** stage III–IV. The blue lines represent the low-risk groups, the red lines represent the high-risk groups.

to the different groups of patients. Nevertheless, among M0 and M1 stage patients, the difference was not statistically significant due to the small number of M1 stage patients, which required further data to verify. Overall, in the subgroup analyses, we confirmed that the high-risk patients were a worse prognosis than the low-risk patients in all subgroups (all $p < 0.05$).

## Relationships Between Risk Scores and Clinical Variables

Through a comprehensive analysis of the heatmap, we can easily distinguish the high-risk and low-risk FRLncRNAs (**Figure 13**). Specifically, LINC01871, LINC02384, LIPE-AS1, and HSD11B1-AS1 were highly expressed in low-risk groups, demonstrating that these FRLncRNAs were low-risk LncRNAs. On the contrary,

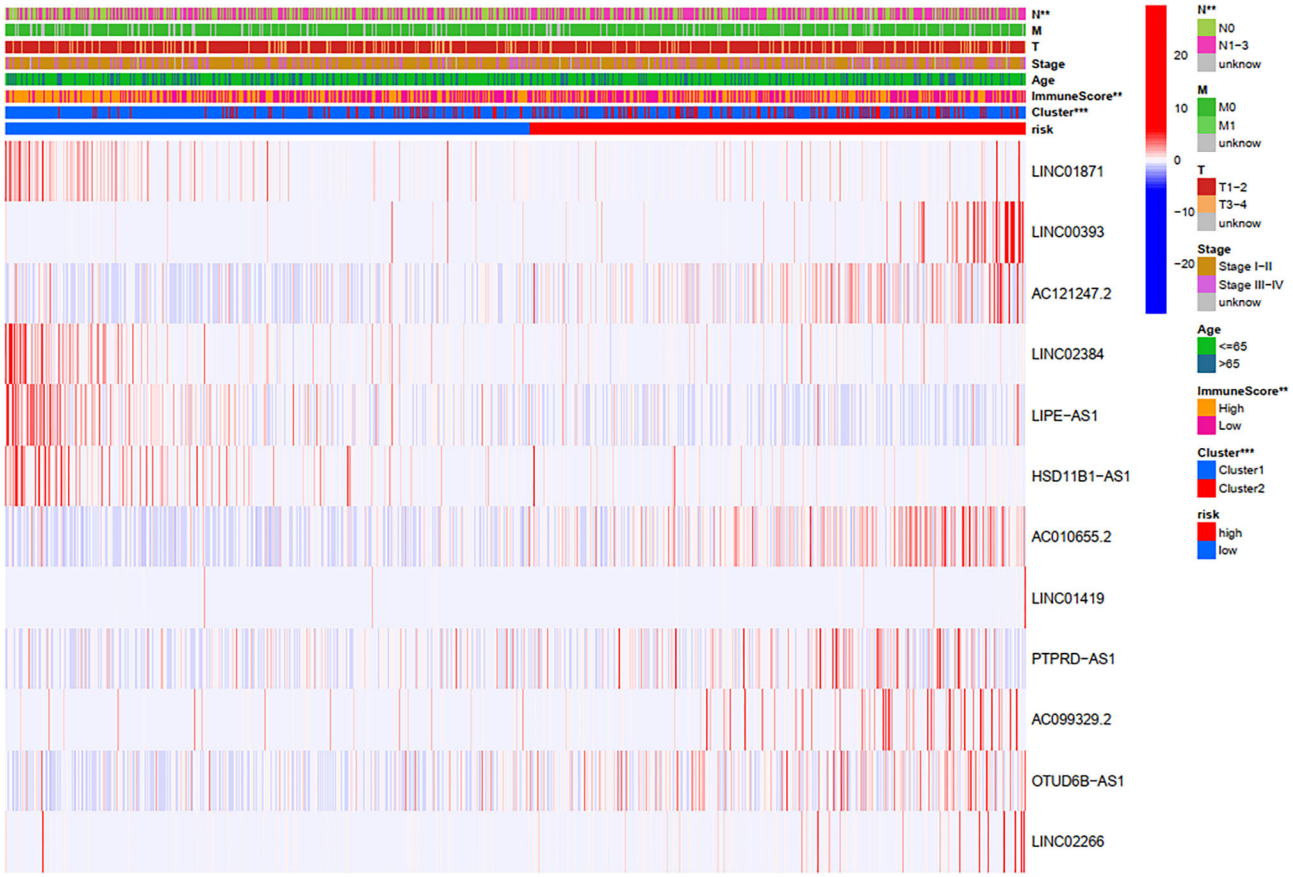


**FIGURE 13 |** Comprehensive analysis of the differences between the high- and low-risk groups.

LINC00393, AC121247.2, AC010655.2, LINC01419, PTPRD-AS1, AC099329.2, OTUD6B-AS1, and LINC02266 were highly expressed in the high-risk groups, demonstrating that these FRLncRNAs were high-risk LncRNAs. At the same time, we could also compare whether the clinical traits were different between the high- and low-risk groups. It could be seen that there were differences among the N stages, immune scores, and clusters ($P < 0.05$). More specifically, patients in the low-risk groups were more likely to be assigned to C1 and had a higher immune score, which were consistent with a better prognosis.

## DISCUSSION

Breast cancer, as the most common female malignant tumor in the world, poses a serious threat to the life and health of women and has become a key global public health problem (1). To improve our understanding of pathogenesis and internal environmental changes in BC, we have made some efforts. Similar to a recent study (27), in this study, we downloaded and integrated the gene expression data of the transcriptome and the clinical information of patients with BC from TCGA dataset, starting with the downstream RNAs of the ferroptosis-related gene. However, unlike the previous study, unsupervised clustering analysis revealed that C1 tended to carry a better prognosis and held a higher infiltration level of immune cells than C2 in our study. Furthermore, GSEA analysis between C1 and C2 unveiled that C1 was enriched in lipid-metabolism-related pathway and immune-associated pathway, which was consistent with the outcomes of cluster analysis. Additionally, 12-FRLncRNA signature involved with LINC01871, LINC00393, AC121247.2, LINC02384, LIPE-AS1, HSD11B1-AS1, AC010655.2, LINC01419, PTPRD-AS1, AC099329.2, OTUD6B-AS1, and LINC02266 could accurately predict the prognosis of patients with BC, which was confirmed by the training set, validation set, and set from GEO database. Specially, PD-L1 was significantly associated with some FRLncRNAs. Combined with cluster analysis, prognostic model, and clinical characteristics, further analysis disclosure that patients in the low-risk groups were more likely to be assigned to C1 and had a higher immune score, which were in line with a better prognosis. These findings are based on ferroptosis, immune microenvironment, and prognosis of BC, which will provide theoretical guidance for the scientific application of ferroptosis and immunotherapy in BC.

According to the comprehensive analysis of 12-FRLncRNA signature, LINC01871, LINC02384, LIPE-AS1, and HSD11B1-AS1 were low-risk FRLncRNAs, whereas LINC00393, AC121247.2, AC010655.2, LINC01419, PTPRD-AS1,

AC099329.2, OTUD6B-AS1, and LINC02266 were high-risk FRLncRNAs. For LINC01871, many studies have confirmed that it may be a protective factor of BC, promoting cancer cells death through many pathways and mechanisms, such as autophagy, which is consistent with our study results (7, 28, 29). Therefore, it can be speculated that IFNG co-expressed with LINC01871 was associated with promoting cell apoptosis by ferroptosis. As for LIPE-AS1, Zhang et al. (30) reported that overexpression of LIPE-AS1 in cervical cancer can promote cell proliferation, migration, epithelial mesenchymal transition (EMT), and inhibit cell apoptosis, which can be reversed by LIPE-AS1 knockdown or mir-195-5p/mitogen activated protein kinase (MAPK) signaling pathway activation.

Regarding LINC00393, Zhao et al. found that BC cells treated with CREBBP/EP300 bromodomain inhibitors can induce the downregulation of H3K27 acetylation level, along with downregulation of LINC00393 expression, which can inhibit the growth of BC cells, indicating it may therefore be a candidate for gene therapy approaches to BC (31). Besides, we also noticed that LINC00393 was coexpressed with SLC7A5, a protein in the amino acid transporter family, which is necessary for the growth of BC cells in a cell-dependent manner (32). In particular, SLC7A11 belonging to the same family is also closely associated with ferroptosis. Studies have shown that the deletion of SLC7A11 gene results in lipid peroxidation, which in turn leads to ferroptosis in some cells or tissues (33–36). In addition, LINC01419 has been repeatedly demonstrated to be upregulated in solid tumors and to promote proliferation and migration of malignant tumors through multiple pathways, such as PI3K/Akt signaling pathway, which is similar to our results and worthy of further study on the association of ferroptosis in BC (37–41). In addition, it has been reported that the overexpression of OTUD6B-AS1 makes hepatocellular carcinoma cells more aggressive through the GSKIP/Wnt/β-catenin signaling pathway (42). As for other FRLncRNAs, there are few studies on ferroptosis or malignant, and further studies are required.

More importantly, the findings of unsupervised clustering analysis showed that there were two different immune microenvironments. We found patients in the low-risk groups were more likely to be assigned to C1 and had a higher levels of immune cell infiltration, such as B cells naïve, plasma cells, T cells CD8, T cells CD4 memory activated, T cells follicular helper, NK cells activated, monocytes, macrophages M1, dendritic cells resting, and neutrophils, whereas the levels of macrophages M0 and macrophages M2 were higher in C2. In short, low-risk patients showed "hot tumor," surrounded by immune-effector cells that are sensitive to immunotherapy, while high-risk patients showed "cold tumor," which impair the effectiveness of immunotherapy (43). Morever, according to the results of GSEA analysis, we found that lipid-metabolism and oxidative stress pathways, such as "adipocytokine signaling pathway," "linolenic acid metabolism," "arachidonic acid metabolism," "fatty acid metabolism," "ether lipid metabolism," and "glutathione metabolism," were enriched in C1, as were immune-related pathways, such as "T cell receptor signaling pathway" and "TGFβ signaling pathway." More interestingly, the studies have shown that when immunotherapy boosts the activity of T cells, it will increase the level of oxidized lipids in tumor cells, leading to the emergence of ferroptosis, which in turn will enhance the killing effect of immunotherapy on cancer (44, 45). Therefore, we can guess the following steps: C1, which is attributed to most low-risk patients, increases the level of lipid oxidation metabolism and induces ferroptosis; ferroptosis heats up the tumor immune microenvironment, activates immune-related pathways, wakes up immune cells, transforms "cold tumor" into "hot tumor," upregulates the expression of PD-L1, enhancing the sensitivity of immunotherapy; the phenomenon of immune mobilization in turn promotes ferroptosis, forming a positive cycle. Collectively, for those tumors with insufficient induction of ferroptosis, the combination of ferroptosis sensitizers and immune checkpoint inhibitors to restore ferroptosis and improve the efficacy of immunotherapy may be a very promising combination therapy strategy. However, further studies are needed to determine the degree of induction and the degree of ferroptosis.

Our study comprehensively analyzed the relationships between ferroptosis, immune microenvironment, and prognosis of BC, which had a certain guiding significance for the designation of clinical immunotherapy combined strategy. However, there were certain limitations that existed in this study. First of all, we only used TCGA data to construct and verify our prognosis model, which lacked both revalidation from other public databases and validation from real-world data. Second, due to the lack of cell experiments or animal experiments to verify the expression of target FRLncRNAs or immune mechanisms, further identification and verification of therapeutic targets were needed. Third, only age and stage were included in the risk score independence analysis, which may increase the error of results due to the lack of clinical information.

## CONCLUSION

In summary, our study defined a novel 12-FRLncRNA signature associated with ferroptosis, which could accurately predict the prognosis in patients with BC. The comprehensive analysis of ferroptosis, immune microenvironment, and patient prognosis depends our understanding of the role of ferroptosis in shaping tumor microenvironment, which was of positive significance for basic research and clinical work in the future.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

## AUTHOR CONTRIBUTIONS

ZX and SJ performed the data analysis. ZX, KF, and JM wrote the manuscript. JM, DT, and KF contributed to the manuscript revision. CY and DT contributed to the literature search and data extraction. ZX, SJ, and KF conceived and designed the

study. All authors have read and approved the final version of the manuscript.

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# Survival Outcomes After Breast-Conserving Therapy Compared With Mastectomy for Patients With Early-Stage Invasive Micropapillary Carcinoma of the Breast: A SEER Population-Based Study

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**Background:** Invasive micropapillary breast carcinoma (IMPC) is a relatively rare pathological type of invasive breast cancer. Little is currently known on the efficacy and safety of breast-conserving treatment (BCT, lumpectomy plus postsurgical radiation) compared with mastectomy in women diagnosed with early-stage IMPC. Accordingly, we sought to investigate the long-term prognostic differences between BCT and mastectomy in patients with T1-3N0-3M0 invasive micropapillary breast carcinoma using data from the Surveillance, Epidemiology, and End Results (SEER) database.

**Materials and Methods:** We retrospectively analyzed 1,203 female patients diagnosed with early-stage IMPC between 2004 and 2015 from the SEER database. The impact of different surgical approaches on patient prognosis was assessed by the Kaplan-Meier method and Cox proportional risk models.

**Results:** A total of 609 and 594 patients underwent mastectomy and BCT, respectively. Compared with patients who underwent a mastectomy, patients in the BCT group were older and had lower tumor diameters, lower rates of lymph nodes metastasis, and higher rates of ER receptor positivity and PR receptor positivity ($p < 0.05$). Kaplan-Meier plots showed that the overall survival (OS) and breast cancer-specific survival (BCSS) were higher in the BCT group than in the mastectomy group. In subgroup analysis, patients with T2 stage in the BCT group had better OS than the mastectomy group. Multivariate analysis showed no statistical difference in OS and BCSS for patients in the mastectomy group compared with the BCT group (hazard ratio (HR) = 0.727; 95% confidence interval (95% CI) 0.369–1.432, $p = 0.357$; HR = 0.762; 95% CI 0.302–1.923, $p = 0.565$; respectively). During the multivariate analysis and stratifying for the T stage, a better OS was found for patients with T2 stage in the BCT group than the mastectomy group (HR = 0.333, 95% CI: 0.149–0.741, $p = 0.007$). There was no significant difference in OS for patients with T1 and T3 stages between the BCT and mastectomy groups ($p > 0.05$).

**Conclusion:** In women with early-stage IMPC, BCT was at least equivalent to mastectomy in terms of survival outcomes. When both procedures are feasible, BCT should be recommended as the standard surgical treatment, especially for patients with T2 disease.

**Keywords: invasive micropapillary carcinoma, SEER, mastectomy, BCT, survival**

## INTRODUCTION

Invasive micropapillary carcinoma (IMPC) is a rare subtype of breast cancer (1). According to the current literature, it accounts for approximately 3%–6% of all breast cancers (2, 3). Fisher et al. (4) first introduced the concept of "micropapillary structures" in breast tumors in 1980, when they observed a "mulberry-like appearance" under electron microscopy. In contrast, the definition of IMPC was first established by Siriaunkgu and Tavassoli in 1993 (5). IMPC has been characterized with low incidence and high malignancy rates and a marked tendency for lymphatic duct infiltration, regional lymph node metastasis, and local recurrence. IMPC is also widely recognized for its specific morphological structure, aggressive biological behavior, and poor prognosis (3, 6, 7). In the latest WHO (2003) classification of breast tumors, IMPC has been classified as a special type of breast cancer (2). Due to its rarity, the impact of surgical modalities on the prognosis of early-stage IMPC has not been determined. Moreover, there is a paucity of recommendations on the choice of surgical modality for IMPC in clinical guidelines.

Over the past 30 years, several randomized trials (RCTs) on BCT and mastectomy have concluded that these two treatments led to the same prognosis in breast cancer patients (8–10). However, these trials were initiated in the 1970s and 1980s. In recent decades, improvements in screening equipment and instruments, as well as in the systemic treatment of breast cancer and radiation therapy, have improved the detection and survival rates of patients with early-stage breast cancer, which has facilitated the gradual replacement of breast-conserving surgery by wide local excision as a better surgical option (11, 12). Recently, several large sample studies from different countries and regions have shown that BCT had higher survival rates than mastectomy for patients with early-stage breast cancer (13–16). Current clinical guidelines recommend breast-conserving surgery for patients with stages I and II breast cancer when contraindications for breast-conserving surgery are ruled out. After downstaging with neoadjuvant chemotherapy, BCT can also be considered for some patients with stage III disease (17). Nonetheless, the prognosis of IMPC patients undergoing BCT and mastectomy is unclear. The high incidence of lymph node metastasis and lymphovascular invasion associated with IMPC makes it challenging for surgeons who choose BCT.

The present study used the Surveillance, Epidemiology, and End-results (SEER) database of the US National Cancer Institute registry to determine the differences in patient survival for each treatment modality. This database can be used to compare the prognostic differences associated with different treatments for various cancers due to its large sample size and long-term follow-up data. Using the SEER database, we sought to assess the survival differences between BCT and mastectomy in T1-3N0-3M0 IMPC patients.

## MATERIALS AND METHODS

### Data Sources and Patient Selection

All patient information, including demographics, diagnosis time, marital status, tumor features, type of surgery, radiotherapy, survival months, and survival status, were obtained from the SEER database. Women diagnosed with unilateral invasive micropapillary carcinoma (ICD-O-3 Code 8507/3) from 2004 to 2015 were selected. The inclusion criteria consisted of breast cancer patients staged T1-3N0-3M0 based on the sixth edition of the American Cancer Commission (AJCC) staging system; breast cancer is the first primary tumor; and patients with complete demographic information and laboratory results for estrogen receptor (ER) and progesterone receptor (PR) positivity. Patients who underwent breast-conserving surgery without radiotherapy were excluded from the study. Finally, 1,203 patients were included in this study. According to the surgical approach, the whole cohort was divided into two groups: breast-conserving therapy (BCT, $n = 594$) and mastectomy ($n = 609$) (**Figure 1**).

### Statistical Analysis

The Pearson chi-square test and Fisher exact probability method were used to compare the characteristics of the BCT and mastectomy groups. The overall survival (OS) and the breast cancer-specific survival (BCSS) of the two groups were analyzed by Kaplan-Meier and log-rank test. In addition, after stratifying for the T and N stages based on the sixth edition of the AJCC staging system, the survival results of the two groups were analyzed and compared. Cox proportional hazards model was used to calculate 95% confidence interval (CI) and hazard ratio (HR) of OS and BCSS. All the tests were bilateral, and $p < 0.05$ was used to denote statistical significance. All analyses were performed using R language software (http://www.R-project.org, The R Foundation).

## RESULTS

### Patient Characteristics

One thousand two hundred three patients with IMPC (T1-3N0-3M0) were analyzed in our study, among which 594 (49.4%) and

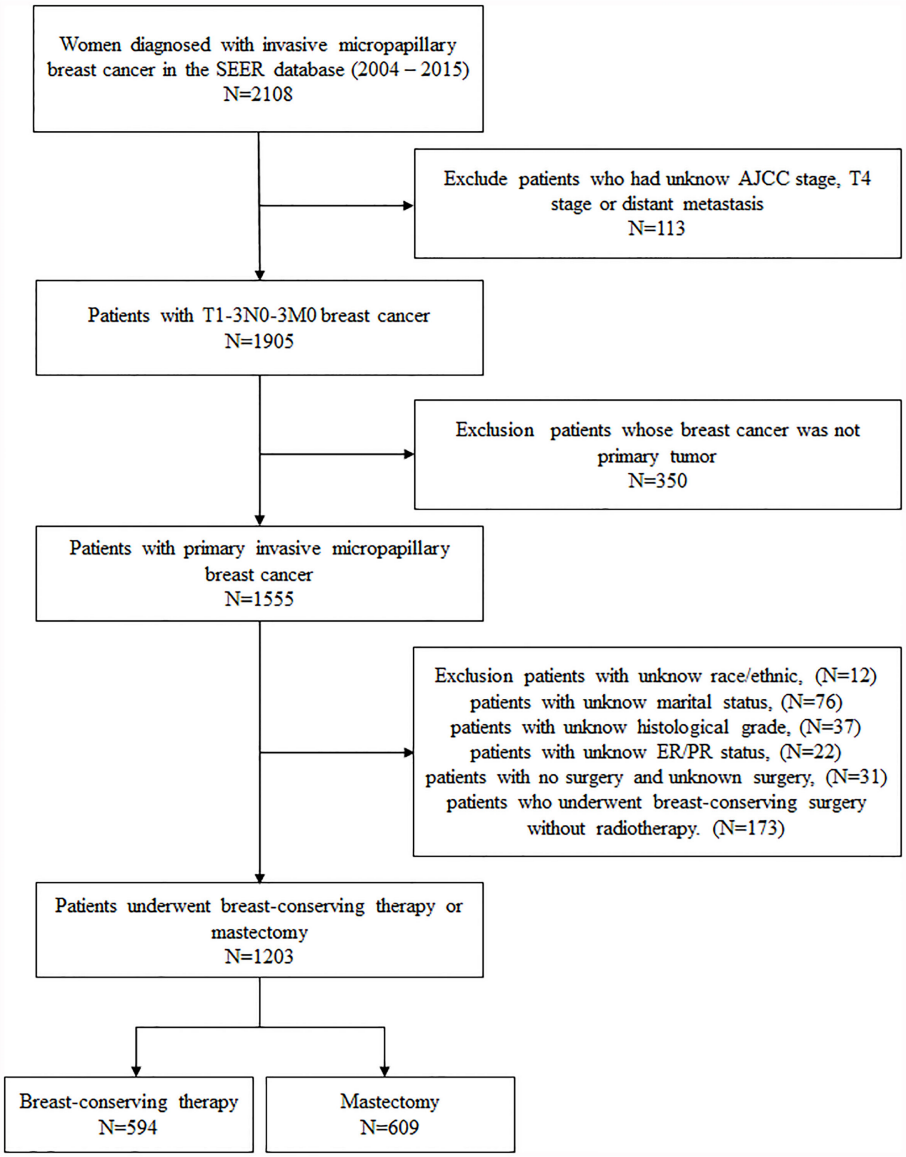


**FIGURE 1** | Flow chart of the study cohort.

609 (50.6%) received BCT and mastectomy, respectively (**Table 1**). The median follow-up time was 57.49 months. Most patients in the BCT group were ≥50 years old (86.2% *vs.* 67.5%), white (78.8% *vs.* 77.0%), and had lower AJCC stage (52.9% *vs.* 23.0%). The BCT group also had a lower proportion of larger-sized tumors (>2 cm) (73.4% *vs.* 42.9%, $p < 0.001$) and low lymph node metastasis rate (64.0% *vs.* 34.2%, $p < 0.001$) and chemotherapy rate (59.3% *vs.* 35.5%, $p < 0.001$) than the mastectomy group. Moreover, a higher percentage of patients with positive ER and PR receptors were found in the BCT group than the mastectomy group (93.3% *vs.* 86.7%, $p < 0.001$; 83.3% *vs.* 75.7%, $p < 0.001$, respectively).

## Survival Outcomes Between Mastectomy Group and BCT Group in Overall and Subgroup Analysis

In our study, Kaplan-Meier curves were used to access the OS and BCSS of patients in the entire cohort and subgroups of patients stratified for T and N stages. Patients who underwent BCT had significantly improved OS and BCSS ($p < 0.05$) than those who received mastectomy (**Figure 2**). After stratifying IMPC patients according to T and N stages, the OS of T2-stage patients who received BCT was better than that of patients who received mastectomy, and there was no statistical difference in other stages. Moreover, the BCSS of IMPC patients treated

**TABLE 1** | Comparison of baseline characteristics of early-stage IMPC between BCT and mastectomy groups.

| Characteristics | Patients, No. (%) | | *p*-value |
|---|---|---|---|
| | BCT | Mastectomy | |
| **Age (years)** | | | <0.001 |
| <50 | 82 (13.8) | 198 (32.5) | |
| 50–64 | 243 (40.9) | 226 (37.1) | |
| 65–80 | 235 (39.6) | 157 (25.8) | |
| >80 | 34 (5.7) | 28 (4.6) | |
| **Race** | | | 0.595 |
| White | 468 (78.8) | 469 (77.0) | |
| Black | 63 (10.6) | 76 (12.5) | |
| Others | 63 (10.6) | 55 (10.5) | |
| **Marital status** | | | 0.492 |
| Married | 352 (59.3) | 349 (57.3) | |
| Single | 242 (40.7) | 260 (42.7) | |
| **Grade** | | | 0.016 |
| I | 56 (9.4) | 45 (7.4) | |
| II | 345 (58.1) | 320 (52.5) | |
| III | 189 (31.8) | 232 (38.1) | |
| IV | 4 (0.7) | 12 (2.0) | |
| **AJCC stage** | | | <0.001 |
| I | 314 (52.9) | 140 (23.0) | |
| II | 235 (39.5) | 261 (42.9) | |
| III | 45 (7.6) | 208 (34.2) | |
| **T stage** | | | <0.001 |
| T1 | 436 (73.4) | 261 (42.9) | |
| T2 | 147 (24.7) | 251 (41.2) | |
| T3 | 11 (1.9) | 97 (15.9) | |
| **N stage** | | | <0.001 |
| N0 | 380 (64.0) | 208 (34.2) | |
| N1 | 175 (29.5) | 219 (36.0) | |
| N2 | 26 (29.0) | 102 (16.7) | |
| N3 | 13 (2.2) | 80 (13.1) | |
| **ER status** | | | <0.001 |
| Negative | 40 (6.7) | 81 (13.3) | |
| Positive | 554 (93.3) | 528 (86.7) | |
| **PR status** | | | 0.001 |
| Negative | 100 (16.8) | 148 (24.3) | |
| Positive | 494 (83.2) | 461 (75.7) | |
| **Radiation** | | | <0.001 |
| No | 0 (0.0) | 390 (64.0) | |
| Yes | 594 (100.0) | 219 (36.0) | |
| **Chemotherapy** | | | <0.001 |
| No | 352 (59.3) | 216 (35.5) | |
| Yes | 242 (40.7) | 393 (64.5) | |

*BCT, breast-conserving therapy.*

with BCT had no significant difference after stratifying for T and N stages (**Figures 3**, **4**).

## Impact of Various Factors on Survival and Stratified Analysis of Overall Survival

Univariate Cox regression model analysis showed that older age (≥65 years old), single status, large-size tumor, lymph node metastasis, and mastectomy contributed to lower OS, while Asian or Pacific Islander and American Indian/Alaska Native race, ER positive, PR positive, and radiation therapy were associated with higher OS (**Table 2**). In addition, single-status patients, higher tumor grade (III), larger tumor size (>5 cm), lymph node metastasis, and treatment with a mastectomy had lower BCSS (**Table 3**); however, positive ER and PR and radiation therapy were protective factors for BCSS. Furthermore, univariate Cox regression model analysis showed that patients who received BCT had superior OS and BCSS compared with those who received mastectomy (HR = 1.590, 95% CI: 1.074–2.356, $p$ = 0.021; HR = 2.395, 95% CI: 1.314–4.364, $p$ = 0.004; respectively). In multivariate Cox regression model analysis, older age (≥65 years old) and radiation therapy were independent risk factors for OS (HR = 2.490, 95% CI: 1.343–4.615, $p$ = 0.004; HR = 0.512, 95% CI: 0.280–0.938, $p$ = 0.030; respectively) but not for BCSS (HR = 1.907, 95% CI: 0.833–4.364, $p$ = 0.126; HR = 0.511, 95% CI: 0.237–1.099, $p$ = 0.086; respectively). Single status and PR positive were independent risk factors for OS and BCSS. Interestingly, patients with N3 stage had worse OS (HR = 2.856, 95% CI: 1.361–5.995, $p$ = 0.006) compared with those with N0 stage, while patients with N1 and N3 stages had worse BCSS compared with those with N0 stage (HR = 2.223, 95% CI: 1.026–4.817, $p$ = 0.043; HR = 3.749, 95% CI: 1.337–10.510, $p$ = 0.012; respectively). Radiotherapy was a protective factor for OS but not for BCSS (HR = 0.512, 95% CI: 0.280–0.938, $p$ = 0.030; HR = 0.511, 95% CI: 0.237–1.099, $p$ = 0.086; respectively). No difference in OS and BCSS was found between the BCT and mastectomy groups (HR = 0.727, 95% CI: 0.369–1.432, $p$ = 0.357; HR = 0.762, 95% CI: 0.302–1.923, $p$ = 0.565; respectively). We further performed a stratified analysis based on T stage. As shown in **Table 4**, in the multivariate analysis stratified by T stage, the OS of the BCT group for T2 stage was better than the mastectomy group (HR = 0.333,

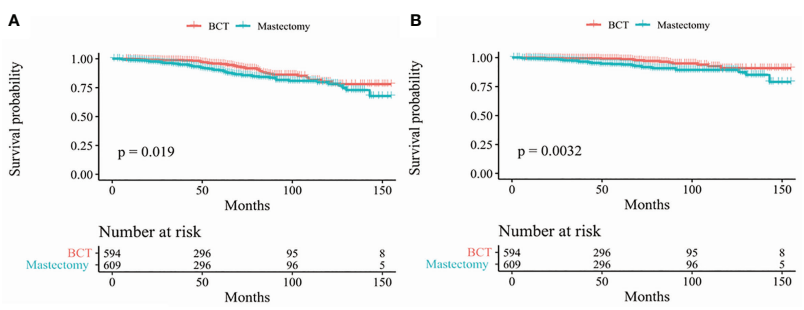


**FIGURE 2** | Kaplan-Meier survival curves **(A)** OS between mastectomy and BCT group in the entire cohort; **(B)** BCSS between mastectomy and BCT group in the entire cohort. OS, overall survival; BCSS, breast cancer-specific survival; BCT breast-conserving therapy.

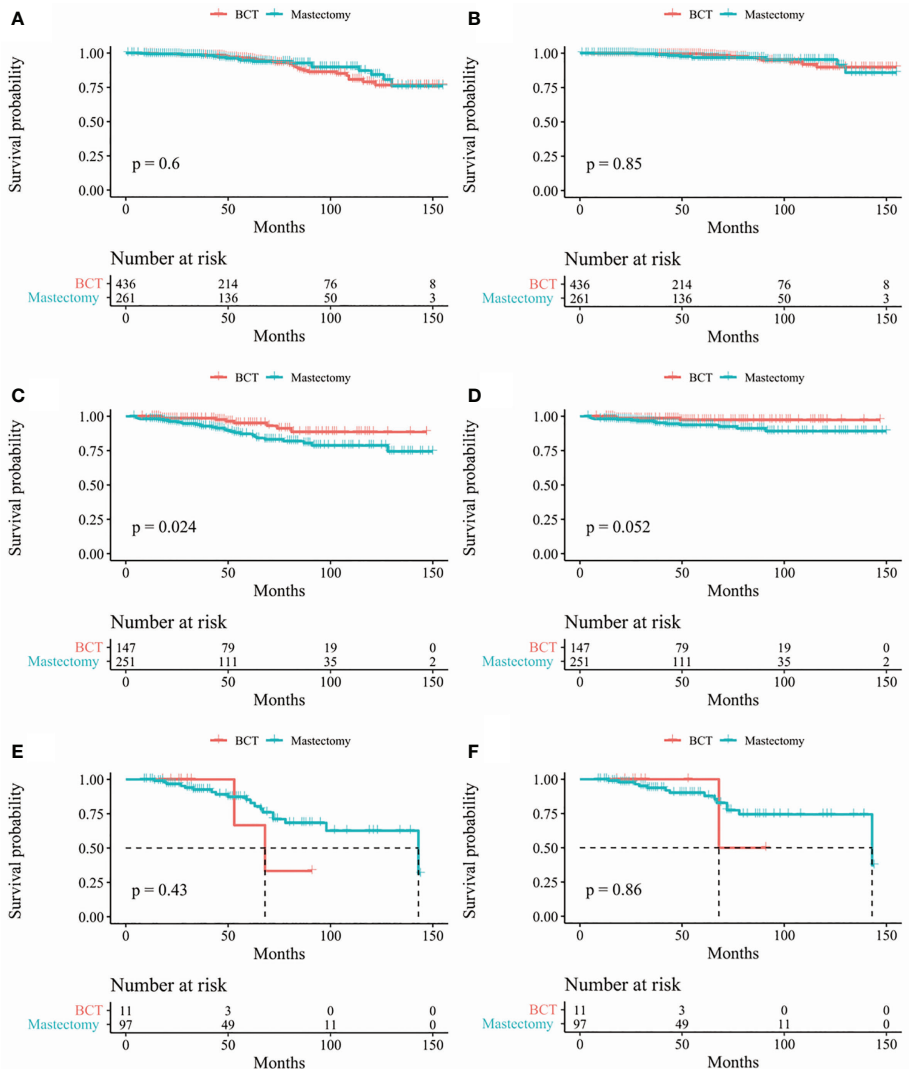


**FIGURE 3** | Kaplan-Meier survival curves of subgroup stratified by T stage **(A)** OS between mastectomy group and BCT group in patients with T1 stage **(B)** BCSS between mastectomy group and BCT group in patients T1 stage; **(C)** Os between mastectomy goup and BCT group in patients with T2 stage **(D)** BCSS between mastectomy group and BCT group in patients with T2 stage **(E)** Os between mastectomy group and BCT group in patients with T3 stage; **(F)** BCSS between mastectomy group and BCT group in patients with T3 stage. OS, overall survival; BCSS, breast cancer-specific survival; BCT, breast-conserving therapy.

95% CI: 0.149–0.741, $p = 0.007$), and no significant difference in OS was found between the BCT and mastectomy groups for patients with T1 and T3 disease (HR = 1.116, 95% CI: 0.608–2.050, $p = 0.722$; HR = 3.328, 95% CI: 0.693–15.974, $p = 0.133$, respectively).

## DISCUSSION

Our results showed that the long-term survival advantage of women with early IMPC receiving BCT is equivalent to those undergoing mastectomy. Interestingly, the OS of patients with T2 stage receiving BCT was better than patients undergoing mastectomy. Our observations were based on data collected from 1,203 women in the SEER database and suggested that when BCT and mastectomy are feasible for early-stage IMPC treatment, BCT should be recommended, especially for patients with T2 stage.

IMPC is a rare histological subtype that predominantly affects women over 50 years old and is associated with a poor prognosis due to its invasiveness (3). The 5-year overall survival rates have been reported to range from 63% to 82.9% (18, 19). Histologically, IMPC consists of small clusters of tumor cells lying within clear stromal spaces which resemble dilated vascular channels. Immunohistochemically, IMPC exhibits an "inside-out" pattern of EMA expression (5, 7, 19). There is no significant difference in imaging findings between IMPC and invasive ductal

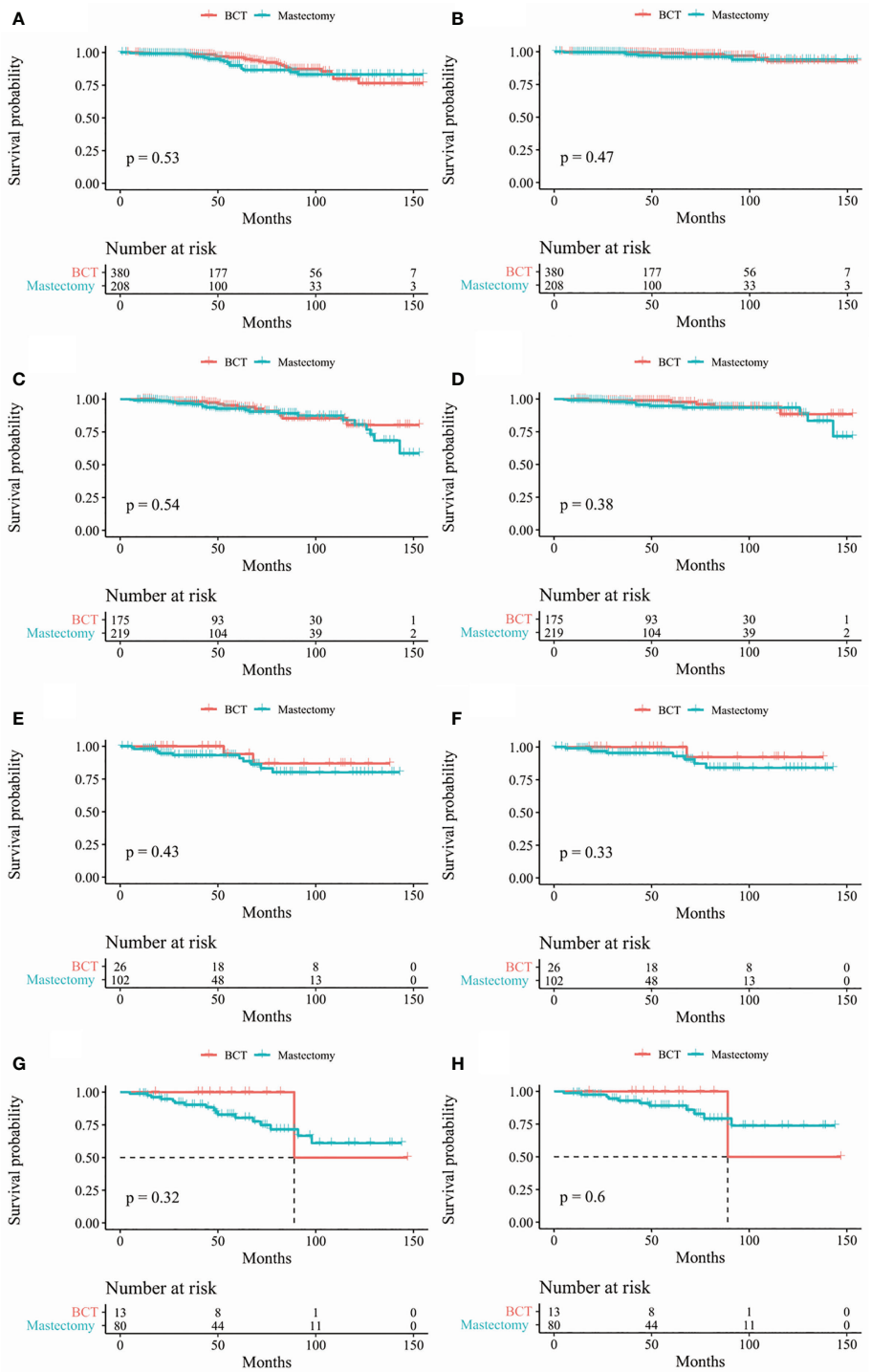


**FIGURE 4** | Kaplan-Meier survival curves of subgroup stratified by N stage **(A)** OS between mastectomy group and BCT group in patients with N0 stage **(B)** BCSS between mastectomy group and BCT group in patients with N0 stage **(C)** Os between mastectomy goup and BCT group in patients with N1 stage **(D)** BCSS between mastectomy group and BCT group in patients with N1 stage **(E)** Os between mastectomy group and BCT group in patients with N2 stage **(F)** BCSS between mastectomy group and BCT group in patients with N2 stage **(G)** OS between mastectomy group and BCT group in patients with N3 stage **(H)** BCSS between mastectomy group and BCT group in patients with N3 stage. OS, overall survival; BCSS, breast cancer specific survival; BCT, breast conserving therapy.

**TABLE 2 |** Univariate and multivariate analysis of OS of patients with early-stage IMPC.

| Characteristics | OS | | | |
|---|---|---|---|---|
| | Univariate analysis | | Multivariate snalysis | |
| | HR (95% CI) | *p*-value | HR (95% CI) | *p*-value |
| **Age (years)** | | | | |
| <50 | Reference | – | Reference | – |
| 50–64 | 1.063 (0.586–1.929) | 0.841 | 1.076 (0.581–1.992) | 0.816 |
| 65–80 | 2.003 (1.143–3.510) | 0.015 | 2.490 (1.343–4.615) | 0.004 |
| >80 | 4.258 (2.122–8.547) | <0.001 | 3.723 (1.702–8.143) | 0.001 |
| **Race** | | | | |
| White | Reference | – | Reference | – |
| Black | 1.471 (0.884–2.449) | 0.137 | 1.126 (0.664–1.911) | 0.659 |
| Others | 0.354 (0.130–0.965) | 0.043 | 0.361 (0.131–1.000) | 0.050 |
| **Marital status** | | | | |
| Married | Reference | – | Reference | – |
| Single | 2.378 (1.606–3.522) | <0.001 | 2.006 (1.321–3.047) | 0.001 |
| **Grade** | | | | |
| I | Reference | – | | |
| II | 0.960 (0.471–1.960) | 0.912 | | |
| III | 1.338 (0.654–2.740) | 0.426 | | |
| IV | 1.836 (0.565–5.966) | 0.312 | | |
| **T stage** | | | | |
| T1 | Reference | – | Reference | – |
| T2 | 1.556 (1.014–2.388) | 0.043 | 1.549 (0.980–2.449) | 0.061 |
| T3 | 3.319 (1.982–5.556) | <0.001 | 3.581 (1.758–7.295) | <0.001 |
| **N stage** | | | | |
| N0 | Reference | – | Reference | – |
| N1 | 1.148 (0.728–1.809) | 0.553 | 1.514 (0.923–2.484) | 0.101 |
| N2 | 1.388 (0.744–2.591) | 0.303 | 1.085 (0.496–2.375) | 0.838 |
| N3 | 2.624 (1.507–4.570) | 0.001 | 2.856 (1.361–5.995) | 0.006 |
| **ER status** | | | | |
| Negative | Reference | – | Reference | – |
| Positive | 0.389 (0.248–0.610) | <0.001 | 0.414 (0.211–0.810) | 0.010 |
| **PR status** | | | | |
| Negative | Reference | – | Reference | – |
| Positive | 0.500 (0.337–0.740) | 0.001 | 0.644 (0.362–1.146) | 0.135 |
| **Surgery** | | | | |
| BCT | Reference | – | Reference | – |
| Mastectomy | 1.590 (1.074–2.356) | 0.021 | 0.727 (0.369–1.432) | 0.357 |
| **Radiation** | | | | |
| No | Reference | – | Reference | – |
| Yes | 0.611 (0.417–0.897) | 0.012 | 0.512 (0.280–0.938) | 0.030 |
| **Chemotherapy** | | | | |
| No | Reference | – | Reference | – |
| Yes | 0.736 (0.503–1.079) | 0.116 | 0.630 (0.390–1.018) | 0.059 |

*OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval; BCT, breast-conserving therapy.*

carcinoma (IDC) as both present as high-density lesions on mammography and enhance significantly with MRI (20). Since most IMPC patients belong to the luminal biological subtype (21), chemotherapy has usually been ineffective in IMPC patients (22). Compared with IDC, IMPC has a greater potential risk of LRR. Therefore, radiotherapy may play an important role in improving LRR of patients with IMPC. A study reported that in patients that did not undergo radiotherapy, the incidence of LRR was significantly higher in the IMPC group than in the IDC group (19). Accordingly, many researchers recommended that patients with IMPC should receive radiotherapy (20, 23). Meng et al. (24) demonstrated that radiation therapy was significantly associated with improved LRR after mastectomy in IMPC. However, the clinical value of BCT for IMPC is still unclear.

In recent years, the optimal extent of breast resection for breast cancer has transitioned from wide resection to narrow surgical resection margins. Halsted's radical mastectomy for breast cancer, which involved removing the whole breast, pectoralis major muscle, pectoralis minor muscle, and axillary lymph nodes, gradually became the standard treatment for breast cancer (25). However, subsequent studies showed that extensive resection did not improve survival rates. Therefore, in recent years, narrowing the extent of breast resection and postoperative adjuvant therapy has been advocated to improve patient survival. BCT which consists of lumpectomy and postoperative radiotherapy has become a standard of care in localized breast cancer (26). During lumpectomy in BCT, the tumor is removed, and the normal breast shape is maintained with minimal tissue damage.

**TABLE 3 |** Univariate and multivariate analyses of BCSS of patients with early-stage IMPC.

| Characteristics | BCSS | | | |
|---|---|---|---|---|
| | Univariate analysis | | Multivariate analysis | |
| | HR (95% CI) | *p*-value | HR (95% CI) | *p*-value |
| **Age (years)** | | | | |
| <50 | Reference | – | Reference | – |
| 50–64 | 0.857 (0.409–1.796) | 0.684 | 0.927 (0.422–2.038) | 0.851 |
| 65–80 | 1.096 (0.522–2.300) | 0.808 | 1.907 (0.833–4.364) | 0.126 |
| >80 | 2.385 (0.893–6.373) | 0.083 | 2.921 (0.933–9.149) | 0.066 |
| **Race** | | | | |
| White | Reference | – | Reference | – |
| Black | 1.129 (0.508–2.511) | 0.766 | 0.661 (0.284–1.535) | 0.335 |
| Others | 0.339 (0.082–1.400) | 0.135 | 0.367 (0.085–1.589) | 0.180 |
| **Marital status** | | | | |
| Married | Reference | – | Reference | – |
| Single | 3.248 (1.802–5.853) | <0.001 | 3.524 (1.843–6.738) | <0.001 |
| **Grade** | | | | |
| I | Reference | – | Reference | – |
| II | 3.075 (0.408–23.154) | 0.276 | 2.936 (0.378–22.797) | 0.303 |
| III | 8.845 (1.208–64.771) | 0.032 | 5.305 (0.692–40.684) | 0.108 |
| IV | 4.274 (0.267–64.401) | 0.305 | 3.182 (0.180–56.346) | 0.430 |
| **T stage** | | | | |
| T1 | Reference | – | Reference | – |
| T2 | 1.815 (0.952–3.462) | 0.070 | 1.336 (0.665–2.885) | 0.416 |
| T3 | 5.827 (2.957–11.485) | <0.001 | 3.696 (1.412–9.675) | 0.008 |
| **N stage** | | | | |
| N0 | Reference | – | Reference | – |
| N1 | 1.922 (0.942–3.923) | 0.073 | 2.223 (1.026–4.817) | 0.043 |
| N2 | 3.090 (1.320–7.235) | 0.009 | 1.355 (0.449–4.093) | 0.590 |
| N3 | 5.515 (2.515–12.094) | <0.001 | 3.749 (1.337–10.510) | 0.012 |
| **ER status** | | | | |
| Negative | Reference | – | Reference | – |
| Positive | 0.225 (0.127–0.398) | <0.001 | 0.349 (0.137–0.887) | 0.027 |
| **PR status** | | | | |
| Negative | Reference | – | Reference | – |
| Positive | 0.319 (0.185–0.551) | <0.001 | 0.557 (0.235–1.317) | 0.182 |
| **Surgery** | | | | |
| BCT | Reference | – | Reference | – |
| Mastectomy | 2.395 (1.314–4.364) | 0.004 | 0.762 (0.302–1.923) | 0.565 |
| **Radiation** | | | | |
| No | Reference | – | Reference | – |
| Yes | 0.502 (0.292–0.866) | 0.013 | 0.511 (0.237–1.099) | 0.086 |
| **Chemotherapy** | | | | |
| No | Reference | – | Reference | – |
| Yes | 1.503 (0.848–2.664) | 0.163 | 0.979 (0.487–1.969) | 0.952 |

*BCSS, breast cancer-specific survival; HR, hazard ratio; 95% CI, 95% confidence interval; BCT, breast-conserving therapy.*

**TABLE 4 |** Univariate and multivariate regression analysis stratified according to T stage.

| T stage | OS | | | |
|---|---|---|---|---|
| | Univariate analysis | | Multivariate analysis | |
| | HR (95% CI) | *p*-value | HR (95% CI) | *p*-value |
| T1 BCT *vs.* mastectomy | 1.175 (0.648–2.131) | 0.596 | 1.116 (0.608–2.050) | 0.722 |
| T2 BCT *vs.* mastectomy | 0.418 (0.191–0.914) | 0.029 | 0.333 (0.149–0.741) | 0.007 |
| T3 BCT *vs.* mastectomy | 1.798 (0.411–7.871) | 0.436 | 3.328 (0.693–15.974) | 0.133 |

*OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval; BCT, breast-conserving therapy.*

Studies have shown that patients that underwent BCT exhibited better physical and mental health and better quality of life (27).

The survival benefits brought by BCT warranted further investigations. Interestingly, randomized clinical trials showed similar survival rates in primary breast cancer patients who received BCT and mastectomy (8–10). Over the past few decades, with increased and early-stage breast cancer screening, the survival rate of breast cancer patients has significantly improved with multiple treatment modalities available, including chemotherapy, radiotherapy, endocrine therapy, and targeted therapy. Large-scale cohort studies have shown higher survival rates in patients with early-stage breast cancer with BCT than with mastectomy. For instance, Hartmann-Johnsen et al. (13) reported better OS and BCSS in women with early-stage breast cancer treated with BCT. The authors emphasized that differences in tumor biology and adjuvant systemic therapy could not fully explain this benefit. Furthermore, a Canadian study (14) on women with locally advanced breast cancer showed better patient outcomes with BCT. Importantly, Mirelle et al. (15) advocated that BCT should be the first choice for most breast cancer patients when both treatments are applicable. In 2016, Marissa et al. (16) controversially reported that BCT improved 10-year OS compared with mastectomy after hierarchical analysis of disease stages and adjustment of confounding variables. Similarly, BCT had a higher overall survival rate than mastectomy for breast cancer in a propensity score matching study based on the SEER database by Wrubel et al. (28). However, most of these studies included invasive ductal breast cancer (IDC) patients. Since IMPC is more prone to lymphatic invasion and higher lymph node metastasis, axillary lymph node dissection and extensive breast resection are recommended to obtain greater resection margins and lower recurrence rates (20, 23, 29). However, some studies found that this approach did not improve prognosis (6, 30). Survival analysis showed that IMPC patients in the BCT group had better OS and BCSS than those in the mastectomy group. In order to eliminate potential selection bias in BCT or mastectomy, further analysis was performed on IMPC patients after stratifying for T and N stages. We found that for breast cancer patients with T2 stage, the OS of the BCT group was better than the mastectomy group. In addition, after the inclusion of significant univariate variables, multivariate Cox regression model analysis showed that older age (≥65 years old), single, larger tumor (>5 cm), and lymph node positive (N3) were associated with poor OS, while ER-positive breast cancer and radiotherapy were associated with good OS. Our study also found that T and N stages were independent risk factors for BCSS in IMPC patients. Interestingly, during multivariate analysis, the OS or BCSS of patients receiving BCT did not improve significantly compared with those receiving mastectomy. After stratifying patients according to the T stage, better OS was found for patients with T2 disease in the BCT group than the mastectomy group, and no significant difference in OS was found between the BCT and mastectomy groups for T1 and T3 disease. According to the NCCN guidelines for breast cancer surgery, patients with tumor diameter less than 3 cm and stage III disease can consider breast-conserving surgery after preoperative chemotherapy (17). From clinical experience, patients with T2 stage disease should generally choose neoadjuvant chemotherapy before BCT; only patients with good responses to neoadjuvant chemotherapy should receive BCT. This approach leads to a better patient prognosis than those undergoing mastectomy. In conclusion, to the best of our knowledge, this is the first study to explore the impact of different surgical methods on the prognosis of early-stage IMPC patients. One main strength of this study was the long follow-up interval, and the large patient population studied, enabling us to provide real-world information on treatment approach selection for women diagnosed with breast invasive micropapillary carcinoma. Our study mainly focused on T1-3N0-3M0 patients, and most of these patients had the opportunity to choose BCT. Overall, our results showed that lumpectomy plus radiotherapy is an effective treatment strategy for invasive micropapillary breast carcinoma patients compared with mastectomy.

We also recognize several limitations of this study. First of all, although we tried to ensure the accuracy of the data; data obtained from the SEER database may have been subjected to selection bias and data input errors. Moreover, tumor-related information was unavailable, including multifocality or multicentricity, molecular typing and secondary surgery rates. However, it is widely acknowledged that patients with multifocal/multicentric tumors are not appropriate candidates for BCT. Therefore, adjusting for these variables was not feasible. Although it is unclear whether the two groups of patients received standard endocrine therapy, according to the positivity rates of ER and PR, we estimated the proportion of patients who underwent endocrine therapy since endocrine therapy is indicated in IMPC patients with positive ER and PR. Accordingly, we believe our findings are reliable. Moreover, no data on local recurrence and disease-free survival rates were available in the SEER database. Indeed, the primary outcome of this study was the survival rate of IMPC patients, which was influenced by other factors, including local recurrence and disease free. However, we could not determine the incidence of lymph node metastasis and tumor thrombus invasion in IMPC patients. Last, we did not analyze the entire IMPC patient population since T4 breast cancer and distant metastatic breast cancer are contraindications to BCT. In a nutshell, we confirmed that BCSS and OS were comparable between BCT and mastectomy in IMPC patients. Surprisingly, patients with T2 stage disease had better OS with BCT compared with mastectomy. IMPC is a special type of rare breast cancer that needs further studies with large sample sizes to investigate the optimal approach for surgical management.

## CONCLUSION

Overall, we demonstrated that prognosis of early-stage IMPC with breast-conserving treatment was at least equivalent to treatment with mastectomy. When both procedures are

applicable, BCT should be recommended as the standard surgical treatment, especially for patients with T2 disease.

## DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. These data can be found here: https://seer.cancer.gov/data/.

## ETHICS STATEMENT

In accordance with national legislative and institutional requirements, this study does not require written informed consent to participate.

## AUTHOR CONTRIBUTIONS

SW was responsible for the design of the project. YZ was responsible for the data analysis. All the authors participated in the writing of the final manuscript. All authors have read and approved the final submission.

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# Giant Ovarian Cysts Treated by Single-Port Laparoscopic Surgery: A Case Series

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**Background:** Ovarian cysts are very common diseases of the female reproductive system. Giant ovarian cysts refer to the tumors with diameters greater than 10 cm. In recent years, due to the development of clinical diagnosis, imaging modalities, and the improvement of patients' cognition of the diseases, the occurrence of giant ovarian cysts has become rare. The purpose of this study was to show a new operation method of single-port laparoscopy to treat giant ovarian cysts.

**Methods:** We report a case series of five patients with giant ovarian cysts who underwent single-port laparoscopic surgery in the gynecology department of the Shengjing Hospital of China Medical University between June 2020 and March 2021. The inclusion criteria were ovarian cysts at least 20 cm in diameter, and cases when the tumor might be malignant were excluded.

**Results:** The patients' mean age was 26.2years. The most common clinical presentation was progressive abdominal distension. Median size of the cysts at imaging was 39.2 cm (range 21–63 cm). All patients underwent single-port laparoscopic surgery, and none of them converted to laparotomy. On final pathological reports, two cysts were serous cystadenomas, and three were mucinous cystadenomas. All patients recovered well and were discharged on time.

**Conclusion:** Giant ovarian cysts can be treated by single-port laparoscopic surgery. In addition to the well-known advantages of laparoscopic surgery (e.g., small pelvic interference, fast postoperative recovery), it can also play the role of perfect cosmetic results, which has more advantages for young women.

**Keywords: giant, ovarian cyst, single-port laparoscopic surgery, case series, gynecologic oncology**

## INTRODUCTION

Female pelvic cysts mostly come from the ovary and are asymptomatic when they are small. The symptoms appear when they reach enormous dimensions. Giant ovarian cysts (GOCs) are tumors larger than 10 cm in diameter or those cysts reaching above the umbilicus (1). Progressive abdominal distension, nonspecific diffuse abdominal pain, and organ compression (constipation, vomiting, and frequent urination) are the main clinical symptoms of ovarian cysts (2–4). Most giant ovarian cysts are treated by surgery. Surgical indications include a rapidly growing or symptomatic cyst, and when its malignant potential cannot be excluded (5). In the past, exploratory laparotomy was the most common surgical method, which had the advantage of minimizing the risk of an intraperitoneal implantation caused by cell overflow in case of an unexpected malignant transformation of the tumor. However, some giant ovarian cysts filled the abdominal cavity and superior reaching the xiphoid process. The abdominal incision reaching tens of centimeters long caused great trouble to patients, especially young women. In recent years, minimally invasive surgery has been widely used in the field of gynecology. Laparoscopy is the choice for most benign ovarian cysts, but the size of the cysts may be a limiting factor. Giant ovarian cysts increase the complexity and difficulty of laparoscopic surgery. Avoiding the leakage of cyst fluid has become a challenge (6). We report five cases of giant ovarian cysts treated by single-port laparoscopy. This method tries to ensure the oncologic safety while treating the disease. The aim of this study is to introduce a new, minimally invasive and effective surgical approach for the treatment of giant ovarian cysts.

## MATERIALS AND METHODS

Five female patients with giant ovarian cysts who underwent single-port laparoscopic surgery between June 2020 and March 2021 were included from the gynecology department of the Shengjing Hospital of China Medical University. The study was approved by the China Medical University Research Ethics Committee. The inclusion criteria: ① All patients were diagnosed as giant abdominal cysts larger than 20 cm in diameter that tend to be benign by pelvic ultrasound, MRI or CT-scan before operation (**Figures 1A–D**). ② The patients had signed the informed consent. ③ The umbilicus was normal. Exclusion criteria: ① Conversion to open surgery or other surgical methods. ② Malignant transformation of cysts. ③ Severe medical system diseases which could not endure laparoscopic surgery. Five patients were confirmed by preoperative imaging (ultrasound, MRI or CT-scan) with giant abdominal masses at least 20 cm, mainly cystic, without obvious solid components, no abnormal increase in tumor markers obviously, showing that the ovarian cysts tend to be benign rather than malignant. Blood tumor markers (CA125, HE4, CA199, CA724, and CEA) were detected for each patient. The patients with complications were consulted in relevant departments to exclude surgical contraindications. In order to eliminate the influence of different surgeons' experience on the surgical results, all patients were completed by a gynecologist who has experience in single-port laparoscopic surgery (author KL). Data were collected with operative time, intra- and post-operative complications, intracystic liquid volume, conversion to laparotomy, and the length of postoperative stay. Approximately 30 days after operation, the satisfaction of patients with abdominal scar was recorded. The score was 1–5 according to the wound recovery based on the subjective evaluation of the patients after the operation, which was the higher the score, the higher the satisfaction.

**Abbreviations:** GOCs, Giant ovarian cysts; MRI, Magnetic Resonance Imaging; CT, Computerized Tomography; CA125, Carbohydrate antigen-125; HE4, Human Epididymis Protein 4; CA199, Carbohydrate antigen-199; CEA, Carcinoembryonic antigen.

### Surgical Procedure

The patients received standardized preoperative nursing preparation and general anesthesia. Single-port laparoscopic surgery was performed using the following techniques. After partial eversion of the umbilicus, a 2–3 cm longitudinal incision was made at the umbilicus (**Figure 2A**). The umbilical incision was lifted, the skin and subcutaneous tissue were incised layer by layer, and the peritoneum was incised after confirming that there was no intestinal adhesion under the incision. The disposable incision protection sleeve (Lookmed, Jiangsu, China) was placed in the incision, the inner ring was placed in the abdominal cavity, and the outer ring was left to the abdominal wall to form a single-port laparoscopic approach access (**Figure 2B**). A giant cyst appeared under the incision and the inside of the cyst was mainly liquid. In order to prevent the adverse effects of sudden drop of abdominal pressure on patients, we used a syringe needle connected with a suction device to slowly suck out the liquid in the cyst (**Figure 2C**). If the cyst divided into several septums, we suck out the liquid in one septum and then used the instruments to lift the wall of the cyst to prevent the leakage of the liquid in the cyst. We changed another septum and continued to suck out the liquid to reduce the pressure of the cyst. When the liquid was sucked out completely, we used silk thread to ligate the incision and returned the cyst to the abdominal cavity (**Figure 2D**).

A sterile glove was connected with the outer ring. The thumb of the glove was cut, and 10 mm trocar (Dike, Guangzhou, China) was placed as the access of a scope and laparoscopic instruments. In order to prevent air leakage and loosening at the joint, a No. 7 silk thread was used to fix and was tied tightly, and the 5 mm (Dike, Guangzhou, China) trocars were inserted into the other two fingers as the instrument port. This is a self-made simple laparoscopic single-port (**Figure 3A**). This is a cost-saving advantage for the patients without affecting the operation.

Carbon dioxide was injected at a pressure of 13 mmHg and a rigid 30° 5-mm laparoscope was inserted (Karl Storz, Tutlingen, Germany). A 30° laparoscope is a better choice because it provides a wide field of vision. Then the standard laparoscopic surgery was performed. Giant ovarian cysts were removed from the umbilicus using an endopouch specimen retrieval bag (Wellead, Guangzhou, China) (**Figures 3B, C**).

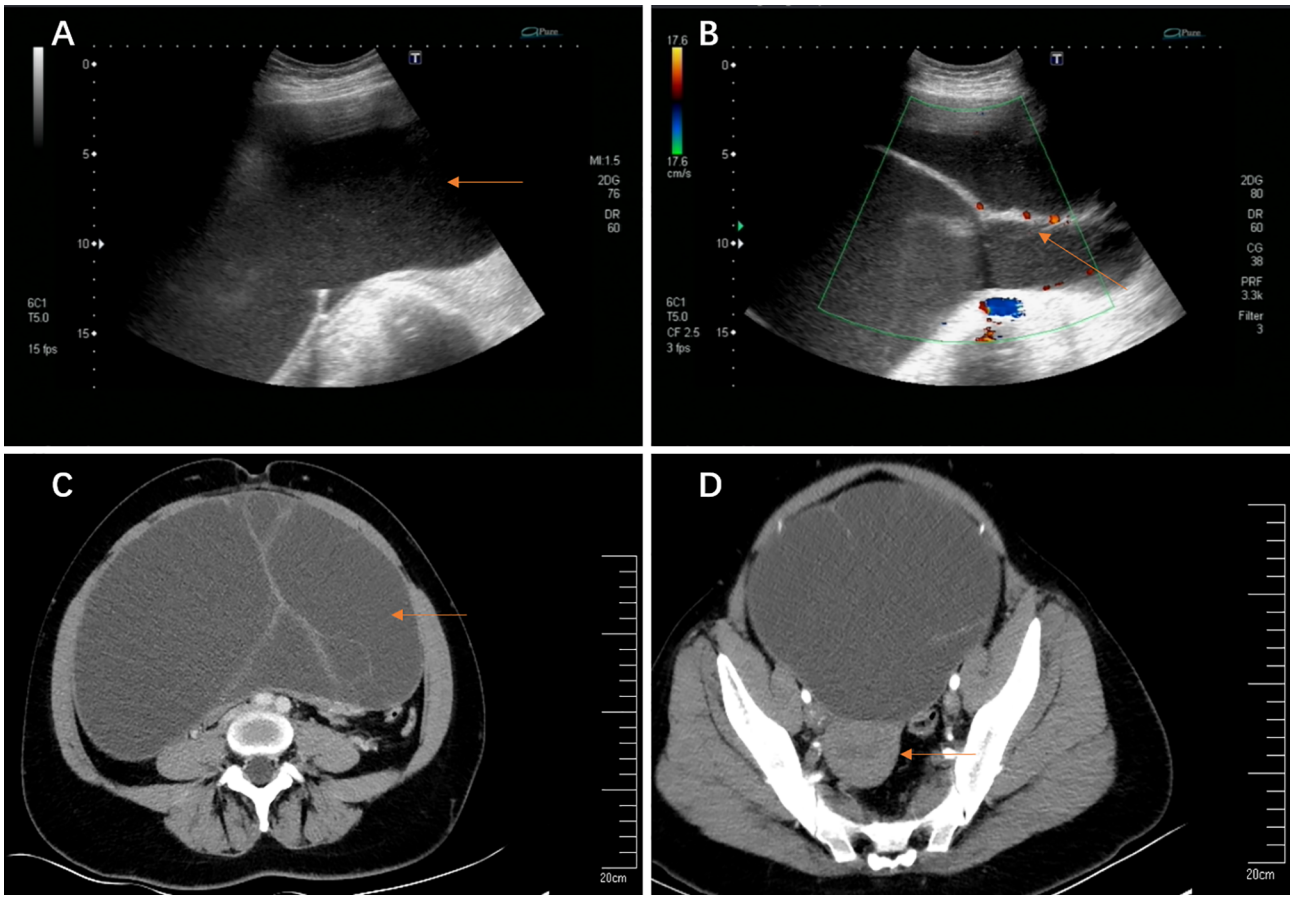


**FIGURE 1** | **(A, B)** Transvaginal ultrasound imaging. **(A)** A giant cyst in the abdominal and pelvic cavity (63.0 cm × 44.0 cm × 13.4 cm); **(B)** The blood flow signal detected at the separation; **(C, D)** Abdomen enhanced CT imaging. **(C)** A giant cyst with septums; **(D)** The uterus was pushed to the back of the pelvis by a giant cyst.

## RESULTS

The study consisted of five female patients and data are shown in **Tables 1**, **2**. The mean age of the operated patients was 26.2 years (range, 19–34 years). The BMI of the five patients ranged from 16.8 to 31.2. According to the BMI calculation results, one patient was thin (patient 3), one normal (patient 4), two obese (patients 1 and 2), and one overweight (patient 5). Coincidentally, all of the patients had no history of gravidity, parity, and previous abdominal operations.

Surgical outcomes are shown in **Table 2**. The most common symptom was progressive abdominal distension (patients 1, 2, 4, and 5), several of which were accompanied by abdominal pain (patients 1, 2, and 5). No obvious abdominal distension occurred in patient 3, mainly due to palpation of abdominal mass. All patients were diagnosed by imaging, ultrasound, MRI or CT-scans. Median size of the cysts at imaging was 39.2 cm (range 21–63 cm), while the maximum was 63.0 cm with the superiors reaching the sword (patients 2). In particular, there were much comorbidities in patient 2. Hypertension occurred 17 years ago. Now oral antihypertensive drugs are used to control blood pressure, and the blood pressure is controlled at 130/80 mmHg. In 2014, she suffered from cerebral thrombosis. The specific location is unknown. She felt numb on the right side of the body at the time of onset, which was improved after a conservative treatment but was left hemiplegic of the right limb. We consulted the anesthesiology, cardiology, and neurology departments before operation to evaluate the safety of the operation and eliminate the operation contraindications. Based on the patient's age and personal will, we decided to perform single-port laparoscopic exploration after discussion.

Four of the five patients presented with normal blood tumor markers. One patient presented with an elevated CA125 of 70.78 (normal range 0–35 mIU/ml) and CA-724 of 8.94 (normal range 0–6.9 mIU/ml) (patient 3). In the postoperative reexamination, the blood tumor markers gradually returned to normal. All patients underwent single-port laparoscopic surgery, and no one converted to laparotomy. Intraoperative suction of intracapsular fluid was in the range of 3,500–16,000 ml (**Figure 3D**).The average volume was 8,700 ml. Four patients underwent unilateral adnexectomy, and one patient an ovarian cystectomy (**Figure 3E**). We had a cosmetic suture of the single-port laparoscopic incision in the patients' navels

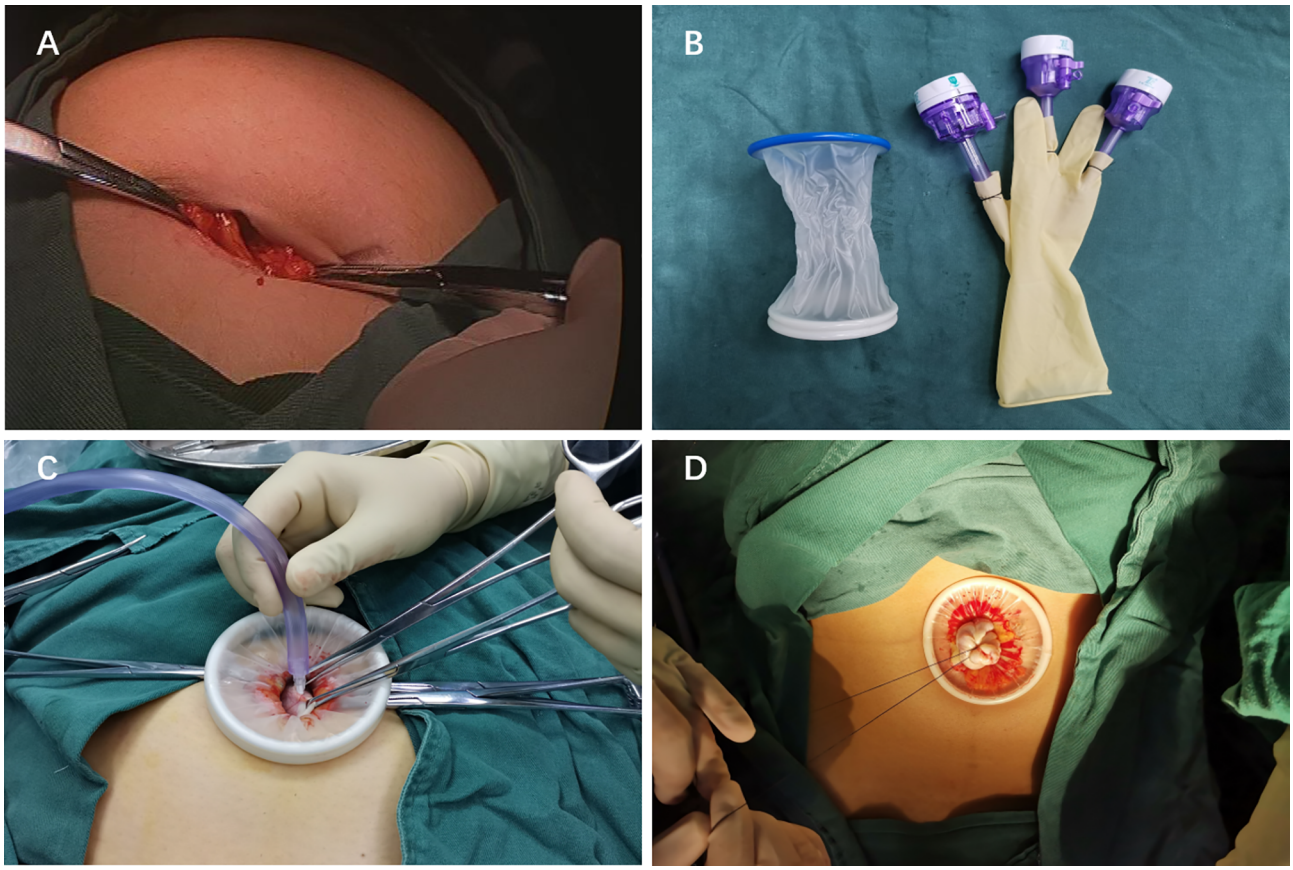


**FIGURE 2** | **(A)** The 2–3 cm longitudinal incision was made at the umbilicus. **(B)** Single-port laparoscopic access. **(C)** A syringe needle connected with a suction device to suck out the liquid in the cyst. **(D)** Ligate the incision in order to avoid the leakage of cyst fluid.

(**Figure 3F**). The average operative time was 85.2 min (range 37–132 min). Neither extravasation of cystic fluid and nor decompression syndrome happened due to the gradual reduction of cystic pressure. Mean blood loss was 26 ml (range 10–50 ml). The average time of hospitalization after operation was 5 days, and such operative method did not increase the post-operative stay. All patients recovered well, and no complications related to the operation occurred. On final pathological reports, two cysts were serous cystadenomas, and three were mucinous cystadenomas. There was no borderline tumor or epithelial ovarian cancer in any of the ovarian cysts operated, but one case reported an active cell proliferation, which should be reexamined. All the patients were satisfied with the abdominal scar 30 days after the operation.

## DISCUSSION

Female pelvic cysts are a very common gynecological disease in women, most of which come from the ovary. During their lifetime, it is assumed that about 7% of women experience a symptomatic cyst worldwide (7). The clinical manifestations appear when the cysts reach enormous dimensions. Giant ovarian cysts (GOCs) are tumors larger than 10 cm in diameter (1). Due to improved imaging techniques, giant abdominal cysts have increasingly become rare. The patients can present with rare complications such as torsion, intestine obstruction, and hydronephrosis in addition to causing non-specific abdominal distension, pain, nausea, and vomiting and changes in defecation habits (8–11). As the nonspecific clinical manifestations of giant ovarian cysts, the differential diagnoses include the giant cysts arising from other intra-abdominal organs (e.g., gastrointestinal, urological, or lymphatic) (12).

The treatment of ovarian cysts depends on the patient's age, the size of the cyst, and its histopathological feature. Excision of the intact cysts for histology is the gold standard (13). Most giant ovarian cysts are benign and are generally treated by surgical excision either by a cystectomy or a salpingo-oophorectomy (14). It is of utmost importance to exclude any possibility of malignant tumor before operation (15). The SRU guidelines propose that within an ovarian or adnexal cystic lesion, multiple thin septations (<3 mm) or an avascular, solid non-hyperechoic nodule are indeterminate characteristics, often found in benign neoplasms. If the cyst is more than 10 cm in size or continues to have indeterminate findings, surgical evaluation should be considered (16).

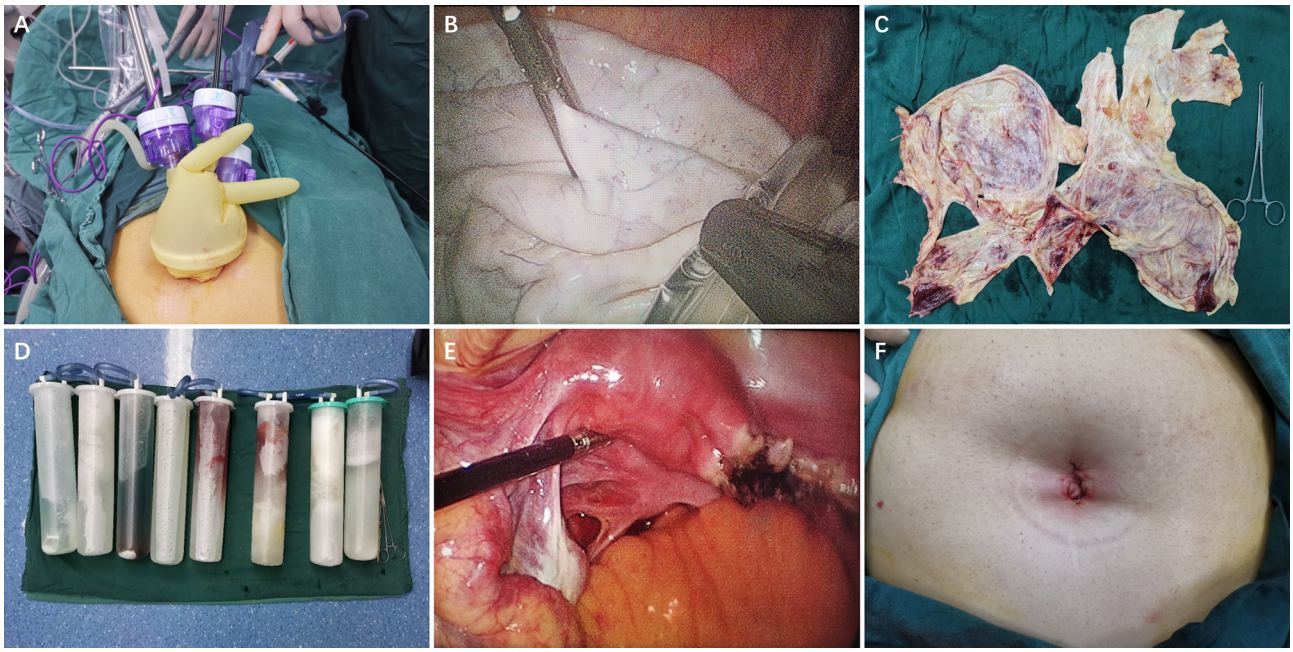


**FIGURE 3** | **(A)** The instruments enter the abdominal cavity through single-port laparoscopic access. **(B)**The excised tissue was put into an endopouch specimen retrieval bag under laparoscope. **(C)** The wall of a giant cyst removed through the navel. **(D)** Intraoperative suction of intracapsular fluid. **(E)** Unilateral salpingo-oophorectomy by laparoscope. **(F)** A cosmetic suture of the single-port laparoscopic incision in patients' navel.

**TABLE 1** | Clinical characteristics of the five patients.

| Characteristics | Age | BMI | Gravidity | Parity | No. of previous abdominal operations |
|---|---|---|---|---|---|
| **1** | 23 | 26.3 | 0 | 0 | 0 |
| **2** | 34 | 25.6 | 0 | 0 | 0 |
| **3** | 19 | 16.8 | 0 | 0 | 0 |
| **4** | 25 | 18.8 | 0 | 0 | 0 |
| **5** | 30 | 31.2 | 0 | 0 | 0 |

In the past, the resection of the cystic mass by an exploratory laparotomy was the preferred management strategy (9). But for laparotomy of benign giant cysts, the huge incision caused trouble to the patients (especially young patients). A study showed that with the development of advanced technology, it was feasible to use laparoscopic surgery to remove giant ovarian cysts on the basis of selecting suitable patients and laparoscopic experts (17). Recently, laparoscopic-assisted excision of these giant cysts had been reported in several literatures (6, 18, 19). Avoiding the leakage of cyst fluid has become a challenge in laparoscopic surgery for treating giant ovarian cysts.

In recent years, single-port laparoscopic surgery has become a hot spot as it uses the natural pores of the navel to hide the surgical incision and has the characteristics of perfect cosmetic

**TABLE 2** | Surgical outcomes of the five patients.

| Patient | Age | Cyst size (cm) | Operative time(min) | Fluid volume in cyst (ml) | Intra-op. blood loss (ml) | Post-op. stay (d) | Conversion to laparotomy | Histology | Post-op. complications | Satisfaction with abdominal scar |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 23 | 32 | 100 | 7,000 | 20 | 5 | No | Serous cystadenoma | No | 4 |
| **2** | 34 | 63 | 75 | 16,000 | 20 | 5 | No | Mucinous cystadenoma | No | 5 |
| **3** | 19 | 21 | 37 | 3,500 | 10 | 5 | No | Mucinous cystadenoma | No | 4 |
| **4** | 25 | 23 | 82 | 4,000 | 50 | 4 | No | Serous cystadenoma | No | 4 |
| **5** | 30 | 57 | 132 | 13,000 | 30 | 6 | No | Mucinous cystadenoma | No | 5 |
| **Mean** | 26.2 | 39.2 | 85.2 | 8,700 | 26 | 5 | – | – | – | 4.4 |

results and fast postoperative recovery. In our study, we used a single-port laparoscope to perform surgery on a slightly larger incision at the umbilicus, which exposed the visual field better and avoided the exudation of liquid in the giant cysts. In order to avoid the impact of sudden drop of intraperitoneal pressure on the patients, we used the method previously described to slowly reduce the fluid in the giant cysts. Facts had proved that this method is effective, and these patients did not appear to have related uncomfortable symptoms. We use the wound protector–retractor to protect the incision and reduce the risk of cell spillage. The endopouch specimen retrieval bag was used to take out the specimen after the resection of the diseased tissue, which reduced the potential risks of the leakage of cells and residual cystic fluid. These measures ensured the safety of the operation. Although giant ovarian cysts are larger than 10 cm in diameter, we still selected cysts larger than 20 cm in diameter for study, which are rarer in clinical practice. We analyzed the general information and surgical outcomes of these patients and found that a single-port laparoscopic surgery did not increase the adverse prognosis of patients. On the contrary, a minimally invasive surgery and perfect cosmetic results accelerated the recovery and satisfaction of patients.

Despite the advantages of single-port laparoscopic surgery, not all giant ovarian cysts are suitable for this type of surgery. We need to evaluate the patient's condition before undergoing an operation rigorously, and it is very important to exclude any possible malignant tumors before operation. Forming an operation triangle in a single-port laparoscopic surgery is difficult due to its limited operation space, relatively concentrated instruments, and mutual interference which propose higher demands to the surgeon. It is necessary for us to improve the safety of surgery through more research.

## CONCLUSION

In the treatment of giant ovarian cysts, it is safe and feasible to perform single-port laparoscopic surgery through the strict screening of suitable patients. This operation method has the same advantages of traditional laparoscopy, and it ensures the safety of operation as far as possible and perfectly improves the cosmetic results, which are particularly important for young women.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by China Medical University Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## AUTHOR CONTRIBUTIONS

LJ conducted a thorough literature review and was the major contributor in writing the manuscript. YH and XZ were responsible for reviewing the literature and collecting the patients' information. KL was responsible for performing the surgery. XM was responsible for providing the figures, screening patients and for performing ultrasound examinations. All authors contributed to the article and approved the submitted version.

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REVIEW
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# Risk Assessment and Preventive Treatment for Peritoneal Recurrence Following Radical Resection for Gastric Cancer

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As the most common recurrence pattern after radical gastric cancer resection, peritoneal recurrence is a major cause of mortality, which affects the prognosis of patients to a very large extent. Peritoneal status and risk of peritoneal recurrence can be evaluated by peritoneal lavage cytology, photodynamic diagnosis, imaging examination, and pathologic analysis. Presently, there is no standard approach for preventing peritoneal recurrence after radical surgery; furthermore, controversies exist regarding the effects of some preventive methods. Among the preventive methods, there are high expectations about the potential of preoperative therapy, surgical skill improvement, hyperthermic intraperitoneal chemotherapy, and postoperative treatment to reduce the incidence of peritoneal recurrence after radical gastrectomy. This study aimed to analyze the results of previous studies on the risk assessment and preventive methods of peritoneal recurrence after radical gastrectomy in recent years. We hope to provide references for better approach to clinical diagnosis and treatment strategies for peritoneal recurrence after radical gastrectomy.

**Keywords: gastric cancer, radical resection, peritoneal recurrence, risk assessment, preventive treatment**

## INTRODUCTION

As a common malignant tumor of the digestive system, gastric cancer (GC) has the fifth highest incidence among malignant tumors worldwide and the third highest fatality rate, and there has been a significant increase in its incidence in East Asia (1). Currently, radical resection is the only curative treatment strategy for GC. However, many patients have recurrence after radical resection, and the prognosis of these patients is extremely poor. Furthermore, GC is mainly associated with the depth of tumor invasion, lymphatic involvement, and Borrmann type. The recurrence patterns after radical gastrectomy are classified as locoregional, peritoneal, and nonperitoneal distant recurrence.

The most common site of first recurrence is the peritoneum (48.8%), then the liver (20.8%), and the locoregional (15.2%) (2). The significant reduction of survival time due to peritoneal recurrence is the leading cause of death (2–4). Therefore, early prevention and detection of recurrence with effective intervention are very important to improve the prognosis of patients with GC after radical resection. Presently, in patients with GC, many therapeutic methods and strategies have been used for the prevention and risk assessment of peritoneal recurrence after radical gastrectomy. To establish a reference value for the formulation of clinical strategies, this study aimed to identify current methods for predicting and preventing peritoneal recurrence after radical resection.

## PREOPERATIVELY

Before surgical operation, it is crucial to evaluate the risk of peritoneal recurrence and identify possible micrometastasis for appropriate treatment modality. Such evaluations can improve the accuracy of diagnoses, to ensure early intervention for high-risk patients, lead to the avoidance of unnecessary additional treatments for low-risk patients, and reduce additional harm from the redundant treatment of patients.

### Peritoneal Cytology

Intraperitoneal free cancer cells (IFCC) play a critical role in the development of peritoneal metastasis of GC (the main cause of failure after radical gastrectomy). Peritoneal lavage cytology, widely regarded as the gold standard for the diagnosis of IFCC, has negative and positive results reported as CY0 and CY1, respectively. Patients with positive peritoneal cytology have poor prognosis; therefore, a positive IFCC is considered an independent adverse prognostic factor. A retrospective review including GC patients with only CY1 status in the absence of obvious peritoneal metastasis reported that all patients had recurrence within 3 years after radical resection, and 92% of these patients had peritoneal metastasis, indicating positive cytology as an important precursor of peritoneal recurrence (5). Several previous studies have demonstrated that serosa infiltration is one of the most important predictors of peritoneal micrometastasis (6, 7). Furthermore, when serosa infiltration or suspected serosa infiltration occurs in GC patients, peritoneal lavage cytology should be implemented to confirm the existence of IFCC. However, GC patients with CY1 status are considered at stage IV, and their prognosis is still poor even after curative surgery for GC (8). Therefore, cytological examinations have a profound influence for GC patients in predicting peritoneal recurrence.

The detection methods of peritoneal cytology mainly include traditional cytology (hematoxylin and eosin staining, HE staining), immunoassay, immunohistochemistry (IHC), and reverse transcription polymerase chain reaction (RT-PCR). The accuracy, sensitivity, and specificity of predicting peritoneal recurrence differ and are 73–91.9, 11.1–80, and 86.4–100%; 72–95, 23–100, and 81–92.9; 54.8–76.7, 22.1–75, and 76.9–97.3%; and 61–89.7, 31–100, and 58.8–95% in traditional cytology, immunoassay, IHC, and RT-PCR, respectively (9). Compared with the remaining three methods, RT-PCR shows some advantages.

The main target of detection by RT-PCR is the carcinoembryonic antigen (CEA). In peritoneal lavage fluid, the sensitivity and diagnostic odds ratio of CEA protein or mRNA to predict peritoneal recurrence are higher than those of traditional cytology; however, traditional cytology has a higher specificity. GC CEA-positive patients are more likely to have peritoneal recurrence after radical resection, with significantly reduced overall survival (OS) (10). A meta-analysis of 117 cases with GC also had a similar conclusion (11). The peritoneal recurrence rate among patients with positive CEA was higher than that among negative-CEA patients. Furthermore, the expression of CEA in peritoneal lavage fluid was closely related to peritoneal recurrence after radical gastrectomy; this is considered the most important prognostic factor for recurrence after curative resection. Some scholars have suggested that the results of traditional cytology are so unstable that the detection of IFCC cannot be guaranteed, and it is necessary to combine them with those of other more sensitive molecular techniques (such as IHC or RT-PCR) to improve the detection rate of IFCC in the abdominal cavity (12).

Although RT-PCR shows advantages in accuracy, sensitivity, and specificity to IFCC detection, its procedure is cumbersome and time-consuming. It is impossible to provide reliable information to the surgeon during operation, which is a great limitation of practicability. The emergence of transcription-reverse transcription concerted reaction (TRC) seems to make up for the deficiency of RT-PCR. As a direct RNA amplification detection method, TRC was developed to detect peritoneal lavage fluid in GC patients (13). Moreover, compared with RT-PCR, TRC has a simpler operation maneuver and a faster detection strategy. The sensitivity (85%) and specificity (100%) of TRC are similar to those of RT-PCR (92 and 100%, respectively), but TRC is significantly faster and can be completed in 1.0–1.5 h (14). A prospective multicenter study of advanced GC (AGC) patients undergoing radical resection revealed that disease-free survival (DFS) and peritoneal recurrence-free survival (RFS) in the positive TRC-CEA group are significantly lower than those in the negative group and that TRC-CEA could be an important prognostic marker to predict survival and peritoneal recurrence in GC with serosal invasion (15). Another study showed that CEA detected by TRC after lymph node resection in radical gastrectomy is an important predictor of prognosis, although it is not closely related to peritoneal recurrence (16).

According to the above-mentioned studies, traditional cytology combined with other detection methods may be the best way to improve the detection rate of peritoneal cancer cells. This can help clinicians to identify the high-risk peritoneal recurrence groups and provide the key basis for the formulation of follow-up therapies.

### Imaging Examination

For GC, $^{18}$F-fluorodeoxyglucose positron emission tomography ($^{18}$F-FDG PET) combined with computerized tomography (CT) ($^{18}$F-FDG PET/CT) is often used to evaluate and predict recurrence

prior to surgery and to monitor recurrence post-surgery, whereas its clinical significance has always been controversial in peritoneal recurrence prediction. A retrospective study involving 279 AGC patients who underwent $^{18}$F-FDG PET/CT before radical resection, with the tumor-to-normal liver uptake ratio (TLR) as the examination parameter, found a remarkably higher 5-year distant metastasis-free survival rate among patients with TLR ≤2.0 compared to that among patients with TLR >2.0 (95.5 *vs.* 68.8%, respectively, $P < 0.0001$); however, TLR had no significant correlation with peritoneal RFS ($P = 0.7$) (17). In addition, the attenuation of $^{18}$F-FDG uptake in the visceral adipose tissue (VAT) was found to be significantly associated with peritoneal RFS and OS, whereas in AGC patients with high VAT attenuation and a standardized uptake value (FDG uptake), peritoneal recurrence is more likely to occur after curative resection (18). In summary, depending on some specific parameters, preoperative PET/CT seems to be capable of being used to assess the risk of peritoneal recurrence after radical gastrectomy and may become an important non-invasive evaluation method. Since the sample size of this research was small and the finding was not very convincing, further studies with larger samples of clinical data to support these are still needed.

## Photodynamic Diagnosis

The diagnosis of peritoneal metastasis in patients with GC has a profound impact on treatment strategies. Currently, staging laparoscopy is routine in clinical settings; however, because some micrometastasis that are invisible to the naked eyes may be missed, eventually, this may lead to inappropriate radical resection in these GC patients. As a new technique for fluorescence imaging of lesions using photosensitive drugs, photodynamic diagnosis (PDD) shows great potential in the discovery of micrometastatic foci, with the commonly used drug being 5-aminolevulinic acid (ALA). ALA-PDD is more sensitive than white light laparoscopy in the detection of peritoneal dissemination, with an increased detection rate of peritoneal metastasis by 21–34% with ALA-PDD (19–21). If only white light observation is used without ALA-PDD detection, about 11% of patients with peritoneal dissemination will be missed, and most of these patients (76.9%) identified by ALA-PDD and confirmed to have peritoneal metastasis had negative cytological results (22). Although ALA-PDD shows obvious advantages in detecting peritoneal metastases that are not visible to the naked eye, its false positive rate is higher (32.3%) (23). ALA-PDD can improve the visualization of the invisible peritoneal metastases, and this helps to determine the peritoneal status of patients with AGC, resulting in GC staging accuracy. However, more large-sample randomized controlled clinical trials (RCTs) are needed to assess the applicability of PDD.

## Neoadjuvant Therapy

Neoadjuvant therapy could induce tumor downstaging and improve the rate of R0 resection for resectable GC (24). The neoadjuvant therapy methods are categorized as neoadjuvant chemotherapy (NAC), neoadjuvant radiotherapy, and neoadjuvant chemoradiotherapy. Radiotherapy is mostly used for esophagogastric junction cancer, while chemotherapy is mainly for GC. A meta-analysis involving 15 RCTs showed that neoadjuvant therapy could significantly reduce the overall mortality of AGC patients at 3 (relative risk, RR = 0.74, $P = 0.005$) and 5 (RR = 0.82, $P = 0.009$) years after radical surgery (25). Nonetheless, the postoperative recurrence pattern of patients who received NAC did not seem to have changed compared with the results of earlier studies, and the peritoneum was still the most common relapse site after radical gastrectomy in these patients (2, 26). Moreover, different types of NAC had no effect on 5-year RFS ($P = 0.236$). A retrospective study also reported no statistical difference in overall recurrence and peritoneal recurrence between NAC and surgery-only groups before or after a propensity score matching (27). Recently, the PRODIGY trial published the results indicating that adding NAC (docetaxel, oxaliplatin, and S-1) to the basic treatment of radical surgery plus S-1 adjuvant chemotherapy could notably improve progression-free survival in patients with resectable localized AGC (hazard ratio, HR = 0.70, $P = 0.023$), although there was no statistical difference in the OS (HR = 0.84, $P = 0.338$) (28). The detailed data related to recurrence were not reported. Surprisingly, some retrospective studies showed that neoadjuvant therapy before radical operation was an adverse factor for the long-term survival of AGC patients (HR = 1.631, $P = 0.006$) (2, 29). In terms of peritoneal recurrence, the proportion of patients receiving neoadjuvant therapy was even higher than that of untreated ones (37.1 *vs.* 32.1%). The above-mentioned conditions may occur because patients who receive neoadjuvant therapy tend to have relatively more advanced stage or high-grade tumors, which may explain the difference between pTNM stage and ypTNM stage (post-neoadjuvant pTNM).

Although many studies have concluded that neoadjuvant therapy has no effect on peritoneal recurrence in GC patients after radical resection (2, 26, 27, 29, 30), the recent findings by Xu et al. seem to have reinforced the confidence in neoadjuvant therapy to reduce peritoneal recurrence (31). By propensity score-matched analysis, Xu et al. found that, for local AGC with serosal invasion, the OS and DFS in NAC-treated patients were significantly better than that in the untreated ones ($P < 0.0001$), and patients who received NAC had fewer postoperative complications ($P = 0.037$). It is exciting to note that the overall recurrence in the NAC group was less than that in the non-NAC group (29.9 *vs.* 63.3%), and peritoneal recurrence significantly decreased (19.0 *vs.* 48.4%). According to the above-mentioned studies, NAC appears to have the potential to improve prognosis and prevent recurrence, especially for patients who are at a high risk of peritoneal recurrence, including serosa-positive patients. Neoadjuvant therapy is recommended for T ≥3 and/or with node-positive GC, according to The Italian Research Group for Gastric Cancer (32). A multicenter randomized phase II trial (NCT02931890) is underway to explore different neoadjuvant therapy regimens (chemotherapy, chemotherapy followed by chemoradiotherapy, and chemoradiotherapy) for the purpose of identifying a comprehensive and objective clinical evaluation (33). At present, the populations among whom neoadjuvant

therapy is being implemented differ between Eastern and Western countries, and no consensus has been reached yet. However, most studies set the treatment range to patients with T ≥3 tumor and N+ tumor.

After diagnosis and staging by routine endoscopy examination, endoscopic biopsy, and contrast-enhanced CT, laparoscopy with/without peritoneal lavage cytology is recommended for patients with stage I B or higher GC or with suspected peritoneal metastasis (32, 34, 35). For GC patients with positive peritoneal cytology or macroscopic peritoneal metastases, it is necessary to change the treatment strategy instead of direct radical operation. Preoperative chemotherapy is needed to improve the possibility of R0 resection to avoid incomplete resection and reduce the risk of cell peritoneal seeding during surgery.

# INTRAOPERATIVELY

## Surgical Maneuver

In addition to the serosa infiltration of gastric tumors which increases the risk of peritoneal dissemination, surgical procedures may also cause cancer cells to enter and penetrate the abdominal cavity from the resection margin, blood, or lymphatic vessels and eventually lead to peritoneal metastasis. Radical gastrectomy includes open surgery, laparoscopic surgery, robotic surgery, and endoscopic procedure; among these, endoscopic procedure is mainly aimed at local early-stage GC. According to the cytological analysis of peritoneal lavage in GC patients undergoing radical resection, the diffusion of tumor cells into the peritoneal cavity after operation is higher than that when the abdominal cavity has just been opened and explored, which suggest that the operation could directly promote the iatrogenic dissemination of tumor cells and increase the possibility of peritoneal metastasis (36). Therefore, surgical methods and related precautions for GC have become important concerns for clinicians to reduce postoperative peritoneal recurrence.

Due to extensive trauma, poor postoperative recovery, and other complications, the traditional open radical resection of GC is rapidly giving way to minimally invasive surgery (MIS). Furthermore, a large number of studies have shown that laparoscopic radical gastrectomy has comparable short- and long-term outcomes compared to traditional open radical gastrectomy and is suitable at all stages for GC curative purposes (37–42). According to these literatures, there is no consensus on whether MIS is superior to open surgery in the short- and long-term outcomes, but MIS is, at least, not inferior to traditional open surgery. A propensity score–matched analysis from an eastern center concluded that the postoperative complications (35.2 *vs.* 40.7%, $P = 0.69$) and 90-day mortality (1.9 *vs.* 3.7%, $P$=1.00) in the laparoscopic gastrectomy (LG) group were comparable to those in the open gastrectomy (OG) group (42). Although there was no significant difference between the two groups in 3-year OS and DFS ($P = 0.34$; $P = 0.51$), the LG group had markedly fewer peritoneal recurrences than the OG group (3.7 *vs.* 27.8, $P < 0.01$). Another recent propensity score –matched analysis from a western center also reported similar results of no statistical difference in OS, DFS, postoperative complications, and mortality between LG and OG groups (43).

Compared with open resection, the postoperative overall recurrence of laparoscopic gastrectomy has limited demerits, and its peritoneal recurrence rate is not higher than that of traditional open surgery (39, 41, 44). KLASS-01, a large RCT, showed that, for patients with clinical stage I GC, the long-term oncological outcomes of laparoscopic distal gastrectomy (LDG) and open distal gastrectomy (ODG) are similar, with no significant difference in peritoneal recurrence between them (1.2 *vs.* 1.0%) (45). Furthermore, the other two RCTs (CLASS-01 and KLASS-02) for locally AGC also achieved similar results with similar 3-year DFS in the LDG and ODG groups and no significant difference between the two groups in peritoneal recurrence (46, 47). Shi et al. studied the long-term tumor outcomes of patients with locally AGC after radical resection and found that the 5-year OS and DFS do not notably differ between the LG and OG groups, with no statistical difference in peritoneal recurrence (LG: 28.6% *vs.* OG: 26.0%, $P = 0.705$) and other types of recurrence, between the two groups (48). Based on the above-mentioned research, laparoscopic radical gastrectomy could achieve short- and long-term outcomes comparable to open surgery while not increasing the probability of peritoneal recurrence but showing significant and more prominent advantages in other aspects, such as reduced intraoperative blood loss and early postoperative recovery. Compared with laparoscopic surgery, robotic surgery, another MIS, has prominent advantages, such as fatigue reduction, high stability, and three-dimensional vision, and has been gradually applied in the treatment of GC. In addition, many studies have also reported better minimally invasive advantages with robotic surgery than laparoscopic surgery in radical gastrectomy, and these two operative methods have similar short- and long-term outcomes as well as postoperative peritoneal recurrence rates (49–53). Regarding the high expense of robotic surgery, its application in the treatment of GC is still not yet popularized. Therefore, laparoscopic resection has gradually replaced traditional open resection to become the mainstream method of radical gastrectomy.

Lymph node dissection is an important part of radical gastrectomy and usually classified as D1, D2, and D3 lymphadenectomy according to the extent of dissection. For resectable GC, D2 lymphatic dissection is mainly recommended (54). Currently, there is insufficient and effective evidence for the relationship between the extent of lymph node dissection and peritoneal recurrence after radical gastrectomy. In the Dutch D1D2 trial, despite the absence of noticeable differences in 15-year OS, DFS, and relapse rate between D1 and D2 groups, the cancer-related mortality rate in D1 group was higher than that in D2 group (48 *vs.* 37%, $P = 0.01$) (55). The patients who received D1 lymphadenectomy showed higher rates of locoregional and liver recurrences than those undergoing D2 lymphadenectomy, but the data related to peritoneal recurrence were not reported in this publication. In another research, Nakanishi et al. found no significant difference in 5-year cumulative peritoneal recurrence

rates between D2 minus and D2 groups for AGC patients with CY0 (29 *vs.* 33%, $P = 0.595$) (56). A retrospective study involving 568 AGC patients reported that the overall recurrences of D2 and D3 patients are comparable (57). Furthermore, there was no statistical difference in peritoneal recurrence rates (14.6 *vs.* 11.6%, $P = 0.319$) and other types of recurrence between the two groups. Similarly, a recently published retrospective cohort analysis reported comparable rates of peritoneal recurrence in the D1 plus and D2 groups (4.4 *vs.* 5.0%, $P = 0.743$) (58). According to the above-mentioned studies, the extent of lymph node dissection during radical resection of GC seems to have no correlation with postoperative peritoneal recurrence. Nevertheless, the propensity score-matched analysis by Hayashi et al. provides some interesting results (59). They reported that the number of retrieved lymph nodes (RLN) is related to the long-term outcome of AGC patients after radical surgery. The RLN ≥40 group had notably longer OS and RFS than the RLN <40 group (HR = 2.11, $P = 0.0057$; HR = 2.35, $P = 0.0001$). Furthermore, compared with the RLN ≥40 group, the peritoneal recurrence rate in the RLN <40 group increased significantly ($P = 0.0007$).

As treatment strategies to prevent peritoneal metastasis after radical gastrectomy, the use of either omentectomy or bursectomy has always been controversial. A multicenter prospective cohort study showed that the incidence of omentum metastasis in curable GC is lower and is only related to later clinical stage and non-curable features, and it suggested that omentum resection is not necessary in radical gastrectomy (60). Sakimura et al. reported that, for patients with AGC who underwent radical resection, there was no significant difference between the omentectomy and non-omentectomy groups in 3-year OS and RFS as well as in overall and peritoneal recurrence rates, which suggest that omentectomy could not improve the survival benefits of AGC patients (61). According to some data from an earlier RCT, the peritoneal recurrence in patients who underwent radical gastrectomy plus bursectomy was less than that of those without bursectomy (8.7 *vs.* 13.2%). Although the 3-year OS in the bursectomy group was better than that in the non-bursectomy group, there was no statistically significant difference between groups (62). A subsequent large retrospective study found that, for AGC patients that underwent radical surgery, additional bursectomy had no significant effect on the OS rate ($P = 0.978$), and there was no significant difference in peritoneal recurrence between the bursectomy and non-bursectomy groups ($P = 0.623$) (63). In 2018, a phase 3, open-label RCT (JCOG1001) that explored the survival benefit of bursectomy for resectable GC was published. The 5-year OS in the non-bursectomy group (omentectomy alone) was 76.7%, compared with 76.9% in the bursectomy group (one-sided $P = 0.65$), with no extra survival benefit from bursectomy. Moreover, based on the JCOG1001 data, the peritoneal recurrence rate in the bursectomy group was also the same as that in the non-bursectomy group (44%), suggesting that bursectomy could not improve peritoneal recurrence (64). In the light of the above-mentioned studies, omentectomy and bursectomy not only failed to prevent peritoneal metastasis or to improve the long-term survival but also increased the operation time, intraoperative blood loss, and complications. Therefore, it seems meaningless to add omentectomy or bursectomy to the radical resection of GC. Although many studies have reported that omentectomy does not improve survival benefits to patients, it is still part of the standard gastrectomy guidelines (32, 35, 65). This may be because it is easier to perform omentectomy than preserve the omentum in GC resection, and omentectomy is beneficial for lymph node dissection. Bursectomy is mainly used in Japan and included in Japanese GC treatment guidelines (65). The fifth edition of the Japanese guidelines refers to the conclusion of the JCOG1001 trial, but bursectomy has not been revised yet (64, 65). The sixth edition of the Japanese guidelines may reinterpret the application of omentectomy and bursectomy.

In the process of radical surgery for GC, blood from intraoperative bleeding easily accumulates in the abdominal cavity, which brings the peritoneal surface directly in contact with blood components, thus activating the extravascular blood cells to produce a variety of cytokines and thereby providing a favorable survival microenvironment for tumor cells that leak into the abdominal cavity. A retrospective study of 540 patients with AGC who underwent radical resection found that large intraoperative bleeding is associated with a high risk of peritoneal metastasis, whereas small bleeding is not, and patients with large intraoperative hemorrhages are more likely to develop peritoneal recurrence (66). Another retrospective research showed a significantly higher peritoneal recurrence rate in patients who received allogeneic blood transfusion during the perioperative period of radical resection for GC than that in patients without allogeneic blood transfusion (22.8 *vs.* 9.3%); however, the rates of metastasis to the liver, lung, and lymph nodes did not change (67). Therefore, surgeons should avoid the higher risks of postoperative peritoneal recurrence related to the development of intraoperative bleeding by minimizing intraoperative blood loss to avoid allogeneic blood transfusion.

## Extensive Intra-Operative Peritoneal Lavage

The mechanism of extensive intra-operative peritoneal lavage (EIPL), a simple adjunctive surgical method, is based on limited dilution to reduce the risk of cancer cell dissemination resulting from surgery. Previous studies have shown that EIPL could effectively reduce the level of cancer cells spreading in the peritoneal cavity during radical resection for GC, and the use of distilled water is as effective as normal saline (36). However, the CCOG1102 trial showed the opposite results of no significant difference in peritoneal relapse-free survival rate ($P = 0.676$) and DFS and OS between the EIPL and non-EIPL groups after radical gastrectomy. In this trial, EIPL could neither reduce postoperative peritoneal dissemination nor improve the prognosis of patients, but it seemed to ameliorate DFS for patients with higher intraoperative blood loss or postoperative abdominal infection (68). In this study, most patients had negative peritoneal cytology results, so, even if there were undetected free tumor cells, washing the abdominal cavity with less saline is sufficient to remove them in the non-EIPL group, which may be the reason why there was no significant difference between the EIPL and non-EIPL groups. In the latest

phase 3, multicenter, large-sample RCT (NCT02140034), the 3-year OS rates in the EIPL and surgery-alone groups were 77.0 and 76.7% ($P = 0.62$), respectively, while the 3-year cumulative incidences in peritoneal recurrence were 7.9 and 6.6% ($P = 0.35$), respectively. On the contrary, EIPL not only failed to reduce peritoneal recurrence and improve patient survival but also significantly increased the incidence of adverse events (69). Wound infections and liver function abnormalities were more common in patients receiving EIPL than in patients undergoing surgery alone (2.0 *vs*. 0.3% and 1.7 *vs*. 0.3%, respectively). Furthermore, the incidence of death due to adverse events in the EIPL group (2.3%) was also higher than that in the surgery-alone group (0.6%). However, another large-scale, multicenter (11 centers) RCT (NCT02745509) in China showed that the postoperative adverse events and mortality in the EIPL group are lower than those of the surgery-alone group (11.1 *vs*. 17.0%, $P = 0.04$; 0 *vs*. 1.9%, $P = 0.02$) (70). In this trial, the long-term results have not been released yet.

## Hyperthermic Intraperitoneal Chemotherapy

Hyperthermic intraperitoneal chemotherapy (HIPEC), a combination therapy with precise temperature control for circulating intraperitoneal perfusion of chemotherapeutic agents, has been widely used in the prevention and treatment of peritoneal metastatic tumors. For patients with serosal invasion in GC, compared with the non-HIPEC group, the survival of HIPEC patients significantly improved ($P < 0.00001$), with a remarkably reduced peritoneal recurrence rate ($P = 0.001$) (71). A retrospective study involving 38 GC patients with serosal invasion showed that the peritoneal recurrence in the HIPEC group is dramatically lower than that in the radical-surgery-alone group (11.1 *vs*. 73.7%, respectively, $P < 0.001$) (72). In another RCT of resectable AGC, the results were similar to the previous RCT results, with much higher peritoneal recurrence in the non-HIPEC group (30%) than that in the HIPEC group (5%) (73). Taken together, HIPEC is an effective method for preventing postoperative peritoneal metastasis in high-risk patients. Presently, the use of HIPEC in most countries is mainly confined to the treatment of peritoneal metastatic carcinoma, which has not been included as a standard practice for the preventive therapy of peritoneal recurrence. Firstly, there may be few medical institutions with equipment and conditions for such treatment. Secondly, the pros and cons of whether the conditions of the patients would still be conducive to accepting HIPEC after severe trauma from radical surgery need to be weighed. Most importantly, there is still a lack of valid evidence from large-sample-size RCTs to support the survival benefits of HIPEC.

# POSTOPERATIVELY

## Pathological Analysis

In addition to the previously mentioned methods for evaluating the risk of preoperative peritoneal recurrence, pathological analysis of the resected tumor specimens also has considerable value. In AGC patients undergoing radical gastrectomy, the depth of tumor invasion was the only risk factor significantly associated with peritoneal recurrence (74). Yoo et al. analyzed the prospective data of 655 patients that underwent radical resection for GC. The time to peritoneal relapse in patients with macroscopic serosal lesions was considerably shorter than that in patients without serosal lesions ($P < 0.001$), and the 5-year peritoneal recurrence rates were 32.8 and 8.7%, respectively. These results suggest that the macroscopic assessment of serosal lesions may be a useful index to predict the risk of peritoneal recurrence after radical resection (75). In accordance with the Japanese Classification of Gastric Carcinoma, tumor infiltrative pattern (INF) is classified into INFa (expanding growth with a distinct border from the surrounding tissue), INFb (an intermediate pattern between INFa and INFc), and INFc (infiltrative growth with no distinct border with the surrounding tissue) (76). Compared with INFa and INFb, INFc patients had significantly more peritoneal metastases; furthermore, INF was found to be an independent risk factor for peritoneal recurrence after radical gastrectomy (77–80). A previous study found that adjuvant chemotherapy could not improve the peritoneal recurrence rate in INFc group but reduced the rate in the INFa/b group (78). In a recent study, Chen et al. employed multiphoton imaging technology to quantitatively analyze the collagen characteristics in the tumor microenvironment from the tissue specimens infiltrating the gastric serosa. They revealed that the features of collagen are related to postoperative peritoneal metastasis in GC with serosal invasion. Furthermore, a collagen nomogram that they constructed to predict the risk of peritoneal recurrence with serosa-positive GC after radical gastrectomy displayed a stronger predictive power than the clinicopathological model (81).

## Early Postoperative Intraperitoneal Chemotherapy

To eliminate the microscopic peritoneal lesions after resection of GC, early postoperative intraperitoneal chemotherapy (EPIC) is administered using mitomycin C and 5-fluorouracil (5-FU) or taxanes through inflow and outflow catheters. This is usually performed 1–5 days after surgery and then repeated subsequently every 24 h. As reported in another study, although the safety of EPIC after radical gastrectomy was acceptable, there was no difference in postoperative survival between patients who received EPIC and those who did not, implying that EPIC could not provide survival benefits to patients undergoing curative resection for GC (82). However, the small sample size of 46 patients was a limitation of that study. A retrospective study based on 245 serosa-positive GC patients who underwent radical surgery found that the 5-year OS- and GC-specific survival rates in the EPIC group were significantly better than those in the non-EPIC group. Moreover, the rate of peritoneal recurrence in the EPIC group is notably lower than in the non-EPIC group (18.5 *vs*. 32.2%, $P = 0.038$) (83). Therefore, EPIC appears to be an effective method for GC patients at a high risk of peritoneal recurrence by improving their survival through reducing peritoneal metastases.

## Adjuvant Chemotherapy

Systemic adjuvant chemotherapy for GC patients after surgery has always been important in clinical studies, and clinicians pay great attention to the formulation of a therapeutic regimen and the effects of different schemes on postoperative recurrence. Adjuvant chemotherapy regimens generally include oral 5-FU as monotherapy (including S-1 and capecitabine) or combined with oxaliplatin. In a phase 2 clinical trial (CCOG0301), the 2-year survival rate was higher among GC patients with positive peritoneal lavage cytology treated with oral S-1 after radical resection than among historical controls (84). All the cases included in this trial had positive peritoneal cytology. Sasako et al. conducted a randomized phase 3 clinical trial to evaluate the effect of S-1 as adjuvant chemotherapy for GC patients after radical resection. They found that the 5-year survival outcomes in the S-1 group are better than that in the surgery-only group. In terms of recurrence, the overall relapse rate in the S-1 group is lower than that in the surgery-only group; in particular, the lymph nodes and peritoneum recurrence rates decreased significantly, and postoperative adjuvant therapy with S-1 could reduce the risk of recurrence by 34.7% (85). The patients enrolled in the study had pathological stage II or IIIA/B with CY0, and the chemotherapy regimen was still S-1 monotherapy. In another phase 3 clinical trial, JCOG9206-2, cisplatin combined with UFT could not improve the overall and relapse-free survival in patients with serosal invasive GC after radical resection (86). According to the data in this trial, adding adjuvant chemotherapy, compared with surgery alone, could not reduce peritoneal metastasis. The patients who participated in the trial had GC with macroscopic serosa-invasive, negative peritoneal lavage cytology without distant metastasis. The postoperative treatment plan was intraperitoneal chemotherapy with cisplatin before abdominal closure, followed by intravenous chemotherapy (cisplatin + 5-FU + UFT). A meta-analysis including 3,897 patients undergoing radical resection of GC showed that the addition of adjuvant chemotherapy significantly reduced the rate of peritoneal recurrence compared with surgery alone ($P$ = 0.001) (87). However, the above-mentioned research only analyzed all the regimens together and did not classify the specific adjuvant chemotherapy schemes in the analyses. Recently, a randomized, controlled phase 3 trial of S-l plus docetaxel adjuvant chemotherapy in patients after radical gastrectomy reported that the 3-year RFS in the S-l plus docetaxel group was better than that in the S-1 monotherapy group ($P$ < 0.001). Although the hematogenous site and node recurrence rates in the combination group are significantly lower than that in the S-1-alone group, there was no statistically significant difference between these two groups in peritoneal relapse (9.3 *vs.* 12.9%, $P$ = 0.092). This suggests that S-1, combined with docetaxel adjuvant chemotherapy regimen, does not improve peritoneal metastasis after radical resection of GC compared with S-1 monotherapy (88).

In accordance with previous studies, there is yet no definite conclusion on the effects of postoperative adjuvant chemotherapy in the prevention of peritoneal metastasis after radical gastrectomy of GC. Drug selection and therapeutic regimen are crucial for the appropriate method, and this still needs to be supported with a large number of clinical studies.

# CONCLUSIONS

The occurrence of peritoneal metastasis after radical gastrectomy seriously affects the prognosis of patients to a great extent, and how to identify patients at a high risk of peritoneal recurrence and develop preventive treatment approaches quickly is vital for the reduction of postoperative peritoneal metastasis. The progression degree of GC is significantly correlated with the resection effect of radical gastrectomy. The greater the progression degree, the lower the possibility of R0 resection that is accompanied by patients at a high risk of peritoneal metastasis. Even if patients with AGC could receive curative surgery and other related treatments, their long-term survival is still relatively poor. Undoubtedly, early detection of tumors cannot only improve the effect of radical resection but also greatly reduce the risk of spread of tumor cells in the peritoneal cavity during operation. Therefore, cancer screening has a more practical significance than any other therapeutic method to avoid postoperative peritoneal metastasis. Presently, many countries with a high incidence of GC, such as Japan and South Korea, have established and formulated their own guidelines for GC screening. There are certain effects on preventing peritoneal metastasis after radical resection of GC through accurate judgment of determining peritoneal status, improvement of surgical procedure, peritoneal lavage, intraperitoneal chemotherapy, and adjuvant chemotherapy

**TABLE 1** | Predictive methods of peritoneal recurrence.

| Timing | Method | | Potential clinical value |
|---|---|---|---|
| Preoperatively | Peritoneal lavage cytology | Traditional cytology (HE staining) | 1. The most important risk predictor |
| | | Immunoassay | 2. Traditional cytology combined with molecular biology techniques such as RT-PCR could improve the detection effect |
| | | Immunohistochemistry | |
| | | RT-PCR | 3. TRC could significantly improve the detection efficiency |
| | | TRC | |
| | $^{18}$F-FDG PET/CT | | Simple and feasible; limited reference value and lack of evidence |
| | Photodynamic diagnosis | | Improve the detection rate of micrometastases and make the staging more accurate, but the false positive rate is high |
| Postoperatively | Pathological analysis | | An important risk predictor of peritoneal recurrence; high feasibility and reference value |

**TABLE 2** | Preventive methods of peritoneal recurrence.

| Timing | Method | | Potential clinical value |
|---|---|---|---|
| Preoperatively | Neoadjuvant therapy | | Certain preventive value for patients at stage T≥3 and N+ |
| Intraoperatively | Surgical maneuver | Open gastrectomy<br>Laparoscopic gastrectomy<br>Robotic gastrectomy | 1. No significant difference in peritoneal recurrence among the three surgical methods<br>2. Considering the intraoperative blood loss and postoperative recovery, laparoscopic resection could be actively selected |
| | | Lymphadenectomy | No definite relevance between the extent of dissection and peritoneal recurrence |
| | | Omentectomy and bursectomy | Be cautious to use bursectomy |
| | | Others | Reducing the amount of intraoperative blood loss could diminish the risk of peritoneal recurrence |
| | EIPL | | It could be considered for patients with massive intraoperative blood loss, while conventional peritoneal lavage for the rest |
| | HIPEC | | Could be considered for patients with a high risk of peritoneal recurrence and permitting physical conditions |
| Postoperatively | EPIC | | Could be considered for patients with a high risk of peritoneal recurrence |
| | Adjuvant chemotherapy | | Doubt in preventive effect |

(**Tables 1**, **2**). Despite all these findings, several prospective multicenter studies are essential to elucidate clinical evidence, promoting the criteria for the prevention of peritoneal recurrence after radical resection in the management of GC.

## AUTHOR CONTRIBUTIONS

LX and HC contributed to the conception and design of the review. LX and SJ collected the related paper and drafted the manuscript. YZ, PZ and EM revised the manuscript. All authors contributed to the article and approved the submitted version.

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# Therapeutic Role of Retroperitoneal Lymphadenectomy in 170 Patients With Ovarian Clear Cell Cancer

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**Introduction:** We evaluated the therapeutic role of retroperitoneal lymphadenectomy in patients with ovarian clear cell cancer (OCCC).

**Materials and Methods:** We retrospectively reviewed 170 OCCC patients diagnosed at two hospitals in China between April 2010 and August 2020. Clinical data were abstracted, and patients were followed until February 2021. Patients were divided into retroperitoneal lymphadenectomy and no lymphadenectomy groups. The Kaplan–Meier method was used to compare progression-free (PFS) and overall survival (OS) between the two groups. Statistical differences were determined by the log-rank test. The COX proportional hazards regression model was applied to identify predictors of tumor recurrence.

**Results:** The median age was 52 years; 90 (52.9%) and 80 (47.1%) patients were diagnosed as early and advanced stage, respectively. Clinically positive and negative nodes was found in 40 (23.5%) and 119 (70.0%) patients, respectively. Of all the 170 patients, 124 (72.9%) patients underwent retroperitoneal lymphadenectomy, while 46 (27.1%) did not. The estimated 2-year PFS and 5-year OS rates were 71.4% and 65.9% in the lymphadenectomy group, and 72.0% and 73.7% in no lymphadenectomy group ($p = 0.566$ and 0.669, respectively). There was also no difference in survival between the two groups when subgroup analysis was performed stratified by early and advanced stage, or in patients with clinically negative nodes. Multivariate analysis showed that retroperitoneal lymphadenectomy were not an independent predictor of tumor recurrence.

**Conclusion:** Retroperitoneal lymphadenectomy provided no survival benefit in patients diagnosed with OCCC. A prospective clinical trial is needed to confirm the present results.

**Keywords: ovarian clear cell cancer, retroperitoneal lymphadenectomy, cancer stage, progression free survival, overall survival**

# INTRODUCTION

Epithelial ovarian cancer (EOC) is the most lethal of all gynecologic malignancies. In 2020, the estimated number of deaths was 13 940 in the USA, which ranks fifth in cancer deaths among women (1). Ovarian clear cell cancer (OCCC) is a lethal histological subtype with an incidence rate ranging from 5%–25% according to geographical area and race (2).

Although the distinct biological and clinical behavior of OCCC differs extensively from serous ovarian cancer, such as younger age and earlier International Federation of Gynecology and Obstetrics (FIGO) stage at diagnosis, greater chemoresistance, and higher rate of thromboembolic complications, the surgical treatment of these different EOC subtypes is similar (2, 3). According to the National Comprehensive Cancer Network (NCCN) clinical practice guidelines for ovarian cancer/Fallopian tube cancer/primary peritoneal cancer (Version 1. 2021, available at NCCN.org), standard surgical staging procedures, including systematic retroperitoneal lymphadenectomy (para-aortic and pelvic lymph nodes) should be performed in ovarian cancer patients with early FIGO stage (apparent FIGO stage IA–IIA). For patients with advanced ovarian cancer involving the pelvis and upper abdomen (FIGO stage ≥ IIB), optimal cytoreductive surgery, including resection of suspicious and/or enlarged nodes, should be performed, while this is not required for patients with clinically negative nodes.

Previous studies have shown inconsistent results regarding the prognostic impact of retroperitoneal lymphadenectomy for ovarian cancer in both early- and advanced-stage patients (4–8). Furthermore, different ovarian cancer subtypes have distinct biological and clinical behavior, which is especially true for OCCC; therefore, the subtypes should be studied separately. We conducted this retrospective study to estimate the prognostic impact of retroperitoneal lymphadenectomy in patients with OCCC.

# METHODS AND MATERIALS

## Patients

This was a retrospective study conducted at Fudan University Zhongshan Hospital and Zhejiang Cancer Hospital between April 2010 and August 2020. Patients who were primarily treated and pathologically diagnosed with OCCC were identified, and their clinical data were collected.

Medical records were abstracted to obtain the patients' age at diagnosis; preoperative value of serum carbohydrate antigen (CA)125 and CA199; preoperative imaging; FIGO stage; preoperative venous thromboembolism (VTE); type of surgery (laparotomy or laparoscopy); Fagotti score; ascites volume; intraoperative exploration; surgical procedures; pathology of dissected lymph nodes; adjuvant chemotherapy; number of chemotherapy cycles; residual disease after primary surgery; and PFS and OS.

Due to the retrospective nature of this study, there were no standards for performing retroperitoneal lymphadenectomy between different surgeons in the two centers. Normally, patients would receive retroperitoneal lymph node dissection when clinically positive nodes were identified according to preoperative imaging or intraoperative exploration. However, for patients with clinically negative nodes, whether to perform lymphadenectomy or not would be determined by the surgeons. Overall, we divided the patients into two groups: lymphadenectomy group and no lymphadenectomy group. Lymphadenectomy group included patients receiving systematic lymph node resection (systematic pelvic lymphadenectomy with or without para-aortic lymphadenectomy or biopsy) and partial lymph node dissection (few patients with enlarged para-aortic lymph node received para-aortic lymph node resection only). Patients did not undergo lymph node resection were included in no lymphadenectomy group. To analyze the role of lymphadenectomy, subgroup analysis was performed stratified by early-stage (FIGO stage IA–IIA) and advanced-stage (FIGO stage IIB–IVB), and also in patients with clinically negative nodes.

The study was approved by the medical ethics committees of both Fudan University Zhongshan Hospital (B2021-368) and Zhejiang Cancer Hospital (IRB-2021-244). PFS was defined as the time from primary surgery to the date of recurrence, and OS was calculated as the time from primary surgery to the date of death or the last follow-up. The last follow-up date was in February 2021.

## Statistical Analysis

The SPSS software package for windows (version 19.0; SPSS Inc., Armonk, NY, USA) was used for statistical analysis. The Chi-square or Mann-Whitney U tests were used to identify differences in the baseline level between lymphadenectomy and no lymphadenectomy group. The Kaplan–Meier method was used to compare survival between groups, and statistical differences were determined by the log-rank test. The COX proportional hazards regression model was applied to identify prognostic factors. A $p$-values of $< 0.05$ was considered statistically significant.

# RESULTS

## Baseline and Clinical Characteristics

We enrolled 170 patients in this study, namely 43 patients from Fudan University Zhongshan Hospital and 127 patients from Zhejiang Cancer Hospital. Clinical characteristics of the 170 patients was shown in **Supplementary Table 1**. The median age at diagnosis was 52 years (range, 30–79 years). More than half of the patients (52.9%) were diagnosed with early-stage disease (FIGO stage IA–IIA). Clinically positive and negative nodes were found in 40 (23.5%) and 119 (70.0%) patients, respectively. In 119 patients with clinically negative nodes, 79 (66.4%) and 40 (33.6%) patients were included in lymphadenectomy and no lymphadenectomy group, respectively. In total, 124 (72.9%) patients underwent lymphadenectomy, while 36 (27.1%) did not. The patients' baseline characteristics in the lymphadenectomy and no lymphadenectomy groups are shown in **Table 1**, and the

**TABLE 1** | Clinical characteristics between lymphadenectomy and no lymphadenectomy group.

| Characteristics | Lymphadenectomy group (n = 124) | No lymphadenectomy group (n = 46) | *P* value |
|---|---|---|---|
| Age at diagnosis | | | |
| ≤50 | 59 (47.6%) | 15 (32.6%) | 0.085 |
| >50 | 65 (52.4%) | 31 (67.4%) | |
| Median preoperative CA125 (U/ml) | 137.4 | 219.0 | 0.430 |
| Median preoperative CA19-9 (U/ml) | 25.2 | 24.5 | 0.212 |
| FIGO Stage | | | |
| Early (IA-IIA) | 67 (54.0%) | 23 (50.0%) | |
| Advanced (IIB-IVB) | 57 (46.0%) | 23 (50.0%) | 0.730 |
| Lymph node status | | | |
| Clinically positive | 37 (29.8%) | 3 (6.5%)[a] | |
| Clinically negative | 79 (63.7%) | 40 (87.0%) | |
| NA | 8 (6.5%) | 3 (6.5%) | 0.001 |
| VTE | | | |
| Yes | 8 (6.5%) | 7 (15.2%) | |
| No | 116 (93.5%) | 39 (84.8%) | 0.123 |
| Fagotti score | | | |
| <8 | 114 (91.9%) | 38 (82.6%) | |
| ≥8 | 10 (8.1%) | 8 (17.4%) | 0.095 |
| Ascites | | | |
| None | 74 (59.7%) | 28 (60.9%) | |
| Yes | 47 (37.9%) | 16 (34.8%) | |
| NA | 3 (2.4%) | 2 (4.3%) | 0.857 |
| Residual disease | | | |
| NGR | 111 (89.5%) | 34 (73.9%) | |
| RD >0 | 10 (8.1%) | 10 (21.7%) | |
| NA | 3 (2.4%) | 2 (4.3%) | 0.028 |
| Chemotherapy | | | |
| Taxane + platinum | 109 (87.9%) | 39 (84.8%) | |
| Other platinum-based chemotherapy | 4 (3.2%) | 3 (6.5%) | |
| Others | 2 (1.6%) | 0 | |
| None | 7 (5.6%) | 4 (8.7%) | |
| NA | 2 (1.6%) | 0 | 0.547 |
| Chemotherapy cycles | | | |
| 0-3 | 28 (22.6%) | 13 (28.3%) | |
| ≥4 | 94 (75.8%) | 33 (71.7%) | |
| NA | 2 (1.6%) | 0 | 0.546 |

*CA125, carbohydrate antigen 125; CA19-9, carbohydrate antigen 19-9; FIGO, International Federation of Gynecology and Obstetrics; VTE, venous thromboembolism; NGR, no gross residual disease; RD, residual disease; NA, not available.*

[a]*Three patients did not received retroperitoneal lymph node resection because of suboptimal debulking surgery in abdominal cavity (residual disease >1cm).*

baseline characteristics were well balanced except regarding residual disease. In the no lymphadenectomy group, patients tended to undergo suboptimal surgery.

## Pathological Characteristics

Of the 124 patients undergoing retroperitoneal lymphadenectomy, 36 (29.0%) patients underwent pelvic lymph node resection, 5 patients (4.0%) underwent aortic lymph node resection, and 83 (66.9%) patients underwent both pelvic and aortic lymph node resection. Postoperative pathology of the dissected lymph nodes showed that 27 (21.8%) patients had positive lymph nodes, and 97 (78.2%) patients had negative lymph nodes. Forty-nine (39.5%) and 72 (58.1%) patients had < 20 and ≥ 20 lymph nodes resected, respectively (**Table 2**).

We next calculated the lymph node metastasis rate according to pT distribution. As shown in **Table 3**, the lymph node metastasis rate was significantly higher when tumor lesions were more extensive, with a rate of 4.3%, 20.0%, and 58.8% for pT1, pT2, and pT3, respectively.

## Survival Analysis

### In the Overall Cohort

The Kaplan–Meier curves shown in **Figure 1** indicate that, in the overall cohort, the estimated 2-year PFS was 71.4% and 72.0% in the lymphadenectomy group and no lymphadenectomy group, respectively ($p$=0.566). The estimated 5-year OS rates were 65.9% and 73.7% in the lymphadenectomy group and no lymphadenectomy group, respectively ($p$=0.669). No significant difference was found between the two groups.

### Subgroup Analysis Stratified by FIGO Stage (Early and Advanced Stage)

We next analyzed the role of retroperitoneal lymphadenectomy separately by stratifying all OCCC patients into early- and advanced-stage groups. The estimated 2-year PFS rates were 89.7% and 100.0% in the early-stage lymphadenectomy group and no lymphadenectomy group, respectively ($p$=0.256). The estimated 5-year OS rates were 92.4% and 100.0% in the early-stage lymphadenectomy group and no lymphadenectomy

**TABLE 2** | Lymphadenectomy characteristics.

| Characteristics | n = 124 |
|---|---|
| Lymph node dissection | |
| Pelvic only | 36 (29.0%) |
| Aortic only | 5 (4.0%) |
| Pelvic and aortic | 83 (66.9%) |
| Lymph node metastasis | |
| Positive | 27 (21.8%) |
| Negative | 97 (78.2%) |
| Number of lymph node removed | |
| <20 | 49 (39.5%) |
| ≥20 | 72 (58.1%) |
| NA | 3 (2.4%) |

*NA, not available.*

group, respectively ($p$=0.263). In advanced-stage patients, the estimated 2-year PFS rates were 50.0% in the lymphadenectomy group and 42.5% in the no lymphadenectomy group ($p$=0.281), and the estimated 5-year OS rates were 36.9% and 46.6% in the lymphadenectomy group and no lymphadenectomy group, respectively ($p$=0.351). The survival curves are displayed in **Figure 2**.

### Subgroup Analysis in Patients With Clinically Negative Nodes

Interestingly, we analyzed the role of retroperitoneal lymphadenectomy in patients with clinically negative nodes. As shown in **Figure 3**, there was no significant difference in the 2-year PFS and 5-year OS rate between the lymphadenectomy group and no lymphadenectomy group ($p$ = 0.378 and 0.777, respectively).

## Univariate and Multivariate Analysis of the Predictors of Recurrence

As shown in **Table 4**, patients with advanced stage, VTE, Fagotti score ≥ 8, ascites, residual disease > 0, and less than four chemotherapy cycles had a shorter PFS by univariate analysis. Multivariate analysis showed that advanced stage (hazard ratio (HR), 3.082; 95% confidence interval (CI), 1.346–7.058), VTE (HR, 2.675; 95% CI, 1.112–6.433), ascites (HR, 2.354; 95% CI, 1.118–4.762), residual disease > 0 (HR, 8.128; 95% CI, 3.342-19.767), and less than four chemotherapy cycles (HR, 1.821; 95% CI, 1.015-3.268) were independent predictors of tumor recurrence, while retroperitoneal lymphadenectomy was not a significant factor influencing tumor recurrence.

# DISCUSSION

Recent studies focusing on the role of retroperitoneal lymph node dissection have emerged following the results of the LION study (4). For advanced ovarian cancer patients, Fang et al. found that systematic lymphadenectomy did not improve survival in patients with no gross residual disease (NGR) or residual tumors measuring < 1 cm (5). Ting et al. showed that retroperitoneal lymph node dissection was not associated with a gain in overall-(OS) and progression-free survival (PFS) for patients with early-stage ovarian cancer (6). Chen et al. showed that retroperitoneal lymph node dissection was not significantly associated with improved prognosis for most stage I EOC patients, but may be necessary for the stage IC subtype (7). Bizzarri et al. showed that pelvic and para-aortic lymphadenectomy improved disease-free survival while having no impact on OS in apparent early-stage ovarian cancer patients (8). In our study, the results suggested that retroperitoneal lymphadenectomy provided no survival benefit in patients diagnosed with OCCC, no matter in the whole cohort or when subgroup analysis were performed stratified by early and advanced stage, or in patients with clinically negative nodes.

Although the results of an earlier study (9) showed that complete surgical staging involving pelvic and para-aortic lymphadenectomy appeared to improve survival in patients with stage I OCCC, more recent research showed no benefit (10). The recent studies including ours seem reasonable for the following reasons: First, for early-stage OCCC patients, the frequency of lymph node metastasis was much lower than other tumor subtypes according to previous studies. Heitz et al. studied the frequency of lymph node metastasis in patients with different tumor stages and histological subtypes who underwent pelvic and paraaortic lymphadenectomy. The results showed that 3.6% of OCCC patients with stage pT1a-pT2aM0 tumors had lymph node metastasis, while the rate was 71.6% in patients with high-grade serous ovarian cancer and 47.4% for high-grade endometrial cancer (11). Mahdi et al. estimated the prevalence of lymph node involvement in stage I OCCC patients from data from the SEER database, and the results showed that 61 (4.5%) of 1359 stage I OCCC patients were upstaged to FIGO stage III (12). In our study, the rate of lymph node metastasis was 4.3% in patients with stage pT1 disease (**Table 3**), similar to findings in these two previous studies. Second, regarding postoperative adjuvant chemotherapy, except for stage IA OCCC patients, for whom observation is feasible, both stage I and stage IIIA OCCC patients should receive postoperative chemotherapy, meaning that

**TABLE 3** | Rates of lymph node metastasis according to pT status.

| pT status | pN1 | pN0 | pNx | Rate of lymph node metastasis[1] |
|---|---|---|---|---|
| pT1 (n=93) | 3 | 67 | 23 | 4.3% |
| pT2 (n=25) | 4 | 16 | 5 | 20.0% |
| pT3 (n=52) | 20 | 14 | 18 | 58.8% |
| Total (n=170) | 27 | 97 | 46 | 27.8% |

[1]*Rate of lymph node metastasis = number of pN1/pN0+pN1.*

*pT, pathologic tumor status; pN, pathologic lymph node status; pN1, regional lymph node metastasis; pN0, no regional lymph node metastasis; pNx, lymph node metastasis not determined.*

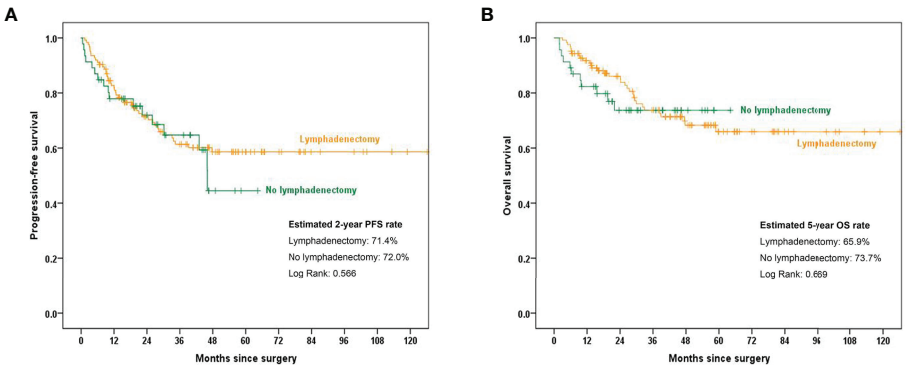


**FIGURE 1** | Kaplan–Meier curves showing 2-year PFS and 5-year OS rates between the lymphadenectomy group and no lymphadenectomy group. **(A)** PFS comparison in the overall cohort; **(B)** OS comparison in the overall cohort.PFS, progression-free survival; OS, overall survival.

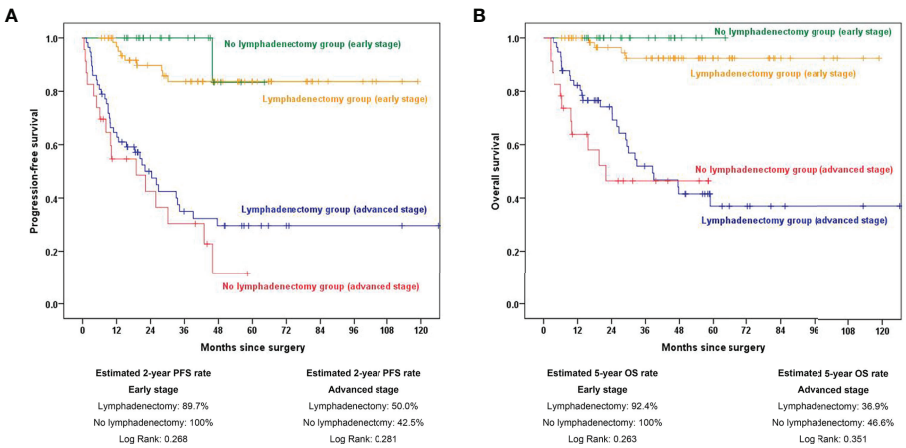


**FIGURE 2** | Kaplan–Meier curves showing 2-year PFS and 5-year OS rates stratified by early and advanced stage between the lymphadenectomy group and no lymphadenectomy group. **(A)** PFS comparison in subgroup analysis stratified by FIGO stage; **(B)** OS comparison in subgroup analysis stratified by FIGO stage. PFS, progression-free survival; OS, overall survival.

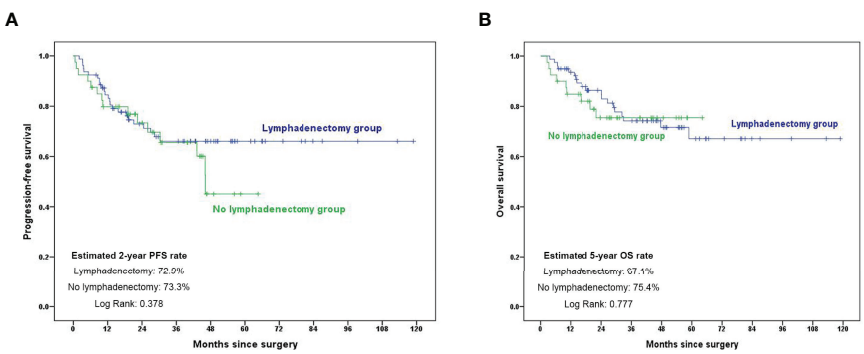


**FIGURE 3** | Kaplan–Meier curves showing 2-year PFS and 5-year OS rates in patients with clinically negative nodes. **(A)** PFS comparison in subgroup analysis in patients with clinically negative nodes; **(B)** OS comparison in subgroup analysis in patients with clinically negative nodes. PFS, progression-free survival; OS, overall survival.

**TABLE 4** | Univariate and multivariate analysis for progression-free survival in all OCCC patients.

| Characteristics | N | Univariate | | Multivariate | |
|---|---|---|---|---|---|
| | | 2-year PFS rate | *p* value | HR (95% CI) | *p* value |
| Age at diagnosis | | | | | |
| ≤50 | 74 | 68.6% | | | |
| >50 | 96 | 73.7% | 0.736 | | |
| FIGO stage | | | | | |
| IA-IIA | 90 | 92.5% | | | |
| IIB-IVB | 80 | 47.8% | <0.001 | 3.082 (1.346-7.058) | 0.008 |
| VTE | | | | | |
| No | 155 | 73.0% | | | |
| Yes | 15 | 54.5% | 0.049 | 2.675 (1.112-6.433) | 0.028 |
| Fagotti score | | | | | |
| <8 | 152 | 77.9% | | | |
| ≥8 | 18 | 0% | <0.001 | 1.764 (0.687-4.525) | 0.238 |
| Ascites | | | | | |
| None | 102 | 87.1% | | | |
| Yes | 63 | 49.4% | <0.001 | 2.354 (1.118-4.762) | 0.014 |
| Retroperitoneal lymphadenectomy | | | | | |
| No | 46 | 72.0% | | | |
| Yes | 124 | 71.4% | 0.566 | 0.557 (0.265-1.168) | 0.121 |
| Residual disease | | | | | |
| NGR | 145 | 81.8% | | | |
| RD >0 | 20 | 8.6% | <0.001 | 8.128 (3.342-19.767) | <0.001 |
| Chemotherapy cycles | | | | | |
| ≥4 | 127 | 88.7% | | | |
| <4 | 41 | 57.1% | 0.011 | 1.821 (1.015-3.268) | 0.044 |

*OCCC, ovarian clear cell cancer; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; VTE, venous thromboembolism; NGR, no gross residual disease; RD, residual disease.*

postoperative adjuvant therapy is almost unaffected by retroperitoneal lymphadenectomy. Furthermore, the rate of lymph node metastasis for all pT1 stage patients (< 5%) may also suggest a lower frequency in OCCC patients with stage pT1A tumors. Therefore, lymphadenectomy may accurately upstage only a small percentage (< 5%) of early-stage OCCC patients, indicating an extremely limited therapeutic role.

Some studies evaluating the number of resected lymph nodes in early OCCC, such as the study by Yuji et al. (13) showed that for patients with stage I OCCC, the group with ≥ 35 resected lymph nodes were correlated with better recurrence-free survival than those with < 35 resected lymph nodes. Harder et al. found a trend toward improved survival when more extensive lymphadenectomy (> 10 nodes) was performed, although there was no statistical significance (12). Matsuo et al. found that adequate lymphadenectomy was associated with a 15%–25% reduction in ovarian cancer mortality compared with inadequate lymphadenectomy (14). In our study, there was no survival difference between patients with < 20 vs ≥ 20 resected lymph nodes (**Supplementary Figure 1**).

A recent study of 410 advanced-stage ovarian cancer patients (including both serous and non-serous cancer) showed no significant difference in 5-year OS and 2-year PFS between the lymphadenectomy group and no lymphadenectomy group, while patients in the lymphadenectomy group had a higher incidence of infection (5). The study included patients with negative (n=288, 70.2%) and positive lymph nodes, and the results indicated no benefit with lymphadenectomy for both the entire cohort and when patients were stratified by lymph node clinical evaluation. ours is the first study investigating the therapeutic role of retroperitoneal lymphadenectomy in advanced-stage OCCC patients. As shown in **Table 3**, almost 60% of patients with stage pT3 OCCC had retroperitoneal lymph node metastasis, which was much higher than in patients with stage pT1 disease. Our results showed a negative prognostic role of lymphadenectomy in these patients.

In addition to advanced ovarian cancer patients undergoing primary debulking surgery, several recent studies have evaluated the role of lymphadenectomy in patients who underwent interval debulking surgery. A systematic literature review from Seidler et al, that included 1094 patients from six retrospective series, suggested no benefit of systematic lymphadenectomy during interval debulking surgery procedure on survival in node-negative, advanced-stage ovarian cancer patients (15). He et al. retrospectively analyzed the role of lymphadenectomy in advanced-stage ovarian cancer patients who underwent interval debulking surgery. Of the 303 patients included in the study, 163 (53.8%) patients achieved NGR, and 127 (41.9%) patients underwent lymphadenectomy. The results suggested no therapeutic value of lymphadenectomy, with both PFS and OS showing no statistical difference between the lymphadenectomy group and no lymphadenectomy group (16). In our study, we did not include patients received neoadjuvant chemotherapy for analysis to avoid bias.

Several limitations existed in our study. The first weakness was the low cases number. A more concrete analysis could be achieved with more cases enrolled, especially when subgroup analysis was performed in the study. Another limitation was that our study included patients with early-and advanced stage, optimal and sub-

optimal surgery, and clinically positive and negative lymph nodes. The heterogeneity of the sample may also weaken the conclusion of our study. However, the results of the current study may provide evidence for designing a randomized clinical trial specifically for patients with ovarian clear cell cancer.

## CONCLUSIONS

In this retrospective study, we found no survival benefit of retroperitoneal lymphadenectomy in OCCC patients, both in the entire cohort and when subgroup analysis was performed. A prospective clinical trial is needed to confirm the present results.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical ethics committees of both Fudan University Zhongshan Hospital and Zhejiang Cancer Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

SY, WG, and JZ contributed to conception and design of the study. PS, HS, and MX organized the database. SY and WG performed the statistical analysis. SY wrote the first draft of the manuscript. WG, PS, and WT wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.754149/full#supplementary-material

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# Palliative Gastrointestinal Surgery in Patients With Advanced Peritoneal Carcinomatosis: Clinical Experience and Development of a Predictive Model for Surgical Outcomes

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**Background:** Palliative gastrointestinal (GI) surgery potentially relieves distressing symptoms arising from intestinal obstruction (IO) in patients with advanced peritoneal carcinomatosis (PC). As surgery is associated with significant morbidity risks in advanced cancer patients, it is important for surgeons to select patients who can benefit the most from this approach. Hence, we aim to determine predictors of morbidity and mortality after palliative surgery in patients with PC. In addition, we evaluate the utility of the UC Davis Cancer Care nomogram (UCDCCn) and develop a simplified model to predict short-term surgical mortality in these patients.

**Methods:** A retrospective review of patients with IO secondary to PC undergoing palliative GI surgery was performed. Logistic regression was used to determine independent predictors of 30-day morbidity and mortality after surgery. UCDCCn was evaluated using the area under the curve (AUC) for discriminatory power and the Hosmer-Lemeshow test for calibration. Our simplified model was developed using logistic regression and evaluated using cross-validation.

**Results:** A total of 254 palliative GI surgeries were performed over a 10-year duration. The 30-day morbidity and mortality were 43% (n = 110) and 21% (n = 53), respectively. Preoperative albumin, age, and emergency nature of surgery were significant independent predictors for 30-day morbidity. A simplified model using preoperative Eastern Cooperative Oncology Group (ECOG) status and albumin (AUC = 0.71) achieved better predictive power than UCDCCn (AUC = 0.66) for 30-day mortality.



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**Conclusion:** Good ECOG status and high preoperative albumin levels were independently associated with good short-term outcomes after palliative GI surgery. Our simplified model may be used to conveniently and efficiently select patients who stand to benefit the most from surgery.

**Keywords: advanced cancer, intestinal obstruction, palliation, palliative surgery, peritoneal carcinomatosis**

# 1 INTRODUCTION

Peritoneal carcinomatosis (PC) is an end-stage presentation of up to 50% of advanced cancer patients with various primary tumors (1, 2). Debilitating gastrointestinal (GI) symptoms due to complex, multilevel intestinal obstruction (IO) are common and may not be adequately palliated with medical or endoscopic therapy alone (3). As such, though infrequently publicized, palliative surgeries make up approximately one-fifth of all surgical procedures performed at any major cancer center annually (4). In fact, most report high rates of clinical success ranging from 80% to 100% after palliative GI surgery for PC-associated IO (5, 6).

Though a direct and effective means of palliation in IO, surgery is associated with significant morbidity risks. A systematic review of 17 retrospective studies on surgical management of malignant bowel obstruction found that serious complications occurred in up to 44% of patients, and mortality rates ranged from 6% to 32% (5). Citing high morbidities and in-hospital deaths among advanced cancer patients undergoing palliative surgery, some physicians adopt a blanket "no surgery" approach in favor of medical treatment alone among palliative PC patients (7, 8). This misconception deprives suitable surgical candidates of a treatment modality that can provide good palliation during end of life. As such, there is a need to identify palliative PC patients who will benefit the most from surgery while adopting discretion when offering a surgical mode of palliation in those in whom poor outcomes are expected.

The UC Davis nomogram was developed to predict 30-day morbidity and mortality among patients with disseminated malignancy who had undergone surgical intervention (9). With the use of data from the American College of Surgeons National Surgical Quality Improvement Program (ACS NSQIP), preoperative factors affecting an individual's risk of perioperative morbidity and mortality were identified. Thirteen and fourteen factors including "do not resuscitate (DNR)" status, age, weight loss >10%, dyspnea, functional dependence, ascites, chronic steroid use, active sepsis, serum creatinine level, serum albumin level, serum white blood cell (WBC), serum hematocrit and acuity of surgical procedure, and procedure type were found to be independently associated with postoperative complications and death, respectively. With the use of the above factors, nomograms predictive of the probability of experiencing a postoperative event were then constructed. While comprehensive, the model has not been independently validated and may be cumbersome when applied in clinical practice due to its complexity.

As such, our study aims to report our clinical experience in palliative GI surgery in the context of PC and evaluate the utility of the UC Davis model in predicting perioperative outcomes in our patient cohort. We also aim to develop a simplified model to predict 30-day morbidity and mortality among patients undergoing palliative surgery.

# 2 MATERIALS AND METHODS

A retrospective review of PC patients who underwent palliative surgery for IO at the Singapore General Hospital was performed from January 2009 to January 2019. Patients with PC from a variety of primary malignancies, including GI, gynecological, hepato-pancreatico-biliary, and others, were included. Patient demographics, perioperative variables, tumor characteristics, and postoperative morbidity and mortality outcomes were obtained from medical records.

The study was conducted with the approval of the ethics board.

## 2.1 Definitions

### 2.1.1 Peritoneal Carcinomatosis and Intestinal Obstruction

All patients had a histologically proven diagnosis of malignancy and histologically or radiologically proven metastases, specifically metastases to the peritoneum, at the time of surgery. IO was defined clinically based on signs and symptoms of obstruction such as abdominal distention, abdominal pain, nausea and vomiting, constipation, inability to pass air, or radiologically on imaging modalities performed (10).

### 2.1.2 Preoperative, Intraoperative, and Postoperative Variables

The following comorbid conditions were determined to be absent or present based on ACS NSQIP criteria (11). Dyspnea was defined as the presence of labored breathing on exertion or at rest. Significant weight loss was defined as weight loss of 10% in the previous 6 months. Preoperative sepsis was defined as a positive bacterial culture identified in addition to two or more of the following criteria: fever, tachycardia, tachypnea, leukocytosis, and anion gap acidosis. Preoperative chemotherapy or radiotherapy was defined as the administration of chemotherapy within 30 days and radiotherapy within 90 days before surgery.

Intraoperatively, the type of surgical procedures was stratified to consider if GI resection, multi-visceral resection, and other abdominal surgical procedures such as adhesiolysis were

performed as per the ACS NSQIP classification. We further collected information on the type of bowel resection, anastomoses, and stoma fashioned. Emergency cases were designated by the primary surgeon after considering the clinical circumstances surrounding palliative surgical interventions.

Data on postoperative complications were collected and included organ-specific complications (hematological, cardiac, respiratory, neurologic, abdominal, and others). This was in line with ACS NSQIP-reported complication codes. Unplanned readmissions and Calvien–Dindo-based classification of major and minor postoperative complications were recorded as well (12).

#### 2.1.3 30-Day Overall Morbidity and Mortality

Morbidity and mortality were considered at 30 days calculated from the date of palliative surgery.

### 2.2 Statistical Analysis

The baseline statistics of the cohort were summarized with a mean (SD) for continuous variables and N (%) for categorical variables. Univariate statistical testing was performed for significant associations between individual preoperative and postoperative variables, with 30-day mortality and 30-day morbidity. We used t-test for continuous variables and chi-square testing for categorical variables.

For the development of the multivariate and simplified multivariate models, the data were split into training and test sets in a 7:3 ratio. Continuous variables were scaled and normalized. Multivariate logistic regression was performed on the training set, with preoperative variables used as predictive factors. The backward method of multivariate logistic regression was used in view of the large number of potential predictors identified. To evaluate discriminative power, the area under the curve (AUC) was evaluated on the test set, with the confidence limits determined by bootstrapping. Sensitivity and specificity were calculated using Youden's method to determine the optimal cutoff point.

UC Davis 30-day morbidity and mortality predicted probabilities were calculated from the UC Davis Nomogram. The AUC was used to determine its discriminatory power and the Hosmer–Lemeshow test (H-L test) for calibration. 95% CIs for the AUC were determined *via* bootstrapping.

Statistical analysis was performed using the Statistical Package for Social Sciences version 24 (SPSS Inc., Chicago, IL, USA), R version 3.6.1, and Python 3.7. Statistical significance was defined at the 0.05 level.

## 3 RESULTS

### 3.1 Baseline Characteristics

A total of 254 palliative GI surgeries were performed among PC patients over a 10-year duration. The median age of our patients was 61.5 (range 52–71). The most common site of primary malignancy was the colon (42.6%). All patients had radiographic or grossly seen peritoneal disease, which was subsequently confirmed on histopathologic specimens; 24.4% had lung metastases, and 31.8% had liver metastases in addition to peritoneal metastases. The demographic and clinical characteristics of the patients are presented in **Table 1**.

### 3.2 Predictors of 30-Day Morbidity and Mortality

The 30-day morbidity after palliative GI surgery was 43% (n = 110). The most common complications included hematologic complications 31.1% (i.e., requiring multiple blood product transfusions), intra-abdominal complications 29.5% (i.e., intra-abdominal sepsis and collections), respiratory complications 22.0%, wound 18.1%, and cardiac 14.1% complications. Minor (Calvien–Dindo grades 1 and 2) and major (Grade 3 onwards) complications occurred in 43% and 57% of patients, respectively. Of the patients, 20.1% and 5.9% had unplanned readmissions and unplanned reoperations, respectively.

Low preoperative albumin and hematocrit, dyspnea, preoperative use of steroids, and preoperative sepsis were predictors of 30-day morbidity on univariate analysis (**Table 2**). On multivariate analysis, preoperative albumin, age, and emergency nature of surgery were found to be independent significant predictors with an AUC of 0.62 (95% CI 0.50–0.76, **Figure 1**).

The 30-day mortality was 21% (n = 53). As all patients had advanced cancer with a prognosis of less than 1 year, it was found that 81% (n = 206) demised within 1 year of palliative surgical intervention. Median survival was 109 days (range 43–265).

The presence of ascites, high Eastern Cooperative Oncology Group (ECOG) status, low albumin, and extent of surgery were associated with higher 30-day mortality on univariate analysis (**Table 2**). Patients with ovarian primaries had a significantly lowered risk of death at 30 days (p = 0.004). On multivariate logistic regression analysis with backward variable selection, only ECOG status and preoperative albumin levels were found to be independent significant predictors (**Table 3**). The final multivariable model had an AUC of 0.77 (95% CI 0.64–0.90, **Figure 1**) and sensitivity and specificity of 0.76 and 0.75, respectively.

### 3.3 Utility of the UC Davis Nomogram in Our Patient Cohort

In the prediction of 30-day morbidity, the UC Davis Nomogram had an AUC of 0.62 (95% CI 0.55–0.69), and the H-L test had a p-value of 0.99. This indicated poor discriminative power but acceptable calibration (**Figure 2**).

In the prediction of 30-day mortality, the UC Davis Nomogram had an AUC of 0.66 (95% CI 0.57–0.75) for 30-day mortality, indicating poor discriminative power. The H-L test had a p-value <0.05, indicating poor calibration.

### 3.4 Simplified Model for 30-Day Morbidity and Mortality Outcomes for Palliative Gastrointestinal Surgery Patients

To develop a simplified model, we entered the significant variables of ECOG and preoperative albumin found on

**TABLE 1** | Demographics and clinical characteristics of palliative GI surgery patients.

| Variable | Mean or N (%) |
|---|---|
| Age | 61.5 (52.3–71) |
| Male | 101 (40%) |
| Smoker | 10 (4%) |
| **Site of primary malignancy** | |
| Lung | 7 (3%) |
| Stomach | 32 (13%) |
| Pancreas | 14 (6%) |
| Colon | 108 (43%) |
| Ovary | 33 (13%) |
| Endometrial | 6 (2%) |
| Cervix | 5 (2%) |
| Others | 49 (19%) |
| Presence of lung metastases | 62 (24%) |
| Presence of liver metastases | 81 (32%) |
| **Comorbid disease** | |
| Hypertension (requiring medication) | 89 (35%) |
| Diabetes (requiring medication) | 49 (19%) |
| Chronic obstructive pulmonary disease | 3 (1%) |
| Myocardial infarction | 5 (2%) |
| Congestive heart failure | 4 (2%) |
| Peripheral vascular disease | 1 (0%) |
| Renal failure | 7 (3%) |
| Dialysis | 4 (2%) |
| **Preoperative clinical characteristics** | |
| Emergency surgery | 172 (68%) |
| Prehospital location (home) | 249 (98%) |
| Independent functional status | 246 (97%) |
| DNR status | 5 (2%) |
| Chemotherapy use (within 30 days) | 51 (20%) |
| Radiotherapy use (within 90 days) | 4 (2%) |
| Weight loss > 10% within 6 months | 79 (31%) |
| Steroid use | 17 (7%) |
| Ascites | 161 (63%) |
| Bleeding disorder | 4 (2%) |
| Dyspnea at rest | 8 (3%) |
| Impaired sensorium | 5 (2%) |
| Pneumonia | 5 (2%) |
| Sepsis | 30 (12%) |
| ECOG status | |
| 0 | 2 (1%) |
| 1 | 141 (56%) |
| 2 | 100 (39%) |
| 3 | 11 (4%) |
| Hematocrit (%) (median, IQR) | 33.9 (30.9–37.5) |
| WBC ($\times 10^9$/L) (median, IQR) | 8.3 (6.2–11.4) |
| Albumin (g/L) (median, IQR) | 31 (27–35) |
| Creatinine (μmol/L) (median, IQR) | 58 (47–81) |
| **Procedure type** | |
| Gastrointestinal resection | 219 (86%) |
| Multi-visceral resection | 22 (9%) |
| Lysis of adhesions | 7 (3%) |
| **Anastomoses** | |
| Any anastomosis | 164 (65%) |
| Gastro-jejunal | 17 (7%) |
| Small bowel–small bowel | 63 (24%) |
| Small bowel–large bowel | 86 (34%) |
| Large bowel–large bowel | 13 (5%) |
| **Stoma** | |
| Any stoma | 91 (35%) |
| Gastrostomy | 4 (2%) |
| Jejunostomy | 4 (2%) |
| Ileostomy | 35 (14%) |
| Colostomy | 46 (18%) |

*GI, gastrointestinal; DNR, do not resuscitate; ECOG, Eastern Cooperative Oncology Group; IQR, interquartile range; WBC, white blood cell.*

multivariable regression into a new logistic model. For 30-day morbidity, the simplified model had an AUC of 0.64 (95% CI 0.51–0.77) and sensitivity and specificity of 0.77 and 0.57, respectively. For 30-day mortality, the simplified model had an AUC of 0.71 (95% CI 0.55–0.86) and sensitivity and specificity of 0.59 and 0.80, respectively. The receiver operating characteristic (ROC) plot summaries comparing UC Davis, multivariate, and simplified models for morbidity and mortality are plotted in **Figure 1**. The model summaries are included in **Table 4**.

To translate the 30-day mortality simplified model into a clinical tool for prediction, we constructed heatmaps of expected risk. The heatmap skeleton was a matrix with ECOG on one axis and preoperative albumin on the other. Preoperative albumin was stratified by rounding off the observed quartile of albumin in the cohort to the nearest 5 g/L. The average expected risk was determined for each cell of the heatmap and colored with a gradient of green to red, with green representing the lowest risk. We constructed a similar heatmap with the empirically observed mortality in our cohort, with the observed mortality aggregated and color-coded for each cell (**Figure 3**).

## 4 DISCUSSION

PC complicated with IO is one of the most common indications for surgical intervention among advanced cancer patients (10). The peritoneum houses intra-abdominal organs and is inevitably involved in disseminated end-stage cancer. Hence, PC represents a "common end point" of most advanced cancers, where patients may develop complex, multilevel IO and thus suffer from progressive inability to tolerate food, intractable abdominal pain, and distension (13, 14). Surgery usually entails bowel resection, bypass, and/or creation of a decompressive ostomy (15). Among our patients, a majority (86%) required GI resection with a frequent need for bowel anastomoses and stoma creation. As such, palliative GI surgery when performed in PC patients represents a unique group where surgery may be extensive and associated with significant postoperative complications (16).

In one of the largest series of palliative surgical procedures, Miner et al. observed at 30-day postoperative morbidity of 29% and mortality of 11% (6). In the context of PC, a systemic review of 17 retrospective studies including 868 patients found that serious complications occurred in up to 44% of patients, while mortality ranged from 6% to 32% (5). Similarly, we found that 30-day morbidity and mortality were 43% and 21%, respectively, among PC patient who had undergone palliative GI surgery. However, while opponents of palliative surgery tend to focus on the high complications rates, many fail to acknowledge the high symptom resolution (up to 80% to 100%) and low "symptom recurrence" rates after surgical intervention. In fact, our study revealed that none of our patients required repeated operation intervention for IO. Defining the parameters of surgical success is thus of

**TABLE 2 |** Predictors of 30-day morbidity and mortality after palliative GI surgery.

| Variable | 30-day morbidity (n = 110) | p-Value | 30-day mortality (n = 53) | p-Value |
|---|---|---|---|---|
| Age | 64 (56–71) | 0.09 | 63 (57–69) | 0.27 |
| Male | 42 | 0.70 | 16 | 0.11 |
| Smoker | 6 | 0.34 | 1 | 0.69 |
| **Site of primary malignancy** | | | | |
| Lung | 3 | 1 | 1 | 1 |
| Stomach | 15 | 0.70 | 7 | 0.81 |
| Pancreas | 8 | 0.41 | 5 | 0.17 |
| Colon | 45 | 0.70 | 24 | 0.64 |
| Ovary | 16 | 0.57 | 1 | **0.004** |
| Endometrial | 4 | 0.41 | 3 | 0.1 |
| Cervix | 1 | 0.39 | 0 | 0.58 |
| Others | 1 | 0.43 | 12 | NA |
| Presence of lung metastases | 29 | 0.56 | 14 | 0.72 |
| Presence of liver metastases | 35 | 1 | 20 | 0.32 |
| **Comorbid disease** | | | | |
| Hypertension | 40 | 0.79 | 53 | 0.12 |
| | 22 | 0.74 | 18 | 1 |
| Diabetes | 0 | 0.26 | 12 | 0.43 |
| Chronic obstructive pulmonary disease | 2 | 1 | 0 | 1 |
| Myocardial infarction | 3 | 0.32 | 1 | 1 |
| Congestive heart failure | 0 | 1 | 0 | 0.58 |
| Peripheral vascular disease | 5 | 0.24 | 0 | 1 |
| Renal failure | 2 | 1 | 1 | 1 |
| Dialysis | | | 0 | 0.58 |
| **Preoperative clinical characteristics** | | | | |
| Emergency Surgery | 69 | 0.18 | 38 | 0.51 |
| Prehospital Location (home) | 109 | 0.39 | 51 | 0.27 |
| Independent functional status | 108 | 0.47 | 50 | 0.36 |
| DNR status | 1 | 0.39 | 1 | 1 |
| Chemotherapy use (within 30 days) | 24 | 0.64 | 9 | 0.69 |
| Radiotherapy use (within 90 days) | 1 | 0.64 | 1 | 1 |
| Weight loss > 10% within 6 months | 33 | 0.79 | 17 | 0.86 |
| Steroid use | 13 | **0.005** | 3 | 1 |
| Ascites | 74 | 0.29 | 40 | **0.05** |
| Bleeding disorder | 3 | 0.32 | 0 | 0.58 |
| Dyspnea at rest | 8 | **0.001** | 2 | 0.67 |
| Impaired sensorium | 3 | 0.65 | 0 | 0.58 |
| Pneumonia | 2 | 1 | 2 | 0.27 |
| Sepsis | 23 | **<0.001** | 4 | 0.34 |
| ECOG status | | 0.13 | | **0.03** |
| 0 | 0 | | 0 | |
| 1 | 54 | | 22 | |
| 2 | 51 | | 26 | |
| 3 | 5 | | 5 | |
| Hematocrit (%) (median, IQR) | 32.7 (29.7–36.1) | **0.003** | 33.8 (30.0–37.1) | 0.23 |
| WBC ($\times 10^9$/L) (median, IQR) | 8.8 (6.5–12.1) | 0.14 | 8.8 (6.3–12.1) | 0.2 |
| Albumin (g/L) (median, IQR) | 29.5 (26–34) | **0.003** | 28 (24–32) | **<0.001** |
| Creatinine (μmol/L) (median, IQR) | 56 (43–76) | 0.58 | 55 (45–73) | 0.94 |
| **Procedure type** | | | | |
| Gastrointestinal resection | 96 | 0.85 | 49 | **0.02** |
| Multi-visceral resection | 10 | | 0 | NA |
| Lysis of adhesions | 2 | | 2 | |
| **Anastomoses** | | | | |
| Any anastomosis | 66 | 0.19 | 29 | 0.1 |
| Gastro-jejunal | 6 | 0.61 | 5 | 0.36 |
| Small bowel–small bowel | 30 | 0.46 | 10 | 0.28 |
| Small bowel–large bowel | 33 | 0.29 | 17 | 0.87 |
| Large bowel–large bowel | 6 | 1 | 2 | 1 |
| **Stoma** | | | | |
| Any stoma | 44 | 0.24 | 23 | 0.2 |
| Gastrostomy | 3 | 0.32 | 2 | 0.19 |
| Jejunostomy | 4 | 0.03 | 2 | 0.19 |
| Ileostomy | 19 | 0.20 | 5 | 0.37 |
| Colostomy | 19 | 0.87 | 11 | 0.55 |

*GI, gastrointestinal; DNR, do not resuscitate; ECOG, Eastern Cooperative Oncology Group; IQR, interquartile range; WBC, white blood cell; NA, Not applicable.*
*Values in bold indicate p < 0.05.*

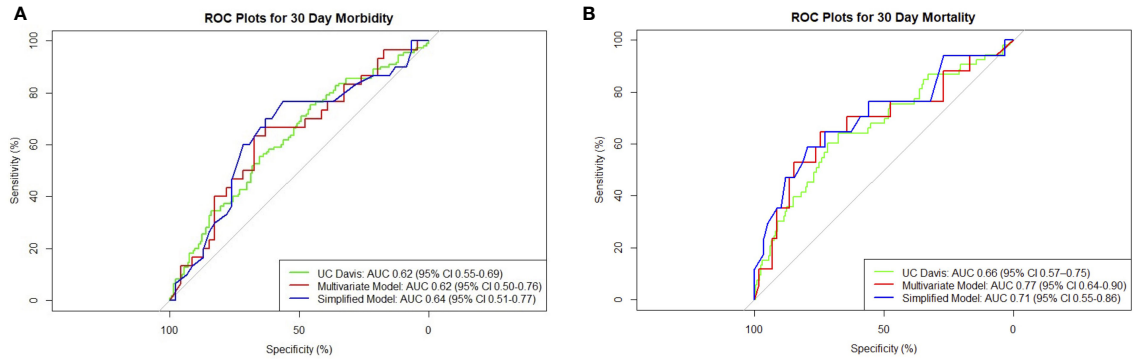


**FIGURE 1** | Composite receiver operating characteristic (ROC) plots for 30-day **(A)** morbidity and **(B)** mortality models.

**TABLE 3** | Model summary of multivariable model for 30-day mortality.

| Variable | Adjusted odds ratio (95% CI) | p-Value |
|---|---|---|
| **Site of primary malignancy** | | |
| Ovary | 0.20 (0.01–1.07) | 0.13 |
| Endometrial | 0 (0–999) | 0.99 |
| **Preoperative clinical characteristics** | | |
| DNR status | 0 (0–999) | 0.99 |
| Impaired sensorium | 0 (0–999) | 0.99 |
| Sepsis | 0.20 (0.02–1.03) | 0.10 |
| ECOG status | 1.59 (1.07–2.38) | **0.023** |
| WBC | 1.50 (0.98–2.33) | 0.06 |
| Albumin | 0.60 (0.39–0.89) | **0.016** |

*DNR, do not resuscitate; ECOG, Eastern Cooperative Oncology Group; WBC, white blood cell.*
*Values in bold indicate p < 0.05.*

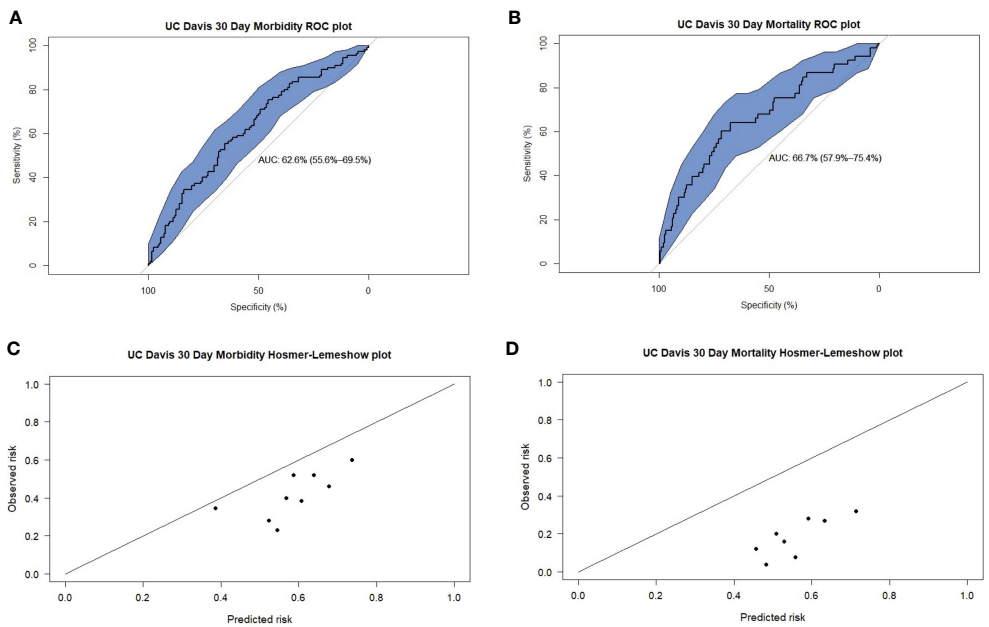


**FIGURE 2** | Receiver operating characteristic (ROC) plots for UC Davis predictions of 30-day **(A)** morbidity and **(B)** mortality. Hosmer-Lemeshow (H-L) plots for UC Davis predictions of 30-day **(C)** morbidity and **(D)** mortality.

**TABLE 4** | Model summary of simplified models.

| Variable | Adjusted odds ratio (95% CI) | p-Value |
|---|---|---|
| **30-Day morbidity** | | |
| ECOG status | 1.26 (0.94–1.71) | 0.12 |
| Albumin | 0.71 (0.52–0.97) | **0.03** |
| **30-Day mortality** | | |
| ECOG status | 1.56 (1.08–2.26) | **0.018** |
| Albumin | 0.60 (0.40–0.89) | **0.012** |

*ECOG, Eastern Cooperative Oncology Group.*
*Values in bold indicate p < 0.05.*

paramount importance—a patient's morbidity or mortality shortly after palliative surgery should not constitute a failure if the wishes of the patients were fulfilled and they had enjoyed a "good" end-of-life experience from their perspective with adequate symptom resolution.

At the time of consideration of palliative surgery among advanced PC patients, the prognosis is often dismal, and predicted survival is less than 1 year. Therefore, it was not surprising that our reported median survival was 109 days and 80% had demised within 1 year after surgery. The importance of identifying factors predictive of short-term mortality is essential to select patients who will benefit the most from palliative surgical interventions. While the existing UC Davis nomogram is comprehensive and useful in predicting 30-day surgical morbidity and mortality, its direct application among PC patients undergoing extensive GI surgery is questionable. The UC Davis cohort is composed of patients who had gone through a variety of surgical procedures, such as vascular, skin and soft tissue, hepatobiliary, and GI interventions. This is distinct from a PC cohort, as the extent of gut manipulation and anastomoses often results in higher rates of perioperative morbidity and mortality. Therefore, when applied to our PC patients, AUC was found to be less than 0.7, representing poor discriminatory power for both 30-day mortality and morbidity outcomes.

Hence, there was a need to devise a risk model that was more applicable to PC patients undergoing palliative GI surgery. Our model comprising ECOG status and serum albumin was found to achieve superior predictive power over the UC Davis model. As such, we advocate the use of this simplified model and translated heatmap as quick clinical tools to aid operative risk discussion.

The impact of preoperative albumin levels on outcomes suggests a role for optimization through preoperative parenteral nutrition in selected PC patients planned for palliative GI surgery. While enteral nutrition has been found to be superior to total parenteral nutrition (TPN) in improving outcomes prior to surgery, this is often not possible in the PC cohort due to multilevel IO (17). In Crohn's disease, characterized by gut failure, not dissimilar to PC patients, TPN given 60 days before major abdominal GI surgery resulted in reduced rates of postoperative complications (18). As such, it is possible that a trial of preoperative TPN can improve albumin levels and lead to improved outcomes among palliative PC patients who do not present with surgical emergencies.

A limitation of this analysis is its inability to account for patients who might have been eligible for palliative surgery but were not operated on because of other factors such as patient decisions or surgeon assessment. We note that our cohort had very few ECOG 0 or 3 patients. Patients may not have been operated on because they were either deemed good candidates for further conservative management (such as ECOG 0 patients, who may have had resolution of obstruction with further nonoperative management) or too poor candidates for surgical management (such as ECOG 3 patients). This can result in paradoxical results, which may be seen when the predicted and observed heatmaps are compared for 30-day mortality. In ECOG 3 patients, those with high albumin >35 g/L experienced lower than predicted risks, while no patients with moderate albumin levels of 25–30 g/L were operated on. Bias arising from surgeon selection of perceived good candidates for surgery may have caused this apparent paradox and may cause overly optimistic risk estimates (such as in the case of the UC Davis model). Further refinement of risk estimates may be possible if further data are collected on such marginal patients.

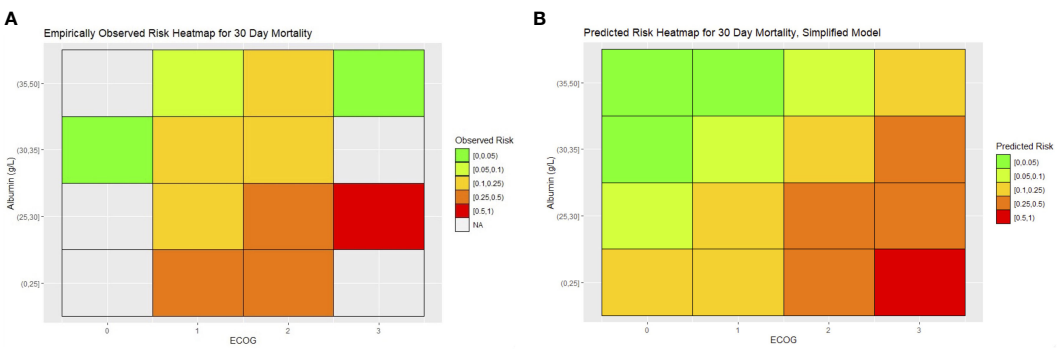


**FIGURE 3** | **(A)** Empirically observed and **(B)** predicted risk heatmaps for 30-day mortality.

In addition, the authors believe that traditional factors used to evaluate surgical efficacy such as the abovementioned postoperative complications and survival fall short of measuring outcomes most meaningful to advanced cancer patients during end of life. Survival reported in quantitative terms without reporting its quality does not attest to overall patient experience and incentivizes surgeons and non-surgeons alike to adopt measures that prolong rather than improve life. Instead, future studies should evaluate measures of quality of life, functional independence, and freedom from symptoms after surgery, which are both clinically important and meaningful to advanced cancer patients undergoing palliative surgery. Therefore, the true "value" of palliative surgery should be considered based on the preferences, expectations, and goals of care of each patient nearing end of life (19).

In conclusion, we found that a good ECOG status and high preoperative albumin levels were independently associated with good short-term outcomes after palliative GI surgery. The UC Davis nomogram showed poor performance in our cohort for the prediction of both mortality and morbidity in our patient cohort. We propose that our simplified 30-day mortality risk model and heatmap may be used as a quick stratification tool for surgeons discussing potential operative risks with patients and that further research will be needed to develop a similar tool for 30-day morbidity.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by SingHealth Centralised Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

Conceptualization: MCCT. Methodology: MCCT, JSMW, and SML. Validation: JSMW, SML, DYZL, CSC, GHCT, and C-AJO. Formal analysis: JSMW, SML, DYZL, CSC, GHCT, and C-AJO. Investigation: JSMW, SML, DYZL, CSC, GHCT, and C-AJO. Resources: CSC, GHCT, C-AJO, and MCCT. Data curation: JSMW and SML. Writing—original draft: JSMW, SML, and DYZL. Writing—review and editing: JSMW, SML, DYZL, CSC, GHCT, C-AJO, and MCCT. Visualization: JSMW, SML, and DYZL. Supervision: CSC, GHCT, C-AJO, and MCCT. Project administration: CSC, GHCT, C-AJO, and MCCT. Funding acquisition: JSMW, CSC, C-AJO, and MCCT. All authors contributed to the article and approved the submitted version.

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METHODS
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# Study Protocol of a Prospective Multicenter Study on Patient Participation for the Clinical Trial: Surgery as Needed Versus Surgery on Principle in Post-Neoadjuvant Complete Tumor Response of Esophageal Cancer (ESORES)

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Ideally, patient-centered trial information material encourages the discussion with the treating physician, and helps patients making trade-offs regarding treatment decisions In a situation of possible equivalent treatment options in terms of overall survival (OS), it can make it easier to weigh up advantages and disadvantages. Preferences for choice of treatment in esophageal cancer (EC) are complex, and no standardized assessment tools are available. We will explore patient's factors for treatment choice and develop a comprehensive patient information leaflet for the inclusion into randomized controlled trials (RCT) on EC. We conduct a cross-sectional, observational study based on a mixed-methods design with patients suffering from non-metastatic EC with post-neoadjuvant complete response after neoadjuvant chemotherapy (nCT) or neoadjuvant chemoradiation (nCRT), to develop patient-centered trial information material. This pilot study is performed in a concept development phase and a subsequent pilot phase. We start with patient interviews ($n$ = 10–15) in the concept development phase to evaluate patients' needs, and develop a Preference and Decision Aid Questionnaire (PDAQ). We pre-test the PDAQ with another $n$ = 10 patients with EC after nCT or nCRT, former patients from a self-help organization, and $n$ = 10 medical experts for their comments on the questionnaire. In the pilot phase, a multicenter trial using the PDAQ and additional measures is carried out ($n$ = 120). Based on evidence of a possible equivalence in terms of OS of the treatment options "surgery as needed" and "surgery on principle" in patients

with post-neoadjuvant complete response of EC, this pilot study on patient participation is conducted to assess patient's needs and preferences, and optimize patients' inclusion in a planned RCT. The aim is to develop patient-centered trial information material for the RCT to increase patients' consent and compliance with the randomized treatment. The trial is registered at the German Clinical Trials Register (DRKS00022050, October 15, 2020).

**Keywords: patient participation, esophageal cancer (EC), patient-centered, study information, psycho-social needs, informed consent**

## INTRODUCTION

Patient-centered health care considers patients and professionals as partners and has its focus on the individual patient's treatment preferences and needs. Thus, patients should be treated as partners with solidarity, empathy, and collaboration, but also with responsibilities (1). An essential point of a modern high-quality health care system is the treatment decision-making process, in which the patient is actively involved by getting relevant information in terms of treatment options. Treatment decision for patients with cancer is a complex task and requires a patient-oriented information process. In the process of providing information, it has to be considered that the patient is in a highly distressed situation in consequence of the diagnosis. Balancing of risks and benefits aimed to reach an understanding of the patient in a difficult situation of treatment options is an important challenge in the process of informed consent in clinical trials. This is regarded as essential also for the information process in randomized trials, and it is anticipated that it will improve the recruitment process and consent to randomization (2). Ideally, patient-centered trial information material encourages the discussion with the treating physician, and helps patients making trade-offs that reflect their own values and preferences (3). In consequence, patient-centered trial information material should include evidence-based information on disease and treatment options, postoperative mortality and morbidity, intermediate and long-term outcomes, side-effects, and burdens to daily life of respective treatment options (4). Educational material should be included, addressing risks and benefits of treatment options. The ethically optimal procedure is one that empowers patients to make preference-sensitive decisions consistent with their goals, values, and preferences (5). Even though shared decision making is not the envisaged process in randomized trials, value clarification is important. The decision to take part in a randomized clinical trial is driven by subjective and intuitive behavior [e.g., feeling of discomfort and vulnerability (2, 5, 6)] and has also psychological, social, and emotional factors (7). The tools of shared decision making processes are useful to gain a more comprehensive understanding of patients' attitudes towards treatment and clinical trials in general, and to take this information into account in the study information material.

The standard of care for patients with non-metastatic esophageal cancer (EC) after neoadjuvant chemoradiotherapy (nCRT) or neoadjuvant chemotherapy (nCT) is principal surgery, 4–8 weeks after nCRT/nCT (8–10). Evaluations of health-related quality of life (HRQoL) for patients who had EC treated with nCRT showed detrimental effects in physical functioning, odynophagia, fatigue, weight loss, and global quality of life in those 4–6 weeks prior to surgery (11). Rapidly physical functioning, odynophagia, and sensory symptoms were restored to pretreatment levels respectively 4–10 weeks after nCRT (11). After surgery role and social function, fatigue, diarrhoea, appetite loss, nausea and vomiting became substantially worse compared to a reference population. Overall HRQL in long-term survivors after esophagectomy did not improve between 6 months and 3 years after surgery, and was worse than that in a comparable reference population (12).

Another as equivalent hypothesized treatment option following nCRT or nCT in terms of overall patient survival is close surveillance with surgery only as needed in persisting or recurring loco-regional tumor (13). A survival disadvantage of delayed surgery in case of local tumor relapse appears unlikely in a protocol of close surveillance of clinical complete response (cCR) (10, 14). Moreover, HRQoL can be restored to levels before treatment after 4–10 weeks after completion of nCRT (11). In a comparative analysis, 36 patients who underwent nCRT and surveillance were matched to 36 patients who underwent nCRT followed by direct surgery. Estimated median overall survival (OS) was equivalent in the surveillance group than in the standard surgery group (58 months, vs. 51 months, $p = .28$). All patients in the surveillance group with loco-regional recurrence in the absence of distant metastases underwent surgery as needed with excellent outcome (median OS 58 months). Moreover, distant metastasis rate was comparable in both groups (active surveillance: 31% vs. standard surgery: 28%) (10). Additionally, we conducted a systematic scoping review of all available studies on the comparison of "surgery as needed" versus "surgery on principle" (15). The results suggest that both post-neoadjuvant treatments are feasible to evaluate in a prospective and comparative clinical trial for complete clinical responders without compromising on OS. Thus, post-neoadjuvant identification of patients with pathological complete response (pCR) followed by closed-meshed surveillance and surgery as needed in case of local tumor recurrence might be a treatment alternative to surgery on principle for patients with post-neoadjuvant pCR. Before practice in routine clinical pathways this has to be evaluated by prospective randomized controlled trials (RCTs). Quality of life is expected to be clearly improved in this group of patients. Omission of esophagectomy reduces length of therapy, complication rate, and time of hospital stay resulting in a reduced treatment cost and faster return to socioeconomic productive work life of the patients.

Clinical response evaluation in the subsequent RCT comprises esophagogastroscopy to locate mucosal tumor, residual or scarred lesions, endoscopic deep biopsies of tumor area to obtain proof or exclusion of residual tumor, endoscopic ultrasound plus fine needle aspiration (FNA) of suspicious lymph nodes to proof or exclude of residual tumor, pathology workup of biopsies and FNA aspirates and F18-FDG-PET CT (whole body) for radiographic/metabolic targeting of loco-regional/distant disease. Clinical response evaluation is done 4-8 weeks after completion of neoadjuvant treatment. In case of clinical histology-proven positive tumor status and/or loco-regional metabolic positive lymph nodes without distant metastasis after clinical response evaluation ("non-CR"), treatment is surgery (Esophagectomy). Patients without histologic evidence of local residual disease, without loco-regional metabolic positive lymph nodes and without evidence for distant metastasis will be considered to be clinically complete responders ("clinical CR") and will be to directly proceed with consecutive close-meshed surveillance visits with surgery only in the event of a local tumor recurrence.

In the situation of possible equivalent treatment options in terms of OS, patient-centered trial information can make it easier to weigh up the advantages and disadvantages of the alternatives. This understanding is the prerequisite for an informed decision. Treatment options can be described by discrete attributes, and the value of the treatment options depends on the nature and level of these attributes. A prospective study showed that 5-year OS, long-term HRQoL, and the chance that esophagectomy is still necessary influenced patients' preference for either active surveillance or planned surgery after nCRT for esophageal cancer (16). A study among patients who had undergone esophagectomy concluded that patients are willing to trade-off 16% of their 5-year survival chance to achieve an improvement in early outcomes (17). Using regression coefficients (β) as measures for the relative importance of attributes, a patient survey assessing preferences of patients towards surgery for preoperative esophagogastric cancer evaluated that patients preferred a better quality of life (QoL) ($\beta = 1.19$), higher cure rate ($\beta = 0.82$), and lower morbidity ($\beta = 0.70$) over treatment in a specialist hospital ($\beta = 0.26$) (4).

The factors influencing patients' treatment preferences for choice of treatment in esophageal cancer are complex, and no standardized assessment tools are available.

## Aims and Purpose of the Study

The purpose of the study is to develop patient-centered trial information material to be used in the planned RCT designed to compare the treatment regimens "surgery as needed" and "surgery on principle" in patients with post-neoadjuvant complete response of EC with respect to OS. The aim of this study is to improve the recruitment of patients in the planned RCT and to improve their consent to randomization and their adherence to the randomized treatment. In a first step we will assess patients' information needs and values in terms of the two treatment options of the envisaged RCT by qualitative interviews. Based on these results a Preference and Decision Aid Questionnaire (PDAQ) will be designed in the first part of the project (i.e., development phase). After pre-testing this questionnaire will be used in a survey in the second part of the project (i.e., pilot testing phase) for the evaluation of patients' preferences and analysis of associations with fear of progression, depression, anxiety, health-related quality of life and disease related social support.

Further, the pilot phase will provide information on the likelihood of patients consenting to participation in the RCT, accepting randomization, and about their compliance with treatment allocation. Hereby we intend to improve the inclusion rate and to optimize the estimations on patient refusal rate, drop-out rate, and cross-over-rate of the envisaged RCT.

# METHODS

In a cross-sectional, observational study we are assessing patients' needs and preferences towards the treatment options of the planned RCT "Surgery as needed versus surgery on principle in patients with post-neoadjuvant complete tumor response of esophageal cancer (ESORES)" in two consecutive phases: (1) A development phase and (2) a pilot testing phase.

We start with detailed qualitative patient interviews ($n = 10–15$) in the development phase. Patients who had already undergone nCT or nCRT for EC and partially also surgery are asked for their needs, preferences, and attitudes towards choice of treatment. Particularly, patients are asked regarding their potential willingness to participate, to accept randomization, and to comply with the treatment to which they will be allocated.

Additionally, $n = 10$ medical experts in the field of EC treatment (i.e., 3–5 clinicians out of the field of EC treatment, 3–5 nurses, and psycho-oncologists) are asked regarding their experiences with patients in terms of patients' attitudes towards treatment choices, preferred treatment option, and the reasons for it.

With respect to patient participation, $n = 2$ members of an adequate Peer-Support Organization (18) are asked to review the interview guidelines, the interview statements, and the provisional PDAQ, and to give comments on them.

Based on the information regarding patients' goals and attitudes, peer-support group members and medical experts' attitudes, the final PDAQ will be constructed. In the subsequent pilot phase, $n = 120$ patients with EC after nCT or nCRT are asked to fill in the PDAQ in a multicenter trial in order to develop patient-centered trial information to serve as study material in the envisaged RCT. The specific study phases are depicted in **Table 1**. Furthermore, details about the study procedures of the pilot phase are provided in **Figure 1**.

## Participants

In the development phase patients' screening for trial eligibility will be performed at the Medical Center University Freiburg. In the pilot phase the PDAQ survey will be performed multicentric in total five specialized centers in Germany. Eligible patients according to the inclusion criteria will be identified through medical records and will be patients after neoadjuvant treatment and before or after surgery. Patients, who participated in the pilot phase, will not participate in the consecutive RCT.

Patient participation in the study is voluntary and patients can withdraw their consent to participate at any time during the

**TABLE 1** | Study procedures.

| Phase | Study Procedures |
|---|---|
| **Development phase (0–7 months)** | Interview guideline<br>Patient eligibility<br>Enrollment, study information, and informed consent<br>Disease specific treatment data of study condition (EC)<br>Interviews<br>Patients in individual interviews (*n* = 10–15)<br>Medical experts (*n* = 10)<br>PDAQ<br>Development<br>Pre-testing (*n* = 10 patients; *n* = 2 patients advocates)<br>Adaption |
| **Pilot phase (8–20 months)** | PDAQ Survey (*n* = 120 patients including)<br>Age<br>Gender<br>Education<br>FoP-Q-SF<br>PHQ-9<br>GAD-7<br>EORTC-QoL-C30<br>OES18<br>Trial information material<br>Disease specific treatment data of study condition (EC) |

*PDAQ, Preference and Decision Aid Questionnaire; FoP-Q-SF, Fear of Progression Questionnaire Short-Form; PHQ-9, Patient Health Questionnaire-9; GAD-7, Generalized Anxiety Disorder Screener-7; EORTC-QoL-C30, European Organization for Research and Treatment of Cancer's Core Quality of Life Assessment; OES18, Oesophageal short module of the EORTC questionnaire.*

study without incurring disadvantages in treatment. Patients will be given sufficient time to read and understand the study information, to review the information, ask questions, and receive satisfactory answers from the trial physician. Subsequently, patients will be asked to sign the informed consent after completed information process. Patients eligible to participate in the study (development phase and pilot phase) have to fulfill the following inclusion criteria:

## Inclusion Criteria

Diagnosis of non-metastatic EC, including both epidemiologically relevant histologies of EC esophageal adenocarcinoma (EAC) and adenosquamos carcinoma and squamous cell carcinoma (ESCC) according to the Universal Integrated Circuit Card (UICC) definition.

Scheduled or running treatment by western standard of care multimodal treatment schemes (nCT plus surgery and nCRT plus surgery).

- Age > 18 years.
- Patients before or after surgery.
- Ability to read and understand German.
- Willingness and ability to give informed consent before study entry.
- Patient's written informed consent has been obtained.

## Exclusion Criteria

Patients who meet the following exclusion criteria cannot participate in the trial:

- No written consent available.
- Patients with gastric cancer.
- Patients with tumors of the cervical esophagus.
- Co-morbidity with contraindication for esophageal surgery.
- Patient without legal capacity who is unable to understand the nature, significance, and consequences of the study.
- Concurrent medical or psychiatric condition that might preclude participation in the study according to investigator assessment.

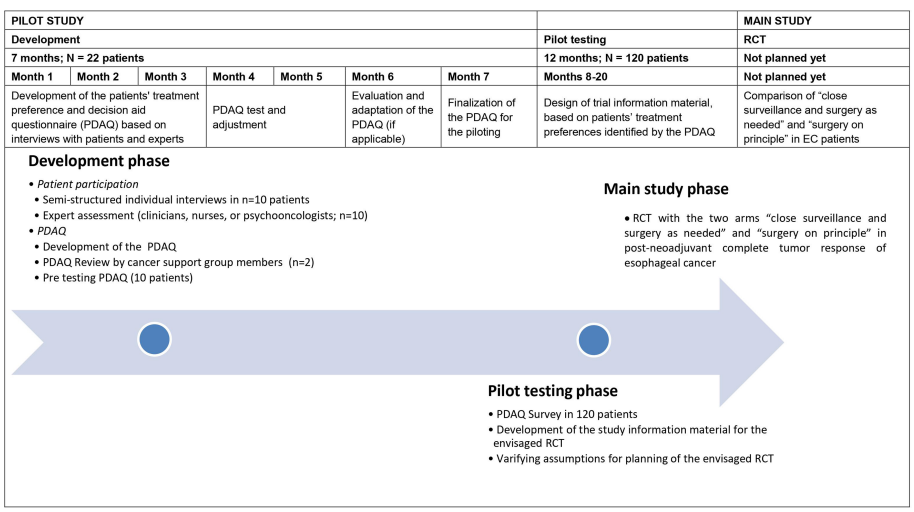


**FIGURE 1** | Flow Chart.

- Cognitive or other type of impairment (such as severe psychiatric disorders and severe cognitive disorders that would interfere with completing paper-pencil questionnaires).
- Simultaneous participation in other studies which could interfere with this study and/or participation before the end of a required restriction period.

## Study Procedures

### Development Phase

Patients' will be asked to provide basic demographic data regarding age, gender, ethnicity, highest level of education, employment status, and marital status. Medical data such as current health status (Eastern Cooperative Oncology Group [ECOG] performance status), time since diagnosis, tumor type (adenocarcinoma, squamous cell carcinoma), clinical stage (cT/cN category), pathological stage (pT/pN category and tumor regression staging), and previous and actual medical treatment is taken from the medical documentation.

Based on patient preferences identified from literature review and expert opinion, relevant issues will be phrased for structuring the interviews.

For patients to be able to evaluate treatment options, they need to have adequate knowledge of treatment opportunities and realistic expectations of potential benefits and harms. Therefore, the interviews assessing patients' treatment preferences, could start by repeating the information about both the "surveillance and surgery as needed" and the "surgery on principle" procedures including a detailed description of respective advantages and disadvantages. It should be stressed, that it is unknown which of the two procedures is superior and that the advantage of the "surveillance and surgery as needed" method is that post-treatment recovery might be quicker and less impairment of long-term HRQoL might be apparent. The advantage of the "surgery on principle" procedure might be improvement of local tumor control and, therefore, improved disease-free survival and the possibility for pathohistological examination of the surgical specimen (19).

Even though patients have no choice of treatment in an RCT, it is important to evaluate their expectations regarding treatment choice along with their constraints regarding RCT. Therefore, the interview guide includes patients' concerns of clinical trials that might be:

- Feeling "left out" and cannot decide by myself which treatment I would like to choose (in a RCT)
- Feeling emotionally challenged in the expectation maybe not to be randomized in to my preferred treatment arm
- Feeling satisfied to be part of medical research that can help improving my or other patients' treatment situation in the future
- I prefer taking part in a clinical trial, to be one of the first people to benefit from a new treatment, knowing there's also a chance that the new treatment turns out to be no better, or worse, than the standard treatment

The interview will end with the question: "If you are randomly assigned to group A, would you accept the assignment or request a group change?"

The individual patient interviews will explore additionally patients' need for information, their expectations regarding treatment, their subjective experiences, and their individual actions regarding decision making.

Surgeons/medical experts might also have treatment preferences that hinder the study information process and might contribute to recruitment bias. Therefore, medical experts/recruiters of the planned centers are asked regarding their attitudes towards both treatment options to ensure that they objectively inform patients about risks and benefits. Further the specialists are asked to focus on two basic questions: (a) are issues included in the preliminary study information material which they consider irrelevant for this patient group and if so, why do they consider these issues irrelevant? and (b) are there issues missing from this information material that the specialists consider relevant and if so, why do they consider these issues relevant?

A project team member with experience in qualitative research methods will conduct the interviews with patients and medical experts (i.e., 3–5 clinicians out of the field of EC treatment, 3–5 nurses, and psycho-oncologists) using the interview guides. Duration of the interviews are calculated approximately 30 minutes. Members from an adequate cancer support group will be asked to provide a review of those gathered views and opinions.

In the development phase, the qualitative analysis of the interviews will lead to a list of issues representing the insights of patients' preferences towards treatment choice. Both, the qualitative analysis of the patients' interviews (including former patients from the cancer support group) and the medical experts' opinion will lead to an adaptation of issues, if applicable. A preliminary PDAQ questionnaire will be developed from the list of issues and 10 patients are asked to give their comments on the phrases using a think-aloud technique. These findings will lead to the final PDAQ with phrased items that can be evaluated for relevance and importance.

### Pilot Phase

After pretesting the preliminary version of the PDAQ will be revised. The final PDAQ will assess patients' needs, preferences, and its influencing factors towards the choices of treatment. The results of the questionnaire survey in the pilot testing phase will provide information for adapting the informed consent material to the patients' needs and preferences for the main study. Patients' information needs and values identified by the PDAQ will be transferred in the proven format of decision-support to be easy to understand, well-structured, clear, and helpful, which will serve as study information material in the envisaged RCT. The medical expert opinion as well as the review of former patients from the cancer support group are included in developing the study information material.

In the pilot phase, the survey includes the PDAQ questionnaire, captures sociodemographic and medical information and the following standardized instruments:

- FoP-Q-SF: The Fear of Progression Questionnaire Short-Form [FoP-Q-SF (20)] is a concise standardized psychological instrument to measure the fear of progression

(FoP) in chronically ill patients (cancer, rheumatic diseases, and diabetes mellitus). The questionnaire consists of 12 items and covers the factors: *affective reactions*, *partnership/family*, *occupation*, and *loss of autonomy*.

- PHQ-9: The Patient Health Questionnaire-9 [PHQ-9 (21)] is a brief and validated measure of depression severity. It consists of 9 items and covers the 9 depression criteria of the Diagnostic and Statistical Manual of Mental Disorder IV (DSM-IV). For each item, the patient has to choose from 0 ("not at all") to 3 ("nearly every day"). Thus, the maximum score is 27.
- GAD-7: The Generalized Anxiety Disorder Screener-7 [GAD-7 (22)] is a standardized 7-item self-report anxiety questionnaire assessing the anxiety symptoms: *nervousness*, *inability to stop worrying*, *excessive worry*, *restlessness*, *difficulty in relaxing*, *easy irritation*, and *fear of something awful happening*. Similar to the PHQ-9, the total score is calculated by adding together the scores for all items ranging from 0 ("not at all") to 3 ("nearly every day").
- EORTC-QoL-C30: The European Organization for Research and Treatment of Cancer's (EORTC) core quality of life assessment [EORTC-QoL-C30 (23, 24)] is a validated instrument to assess the quality of life of oncological patients. It contains 30 questions in 10 subscales. Furthermore, the EORTC-QoL-C30 has a specific module for EC called OES18 (25) to assess the detailed symptoms of EC-patients.

The survey will be conducted in collaboration with five centers. A final sample of n = 120 patients will be included.

## Trial Information Material

Based on the results of the survey, the information material for the envisaged RCT will be revised. In addition, a check-list for clinicians will be developed to guide the information process.

Recommendations for the design of risk information include graphical displays to increase the effectiveness of risk communication (3); therein simple bar charts are preferred and absolute risks are given rather than relative risks, and comparisons with everyday risks are proposed.

We will base the development of information material on useful design formats that follow the quality standards for patient decision aids for presenting risk information and prediction models [i.e., the SUNDAE (Standards for Universal Reporting of Patient Decision Aid Evaluation) checklist (26), and the IPDAS (International Patient Decision Aid Standard) Collaboration] that include percentiles, ratios, and pictographs (7, 27, 28).

Estimates for OS after nCRT or nCT + esophagectomy can be obtained from an interactive web-based instrument (nomogram), where the individual survival of patients is estimated based on their individual pathological, demographic, and treatment data (29).

It is suggested that clinicians have a toolbox of presentation styles to suit different patients and outcomes (30). It may be that multimodal consultations, incorporating verbal and visual information, presented differently, such as event rates, risk ladders, or bar charts, may maximize patient understanding of different treatment outcomes. Option grid formats can be used to display attributes of treatment options and to answer patients' most relevant questions (16, 17, 31). Patient-relevant questions when making trade-offs with regard to their treatment decision might be for example:

How long will I stay in hospital? (Risk of in hospital mortality)

Which treatment is the best for long-term survival? (5-year survival rate)

What are the chances of cancer coming back in the esophagus? (Risk of relapse)

How long will it take to recover? (Risk of persistent gastrointestinal problems)

How many patients experience physical side effects (e.g., speech pathologies, dysphagia, respiratory restrictions, pain, anxiety, etc.)? (Risk of post-treatment complications)

How many consultations will I have? (Burden of appointments)

The trial information material developed and designed during development and pilot testing phase including a clinician's check-list to guide the information process will be used to provide comprehensive education and information about the randomized controlled trial for patients with non-metastatic EC after nCRT.

## Data Management and Monitoring

During the study, all personal data will be kept separately in the patient identification log (identification of patient and contact details). All patient data will be captured in pseudonymized form. After transcription all audio files will be stored until the end of the project at least for three years and then be deleted. The data management will be performed with REDCap™ Version 9 (redcap@vanderbilt.edu).

Details on data management (procedures, responsibilities, deviations, etc.) will be described in a data management manual which will be continuously updated and maintained during the trial. The technical specifications of the database will be described in a data description plan (DDP). Before any data entry is performed, the trial database and electronic case report forms (eCRFs) will be validated. Site data entry personnel will not be given access to the trial data base until they have been trained and signed an access form.

Statistical Analysis System (SAS) software will be used to review the data for completeness, consistency and plausibility. The checks to be programmed will be specified beforehand in a data validation plan. After running the check programs, the resulting queries will be sent to the investigator for correction or verification of the documented data. Data corrections will be entered directly into REDCap by the responsible investigator, or designated person. Query forms which contain the corrections must be confirmed by the dated signature of the investigator (not the study nurse) in the designated places. Due to the characteristics of the study, no data monitoring committee (DMC) will be included.

## Biostatistical Planning and Analysis

### Development Phase

In this phase the focus lies on qualitative analyses of the patient interviews supported by specific software (MAXQDA). Statistical analyses are confined to descriptive analyses. All qualitative

analyses will be performed at the Endowed Professorship Self-help Research, Interdisciplinary Tumor Center Medical University Freiburg, with the support of the Clinical trial unit if applicable.

### Pilot Phase

The pilot phase mainly has two objectives:

1) The development of patient-centered information material optimally fulfilling the individual needs of the patients with the aim to improve the recruitment of patients for the clinical trial and to improve their consent to randomization between the standard treatment "surgery on principle" and the experimental treatment "surveillance and surgery as needed" and their adherence to the randomized treatment.

2) To verify assumptions for sample size calculation for the planned RCT regarding the rate of EAC vs. ESCC, the rate of nCRT vs. nCT, and the pCR rate after surgery.

With regard to objective 1) the effects of socio-demographic, disease-specific, and psycho-social factors on patients' treatment preference ("surgery on principle" versus "surveillance and surgery as needed") will be analyzed. Additionally to descriptive analyses, univariate and multivariate logistic regression models will be used to identify which factors may be associated with patients' treatment preference. Effect sizes will be quantified by odds ratios with 95%-confidence intervals and tested using the Wald test. P-values will be interpreted in a descriptive sense. Those factors identified as influential with a relevant effect size will then especially be considered in the development of the patient-centered trial information material and check-list for clinicians in the planned RCT.

Sample size was chosen based on feasibility without formal sample size planning based on statistical power calculations. The inclusion of 120 patients from 5 clinical centers within a time period of 6 to 12 months was regarded as feasible. The following statistical consideration only exemplifies the possible conclusions with the chosen sample size: 120 patients would provide 80% power for an identification of a factor influencing patients' preference with an odds ratio of 3 at a significance level of.05, considering adjustment for other correlated factors (variance inflation factor 1.2). With regard to objective 2) descriptive analyses of tumor type, type of neoadjuvant therapy, and of pCR status after surgery will be performed.

## DISCUSSION

Due to evidence of a possible equivalence with regard to OS of the two treatment options "surveillance and surgery as needed" and "surgery on principle" in patients with post-neoadjuvant complete response of EC, this pilot study is aimed to involve patients early in the development of the main trial. Hereby integration of patient's needs and preferences, and optimization of patients' information and inclusion in the planned RCT should be achieved. We use a mixed-methods approach in the concept development and the pilot testing phase of the study.

In our experience patients are interested to be involved in clinical trials and other psychosocial studies. Against the background of an increasing demand for patient participation in clinical trials this study realizes an innovative patient-centered approach to involve patients and patients' mandatories in various stages of the clinical trial. Patients may benefit in participating in the study by helping to create comprehensive study information material and to optimize patient care. Patient's mandatories may help to improve the study material from a patient's perspective. The risks for patients and medical experts in this interview and questionnaire survey are estimated to be very low.

Nevertheless, participation in a patients' evaluation (i.e., interviews and questionnaire survey) to assess treatment preferences regarding both a standard principle surgery therapy and an experimental treatment with active surveillance and surgery as needed might bear the risk that patients get new information towards treatment options they did not have before in their own treatment. Patients might get emotionally affected when they recognize they would have preferred another treatment as they received.

Considering the clinical relevance of identifying factors influencing patients' decision towards one treatment option, it is important to mention specific problems with adherence to the allocated trial treatment in completed RCTs comparing surveillance with surgery on principle in EC patients (17, 32, 33). In the published trials, a striking difference in the compliance to the allocated treatment was to be noticed between the different arms of the trials, with higher rates of non-compliance to the protocol in the surgical-arms. For the ongoing SANO-trial, this factor was included to the study design by using a cluster-randomisation (13). For the planned RCT, we are going to address this issue not alone by specific trial design but also by conducting this pilot study to create patient-centered trial information material. The eligibility criteria of the main trial won`t be affected by the results of the patient's participation study. The study aims to optimize information material for the main trail we expect that this shall improve study recruitment and protocol adherence by creating comprehensive and patient-centred study information material.

## Ethics and Dissemination

The research will be conducted in accordance with the principles of Good Clinical Practice. The study is registered at the German Clinical Trials Register (DRKS00022050, October 15, 2020) and has been approved by the Medical Ethical Committee of Freiburg University Medical Center (No. 20-1037). Any amendments to the protocol will be communicated and re-approved by the ethics committee. The findings of this study will be disseminated widely through peer-reviewed publications and international conference presentations.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethical Committee of Freiburg, University Medical Center. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

Primary sponsor: JHo. Conceptualization: JW, AK, and JHo. Project planning: JW, AK, CS, JHo, JHi, and MC. Writing: AK, MC, and JW. Statistical counseling: CS. Funding acquisition: JW, JHo, and JHi. Editing: JW, AK, CS, JHo, MC, MR, PG, CB, and JHi. All authors provided review of the manuscript. All authors read and approved the final manuscript.

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# Pyroptosis-Related Signatures for Predicting Prognosis in Breast Cancer

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**Background:** Female breast cancer (BC) has become the most common cancer in the world, and its mortality was considerably higher in transitioning vs. transitioned countries. Pyroptosis, an inflammation-dependent programmed cell death mediated by inflammasomes, has been observed in human colorectal tumors and gliomas. However, the characteristics of pyrolysis-related genes and their influence and mechanism on the tumorigenesis and progress of BC were unknown.

**Methods:** Based on the global public database, we used comprehensive bioinformatics analysis to systematically analyze the expression of pyroptosis-related genes in BC and their relationship in tumor progression. In addition, BC patients were divided into two groups, and the clinical features and outcomes could be better predicted by the consistent clustering of pyroptosis-related genes. Least absolute shrinkage and selection operator (LASSO) Cox regression analysis was used to establish a risk score. Then, we further explored the prognostic value and clinical features of pyroptosis genes. Finally, we used the Human Protein Atlas (HPA) platform to identify the expression at protein levels of the key genes.

**Results:** We confirmed that the expression of pyroptosis-related genes was different in BC and normal breast tissues. A high frequency of somatic mutations occurred in BC. In addition, 33 pyroptosis-related proteins interacted frequently. Based on univariate analysis and the LASSO Cox model, five pyroptosis-related genes [including GADMA, interleukin-6 (IL-6), NLR pyrin domain-containing protein 6 (NLRP6), caspase-1 (CASP1), and caspase-9 (CASP9)], were obtained to calculate a risk score. The risk score was identified as an independent risk factor for the prognosis of BC and might play an auxiliary role in clinical classification. The HPA platform confirmed that the expression trends of the key genes were consistent with our previous studies.

**Conclusion:** Pyroptosis had an important effect on the progression of BC. And the pyroptosis-related genes could be used as new prognostic biomarkers and therapeutic targets for BC.

**Keywords: breast cancer, women in oncology, surgical oncology, pyroptosis, LASSO Cox analysis**

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## INTRODUCTION

Breast cancer (BC) has become the first malignant tumor with morbidity and mortality among women in the world (1), which seriously endangers women's life and health. The incidence of BC has increased significantly, and its mortality rate has also shown an upward trend in fluctuation view of the existing research results. The incidence of BC has increased slightly by 0.3% per year since 2012 (2). Therefore, it is urgent and critical to developing powerful prognostic predictors and new therapeutic targets to enhance the prognosis assessment and treatment level of BC.

Pyroptosis, also known as cell inflammatory necrosis, was a new form of programmed cell death closely related to inflammation (3). When the cell pyroptosis occurred, the cell swelled. Before the cell ruptured, a protrusion was formed on the cell, and then a pore was formed in the cell membrane, which made the cell membrane lose its integrity, released the cell content, and triggered an inflammatory response (4). As the morphology changed, the nucleus shrank and DNA broke (5). The pyroptosis process involved many inflammation-related molecules such as gasdermin (GSDM) protein family, NOD-like receptors (NLRs), interleukin (IL) series molecules, caspase molecules, etc. The caspase family belonged to cysteine proteases, which were key enzymes that cause cell apoptosis (6). Once activated by signal pathways, they could degrade intracellular proteins and lead to cell death. Interleukins and related cytokines were the communication means of innate and adaptive immune cells and non-immune cells and tissues (7). Interleukins affected the occurrence, development, and control of cancer. NOD-like receptors were a subgroup of cytoplasmic host pattern recognition receptors (8). NOD-like receptors was involved in the formation of the inflammasome polyprotein complex, which induced the release of interleukin-1β (IL-1β) and interleukin-18 (IL-18) leading to a pro-inflammatory response (9). Gasdermins were a family of intracellular proteins that executed pyroptosis (10, 11). Caspase-1 (CASP1) and caspase-11/4/5 induced pyroptosis through cleavage Gasdermin D (GSDMD). Gasdermin D released its N-terminal domain after being cleaved by CASP1 or caspase-11/4/5 (12). It had the activity of binding membrane lipids and punching holes in the cell membrane, leading to changes in cell osmotic pressure and swelling until the final cell membrane ruptured (13). However, there were few studies on its specific functions in BC. We researched the role of pyroptosis-related genes in BC.

In this study, we firstly conducted a systematic study on the TCGA database and the GSE45628 microarray database to evaluate the difference in pyroptosis-related genes expression between non-specific invasive BC tissues and the paracarcinoma tissues. Then we discussed whether these genes had an impact on the prognosis and constructed a prognostic evaluation model through the least absolute shrinkage and selection operator (LASSO) analysis to help judge the progress and prognosis of clinical patients after standard treatment, including the NLR pyrin domain-containing protein 6 (NLRP6), interleukin-6 (IL-6), CASP1, caspase-9 (CASP9), and GSDMD. According to the relative coefficients, these key genes played a vital part in suppressing cancer in the effect of pyroptosis on BC. Finally, we further studied and validate the expression of the key genes in the Human Protein Atlas (HPA) platform.

## MATERIALS AND METHODS

### Public Databases

We selected the data set according to the following criteria: Inclusion criteria: datasets involving human BC and expression profiling by the array. Exclusion criteria: datasets with a sample size smaller than 10 and datasets without follow-up or metastasis information. We obtained public RNA sequencing (RNA-seq) data and corresponding clinical features including 1,213 BC patients and 130 normal people. The training set came from The Cancer Genome Atlas (TCGA; https://tcga-data. nci.nih.gov/tcga/, $n$ = 1,222, 1,109 BC samples and 113 normal breast samples), the validation set was a separate Gene Expression Omnibus (GEO) microarray dataset (GSE42568, $n$ = 121, 17 normal breast samples, 104 BC samples) (14). The above data were all publicly accessible resources, so ethical approval was not required. The characteristics of BC patients and normal people were shown in **Supplementary Table 1**. The human protein–protein interactions (PPI) were compiled from the Human Integrated Protein–Protein Interaction rEference (HIPPIE) database (http://cbdm.mdc-berlin.de/tools/hippie/hippie_current.txt) (15).

### Consensus Clustering

From the TCGA database, 33 previously reported expressions related to pyroptosis were extracted. The BC Patients without follow-up information were deleted. After that, with the help of the "Consensus Cluster Plus (16)" package, the above steps and 1,000 times repetitions for guaranteeing the stability of clustering. The optimal number of clusters were determined according to the consensus clustering algorithm. At last, two clusters were determined according to the expression profile of pyroptosis-related genes in BC patients.

### Pathway Enrichment Analysis

Using Spearman's analysis and the String online tool (https://string-db.org/), the correlation and interaction of 33 pyroptosis-related genes were evaluated. We used Metascape (http://metascape.org/) to complete the assessment of the biological behavior of different clusters. Gene Set Variation Analysis (GSVA) was used to derive the landmark pathways described in the molecular feature database and conducted pathway analysis to determine the potential mechanisms involved in the molecular cluster of pyroptosis. After that, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed with the help of Gene Set Enrichment Analysis (GSEA), using cluster Profiler, an R/Bioconductor package.

### Composing Risk Score and Verification

In order to construct powerful prognostic characteristics, all molecules related to pyroptosis obtained by non-factor analysis were included in the LASSO Cox regression model. We

incorporated the genes obtained after the initial screening of univariate analysis into the Lasso penalty Cox regression, and the genes related to the prognosis of BC were further analyzed to identify potential prognostic genes. Then, they derived the most suitable lambda value by a five-fold validation through the R package "glmnet" to minimize the average cross-validation error (30). After screening, it was finally determined to use five key genes and their coefficients to construct a prognostic model. The formula format of the risk score was as follows:

$$\text{Risk score} = \sum_{i=1}^{n} Coefi^{*} xi$$

where *Cofi* was the coefficient, *xi* was the pyroptosis-related genes, and the mRNA expression value of the genes. This formula would calculate the risk score of all patients in our study.

## Immunohistochemical Data of Partial Pyroptosis-Related Genes Expression

We used the data provided by the HPA (https://www.proteinatlas.org/) to examine immunohistochemical (IHC) staining of GADMA, IL-6, NLRP6, CASP1, and CASP9 in normal breast tissue and non-specific invasive BC cases. Based on a comprehensive assessment of the staining intensity (negative, weak, medium, or strong) and the proportion of stained cells (<25, 25–75, or >75%), manually scored protein expression in the database.

## Statistical Analysis

Unless otherwise stated, all statistical analyses were conducted by R software (version 3.5.1). We got help from the Student's *t*-test (unpaired, two-tailed) to compare the difference between two independent groups. The correlation between pyroptosis-related genes, risk scores, and clinical characteristics was calculated by the Chi-square test. The Kaplan-Meier analysis of Disease-Free Survival (DFS) and Overall Survival (OS) was performed using a log-rank test according to the best cut-off value. We subsequently performed univariate and multivariate Cox regression analysis to determine the relationship between different variables and clinical outcomes. The *P*-values were corrected for multiple comparisons via the Benjamini and Hochberg (BH). The *P*-value $< 0.05$ was considered statistically significant.

# RESULTS

## Differential Expression of Pyroptosis-Related Genes in Breast Cancer and Paracancer Tissues

For the purpose of exploring the important biological functions of pyroptosis-related genes in BC occurrence and tumor progression, based on GEO and TCGA, 33 pyroptosis-related genes were compared between BC tissues and paracarcinoma tissues in the mRNA and protein levels. First of all, we found that the expression of 33 genes was disordered in BC tissues and paracarinoma tissues in the TCGA dataset (**Figure 1A**), which were verified in GSE42568 (**Figure 1B**). Further analysis of the specific differences indicated that the expression level of five genes (CASP3, CASP6, GSDMD, NOD2, PYCARD) was prominently increased in BC tissues. Another eight genes were observably lower expressed in BC tissues (**Figure 1C**). In addition, we tried to further explain the reasons for the alterations in the expression of pyroptosis-related genes by the somatic mutations of the differential genes. The analysis results showed that 128 out of 986 samples had mutations, which the mutation rate was 12.98%. The rate was relatively high, and somatic mutations of differential genes were common in BC patients (**Figure 1D**). Subsequently, we utilized Pearson correlation analysis to verify the relationship between the expression profiles of 33 pyroptosis-related factors in the TCGA database (**Figure 1E**). As shown in the figure, most of the pyroptosis-related genes were positively correlated, and the expression of AIM2, CASP5, CASP1, CASP4, IL-18, NLRP1, NLRP3, and SCAF11 showed strong correlations in BC. To sum up, the above results showed that there was a large genomic and expression variation of pyroptosis-related genes between BC tissues and paracarinoma tissues. Furthermore, the effect of pyrolysis-related genes on prognosis in BC was shown in **Supplementary Figures 1**, **2**.

## Interaction and Unsupervised Consensus Analysis of 33 Pyroptosis-Related Genes

To achieve the aim of identifying the relation between pyroptosis-related genes and clinicopathological characteristics in BC, we systematically studied the functions, interactions, and correlations of pyroptosis-related genes. It could be seen from the figure that there was a strong relationship in the PPI network (**Supplementary Figure 3A**). Univariate analysis showed that IL-18 is a potential protective factor, and TIRAP was a potential risk factor for OS (**Supplementary Figure 3B**). In addition, CASP9 was a potential protective factor for DFS in pyroptosis (**Supplementary Figure 3C**).

For the purpose of exploring the relationship between 33 pyroptosis-related differently genes (DEGs) and BC subtypes, we performed an unsupervised and consistent cluster analysis based on the TCGA cohort, by changing the clustering variable ($k$) from 2 to 6 (**Supplementary Figures 4A–D**). The result showed that the intra-group correlation was highest at $k = 2$, while the inter-group correlation was low, and could separate the samples in the TCGA data set relatively stable, which indicated that BC patients could be well-divided to two clusters (**Supplementary Figure 4E**). We also showed the classification of each sample (column) under the different number of clusters ($k$). When $k = 2$, most samples were divided into light blue and dark blue two parts (**Supplementary Figure 4F**). Therefore, we divided the training set of BC patients into cluster 1 (C1) and cluster 2 (C2). Next, the differential gene transcription profile and clinicopathological characteristics analysis were both displayed in the heatmap, which indicated that there was a significant separation between C1 and C2 of pyroptosis-related molecules. At the same time, we found that the clinicopathological characteristics were significantly correlated with differential genes related to pyroptosis. Among them, the

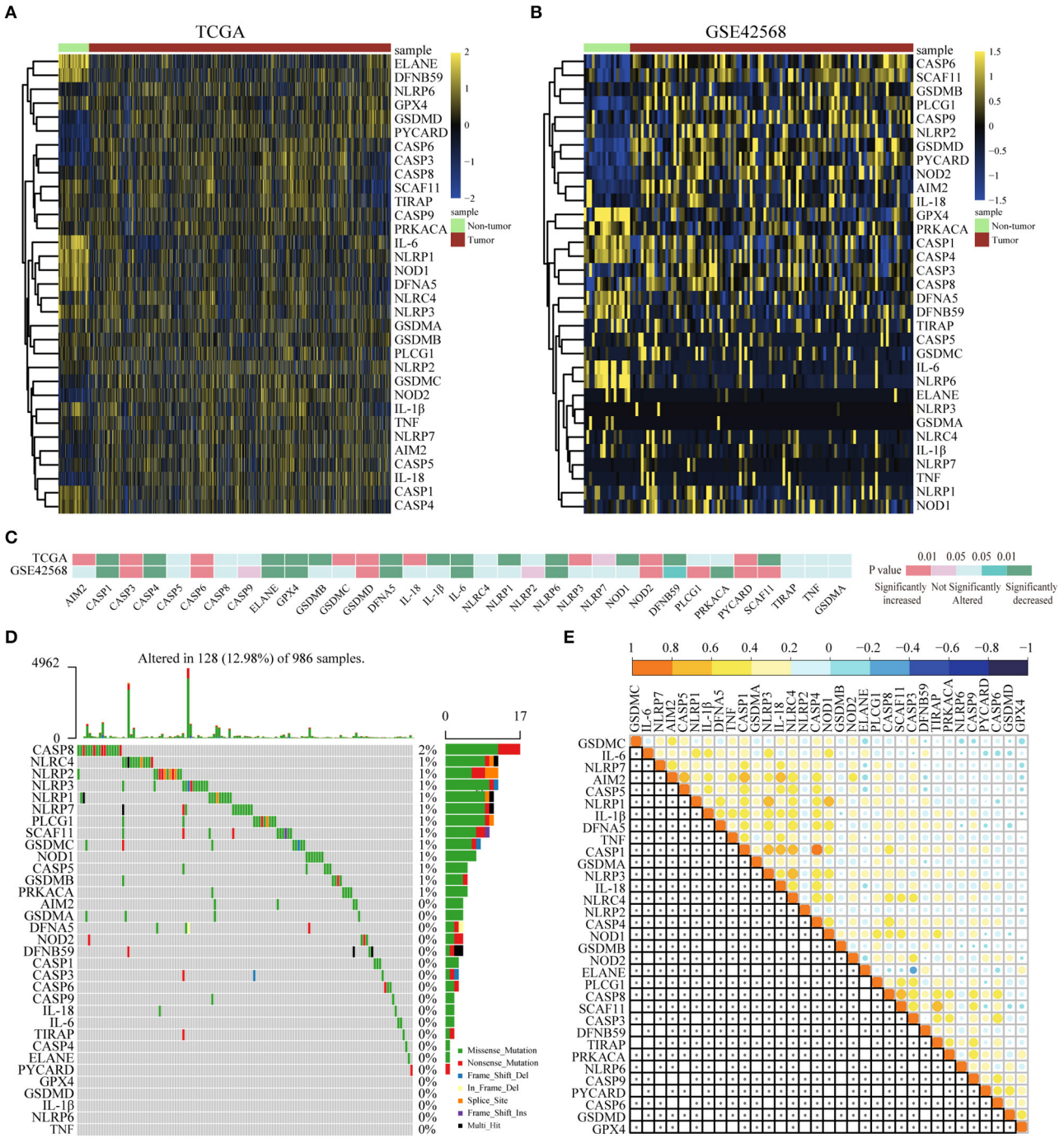


**FIGURE 1 |** Differential expression of pyroptosis-related genes in BC and paracancer tissues. **(A)** The expression of pyroptosis-related genes in BC and paracancer tissues in the TCGA database. **(B)** The expression of pyroptosis-related genes in BC and paracancer tissues in the GSE45628 database. **(C)** The mRNA expression status of pyroptosis-related genes in BC. **(D)** The somatic mutation frequency in pyroptosis-related genes of the TCGA database. **(E)** Pearson's correlation analysis of the expression of 33 pyroptosis-related genes in BC. BC, breast cancer.

expression level of Human Epidermal Growth Factor Receptor 2 (Her-2) might be different in the two groups, which needed to be further verified (**Figure 2A**). In the prognostic analysis, the OS and DFS of the two groups were markedly different. Cluster 2 presented a potentially better prognosis, but the difference was not significant in the field of statistics (**Figures 2B,C**). Furthermore, t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction analysis showed that the cluster

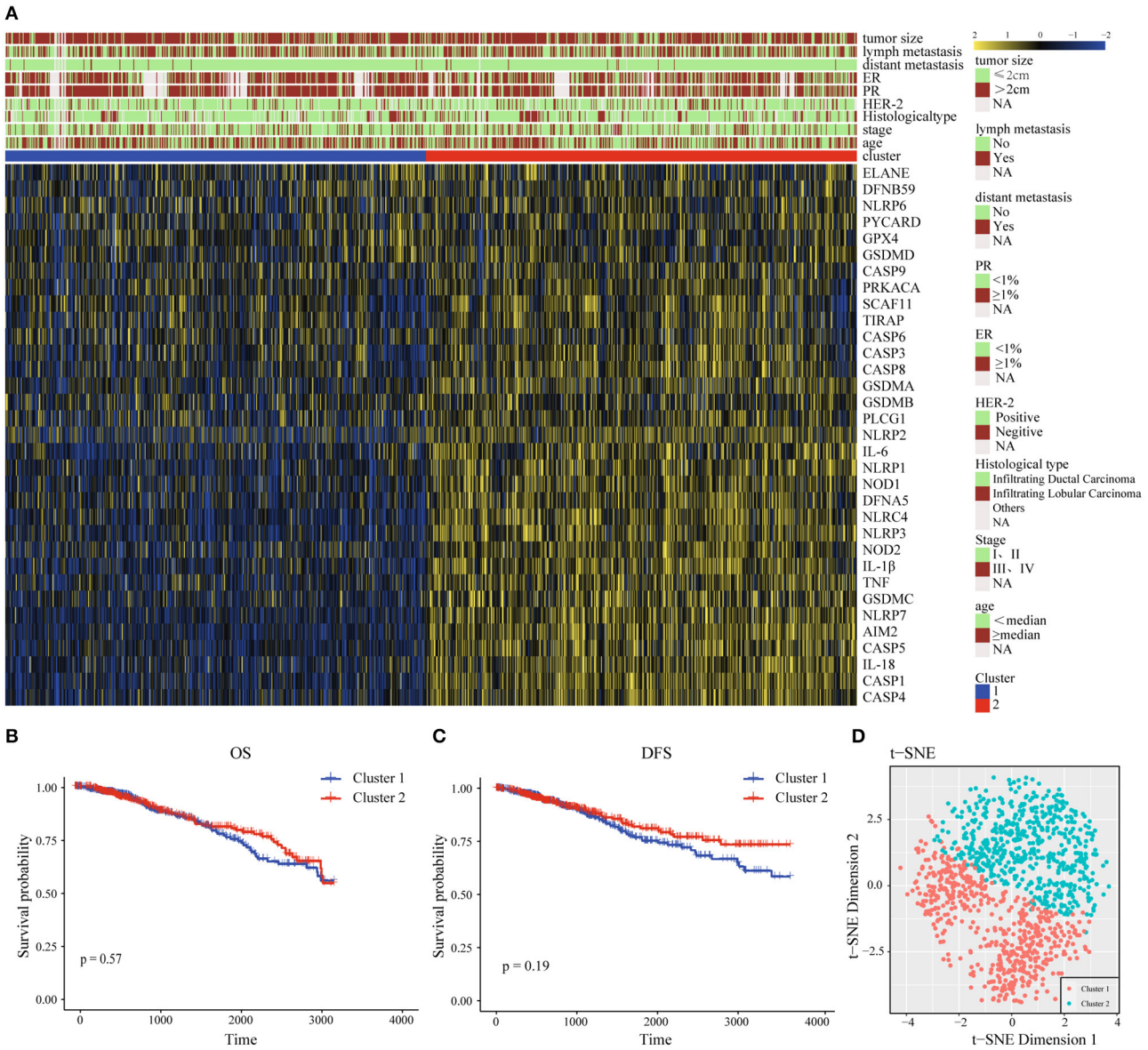


**FIGURE 2 |** Interaction and unsupervised consensus analysis of these pyroptosis-related-genes. **(A)** The relationship between the clinical features and the expression levels of pyroptosis-related genes in the two clusters of BC. **(B)** Overall Survival analysis of two clusters of BC patients. **(C)** Disease-Free Survival analysis of two clusters of BC patients. **(D)** t-SNE plots of TCGA-BC RNA-sequence profiles for the two clusters. t-SNE, t-distributed stochastic neighbor embedding; DFS, Disease-Free Survival; OS, Overall Survival; HER-2, Human Epidermal Growth Factor Receptor 2; PR, progesterone receptor; ER, estrogen receptor.

was divided into two discrete clusters, C1 and C2 had different transcriptome characteristics (**Figure 2D**). These results indicated that pyroptosis might be correlated with the malignant progression and clinicopathological characteristics of BC.

## Functional Annotation of Different Subgroups

To achieve exploring the different clinicopathological characteristics and prognosis of the two clusters of BC, we further investigated whether the genes screened by different subgroups had different biological processes. The top 200 most significant differentially expressed genes of pyroptosis-related genes clusters based on the *P*-value were selected to annotate the biological function. Metascape analysis of the upregulated genes revealed that "Cytokine-cytokine receptor interaction," "Natural killer cell mediated cytotoxicity," "positive regulation of interleukin-23 production," "NOD-like receptor signaling pathway," "response to interferon-gamma," "cellular defense response," and other inflammatory responses and immune-related pathways are significantly enriched in cluster 2, which might be closely related to the progression and distant metastasis

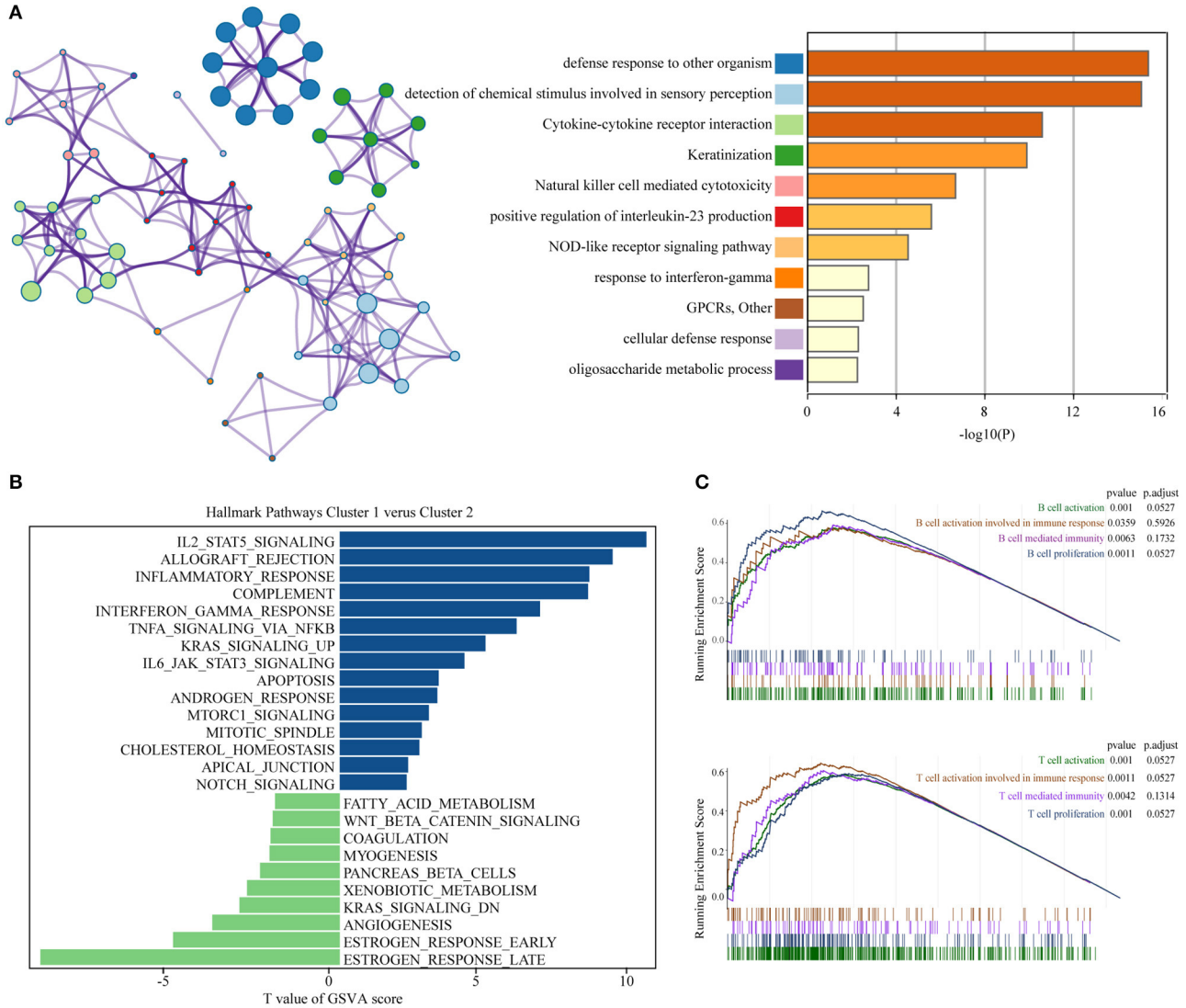


**FIGURE 3 |** Functional annotation of different subgroups. **(A)** Significantly enriched top 20 pathways and interactions. **(B)** The GSVA between pyroptosis-related genes clusters1 and clusters2 using hallmark gene sets. **(C)** B cell and T cell related immune pathways are shown in GSEA. GSEA, Gene Set Enrichment Analysis; GSVA, Gene Set Variation Analysis.

of BC. The first 20 signally enriched bioinformatics pathways were listed in **Figure 3A.** We used GSVA to determine the degree of enrichment of different pathways between C1 and C2, the results showed that the inflammatory pathways such as "inflammatory response," "interferon-γ response," "TNF-α-NF-κB signaling," "IL2-STAT5 signaling," "IL6-JAK-STAT3 signaling" were significantly activated in C2 patients (**Figure 3B**). Then, we used GSEA to evaluate the activation of specific response pathways; and the analysis results pointed out that "B cell activation," "B cell activation involved in immune response," "B cell mediated immunity," "B cell proliferation," and the corresponding immune response of T cells were activated in C2 (**Figure 3C**). We explored the effects of pyroptosis-related genes on the prognosis. In general, the pyroptosis process promoted the activation of multiple inflammatory pathways, which might affect the tumorigenesis and progress of BC. In the cause of further exploring the specific effect of the pyroptosis process on the prognosis of BC, we further developed a prognostic model.

## The Prognostic Value of Pyroptosis-Related Genes and the Construction of Risk Score Model

After we have known the relationship between pyrolysis-related genes and BC progression, we further clarified the influence of 33 pyroptosis-related genes on the prognosis of BC with the help of univariate analysis in the TCGA training dataset. A total of nine genes including IL-18, IL-6, CASP1, CASP4, CASP9, NLRP6,

NLRP3, PYCARD, GSDMA were selected according to *P*-value $< 0.2$ for further analysis. The prognostic risk score features were constructed through the LASSO regression model based on the minimum criteria, including NLRP6, IL-6, CASP1, CASP9, and GSDMA (**Figure 4A**). By using the five genes screened and corresponding coefficients to construct a risk score feature, and the risk score formula was as follows: Risk score = (−0.0318 × the expression of NLRP6) + (−0.0499 × the expression of GSDMA) + (−0.0234 × the expression of IL-6) + (−0.0114 × the expression of CASP1) + (−0.3714 × the expression of CASP9), In our risk score, the five selected genes were tumor-suppressor genes.

To learn more about the prognostic value of the risk score, BC patients were divided into high-risk group and low-risk group based on the median of the risk score in the TCGA database. The results indicated that the high-risk score group was associated with a higher mortality rate (**Figure 4F**). Survival analysis based on OS and DFS clarified that high-risk score group had a worse prognosis than those with low-risk scores (**Figures 4B,D**). At the same time, we named the GSE42568 dataset as the validation set to achieve the aim of verifying the prediction power of the risk score, and the results of the validation set and the training set were the same (**Figures 4C,E,G**).

## The Relationship Between the Risk Score and Clinical Characters in Breast Cancer Patients

Next, we further researched on the correlation among the pyroptosis-related genes and risk scores and the clinical features of BC. The heat map represented the expression and clinical characteristics of pyroptosis-related genes in high- and low-risk patients (**Figure 5A**; **Supplementary Tables 2, 3**). We found that age, TNM stage and distant metastasis had a close relationship with the high-risk score. Subsequently, we assessed the difference between the risk score and each clinical feature. It could be seen from the histogram that triple-negative breast cancer (TNBC) had the highest risk score, the highest risk of disease progression and recurrence, and the worst prognosis. The results of this analysis were consistent with previous studies of TNBC. The results of the other three subgroups Her-2, Luminal-A, and Luminal-B were also consistent with the National Comprehensive Cancer Network (NCCN) guidelines. The risk score we designed could help determine the clinicopathological classification of BC. If the risk score was higher, the clinical classification was closer to the TNBC or Her-2 subtype. In addition, we found that a higher risk score meant a worse prognosis (**Supplementary Figure 5**). Conversely, the lower the patient's score, the more likely the classification result was Luminal-A or Luminal-B subtype (**Figure 5B**). We found age and lymphatic metastasis were important factors affecting risk scores (**Figures 5C,D**). Then, univariate and multivariate Cox regression analyses were conducted on the TCGA dataset. We observed that estrogen receptor (ER), progesterone receptor (PR), tumor size, age, lymph metastasis, TNM stage, and distant metastasis were significantly correlated with prognosis by the univariate analysis (**Figures 5E,F**). Then, the risk score remained strongly associated with the DFS by multivariate analysis (**Figures 5G,H**). The above showed that the risk score established by pyroptosis molecules was an independent predictor of the prognosis and progression of BC.

## The Expression Level of Five Key Pyroptosis-Related Genes in Breast Cancer Patients

To further research the five genes included in the risk score, we identified these in the TCGA database, and the results showed that three genes including CASP1, IL-6, and NLRP6 were significantly decreased in the BC patients at the RNA level (**Figure 6A**). While there was no significant difference in CASP9 and GSDMA between BC and paracarcinoma tissues. Then, we used the HPA database to evaluate the expression difference at the protein level. The results indicated that the protein levels of NLRP6, IL-6, CASP1 in BC tissues were significantly decreased vs. normal tissues (**Figure 6B**). The expression level of CASP9 and GSDMA in BC and paracarcinoma tissues was similar. The results at the protein level were consistent with the RNA expression data we found earlier.

# DISCUSSION

Although improvement has happened in therapeutic effects and prognosis these years, progression and metastasis are still the main causes of death in BC patients (17). Existing treatment and testing methods can not cover all BC patients, and carcinogenesis, malignant progression, and recurrence involve complex multistep processes and still to be fully elucidated. Thus, it is crucial to understand the potential in review mechanisms involved, to develop powerful prognostic predictors, and explore novel therapeutic targets. After clarifying the differences in the expression of pyroptosis-related genes in BC and paracarcinoma tissues, in order to quantify the impact of pyrosis on the progression, a risk score was constructed to evaluate the prognosis of patients. At the same time, a part of the possible mechanism was discussed.

In this study, we firstly reported the expression levels of 33 pyroptosis-related genes in BC and paracarcinoma tissues and identified that they were differentially expressed. The analysis results showed that the expression of five molecules such as CASP3, CASP6, GSDMD, NOD2, PYCARD in BC tissues was higher than that in adjacent tissues. Then, we found that somatic mutations of pyroptosis-related factors were more common in BC. Many previous studies have confirmed that pyroptosis-related genes had a close relationship with the tumorigenesis and progression in many cancers. Interleukin-6-mediated activation of STAT3 in fibroblasts played a key role in driving colorectal tumors (18). However, there was no significant difference in clinical characteristics between the two clusters which were figured out through the consensus clustering analysis. In order to further evaluate the prognostic value of these pyroptosis-related genes, a five-genes risk signature was constructed based on univariate analysis and LASSO Cox regression analysis in the TCGA database, and we validated

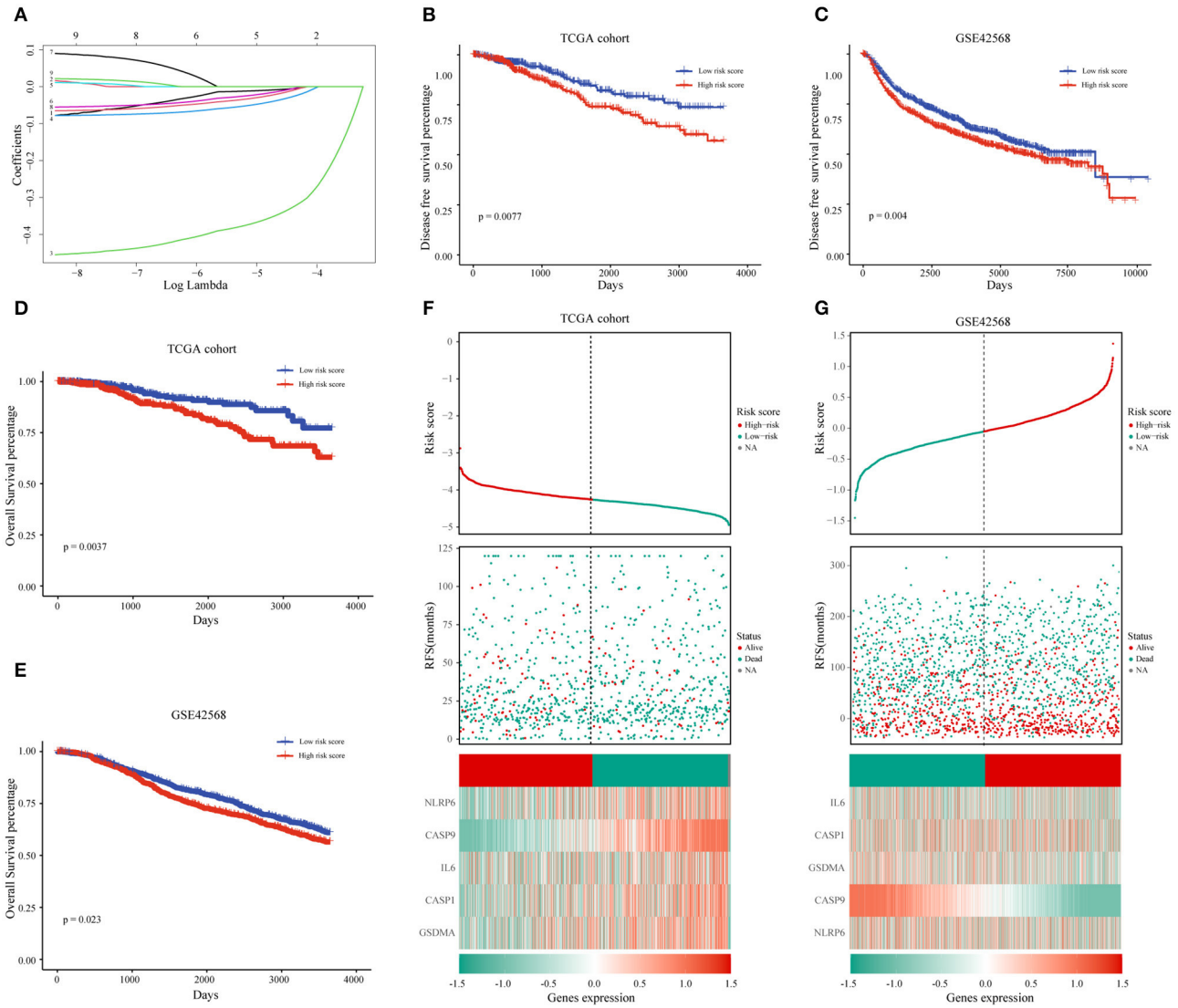


**FIGURE 4 |** The prognostic value of pyroptosis-related genes and the construction of risk score model. **(A)** The coefficients of five pyroptosis-related genes in LASSO Cox regression were calculated. **(B,D)** Kaplan-Meier analysis of OS and DFS in patients with low- and high-risk scores in TCGA dataset. **(F)** BC patients with high-risk scores had a worse prognosis in the TCGA database. **(C,E,G)** The same analysis method is used to verify the risk score characteristics in the GSE42568 data set. TCGA, The Cancer Genome Atlas; DFS, Disease-Free Survival; OS, Overall Survival.

it in a GEO dataset with good performance. And the DEGs between the high- and low-risk group had a close relationship with immune-related and inflammatory response pathways by the functional analysis. Comparing the degree of pathway enrichment between the low-risk group and the high-risk group, we found that the high-risk group reduced specific immunity and non-specific immunity-related pathway activities versus the low-risk group.

The risk score we constructed contains five pyroptosis-related molecules including IL-6, CASP1, CASP9, NLRP6, GSDMA, etc. Interleukin-6 influenced the proliferation, differentiation and anti-apoptosis of common malignant tumor cells. Overexpression of IL-6 and its receptors were usually found in BC, prostate cancer, and oral squamous cell carcinoma. Interleukin-6 could activate the potential autocrine/paracrine Notch-3/Jagged-1 ring to improve the self-renewal of breast stem cells (19). Interleukin-6 and its downstream effector STAT3 constituted a key carcinogenic pathway (20), so targeting IL-6 may bring benefits to BC patients. Previous studies confirmed that the apoptosis caused by CASP1 involves the Bid-caspase-9-caspase-3 axis, which could lead to pyroptosis mediated by GSDME. Caspase-1 is involved in pyroptosis. The effect was clearer than in apoptosis (21). Recently, it was discovered that caspase-1 mediated GSDMD proteolysis, the formation of GSDMD membrane pores, cell lysis, and pyroptosis. Gasdermin D was a substrate of caspase-11 and CASP1 and mediates cell

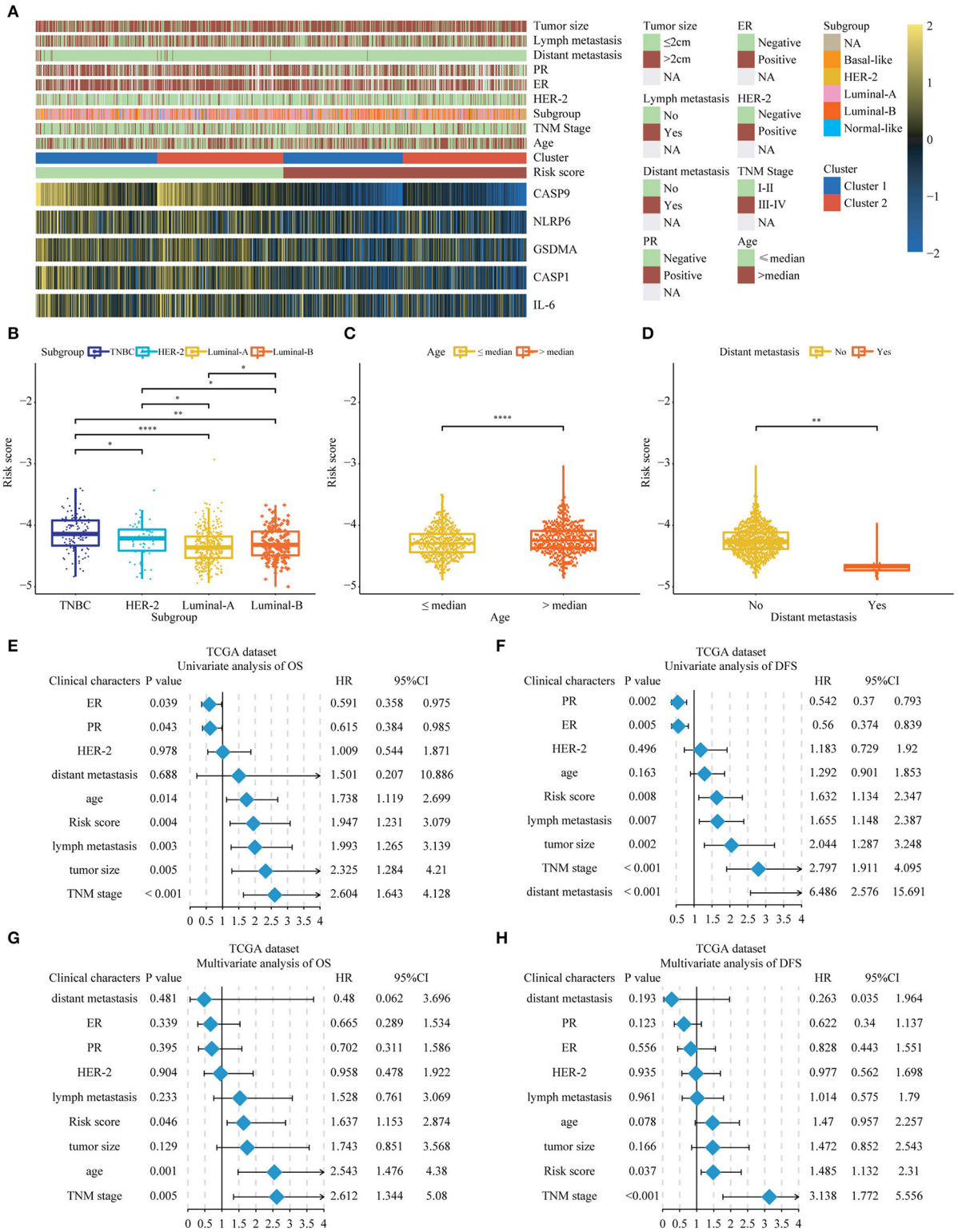


**FIGURE 5 |** The relationship between the risk score and clinical characters in Breast cancer patients. **(A)** The relationship between risk score, cluster 1, cluster 2, and 5 key genes and clinical case characteristics of BC patients. **(B–D)** The relationship between risk score and clinical characteristics, including PAM50 classification, age and distant metastasis. **(E–H)** Through univariate and multivariate analysis, the risk score was an independent risk factor for the prognosis of breast cancer in the TCGA database. *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$. TNM, Tumor Node Metastasis; DFS, Disease-Free Survival; OS, Overall Survival; TNBC, triple-negative breast cancer; HER-2, Human Epidermal Growth Factor Receptor 2; PR, progesterone receptor; ER, estrogen receptor; TCGA, The Cancer Genome Atlas.

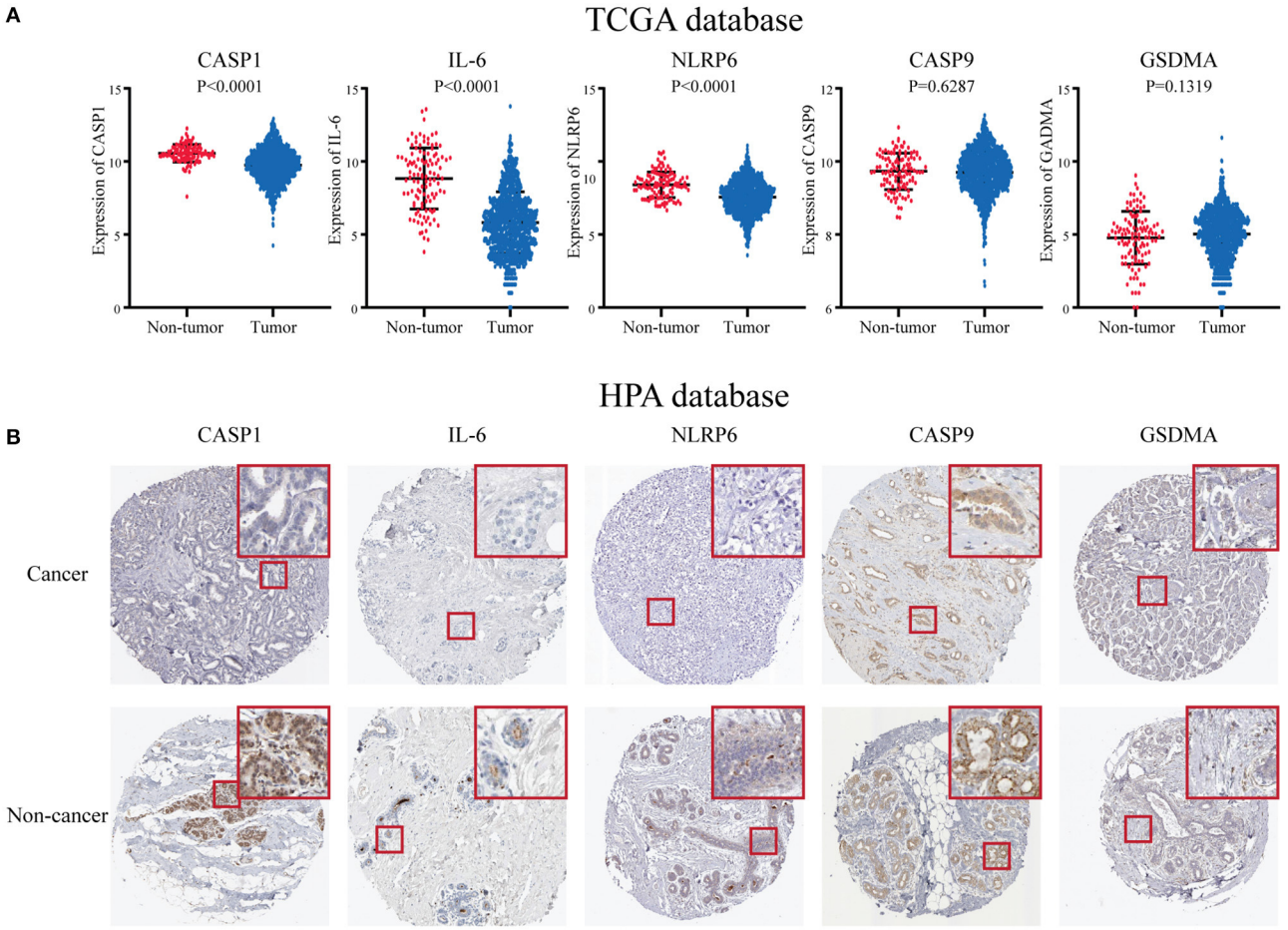


**FIGURE 6 |** The expression level of five key pyroptosis-related genes in breast cancer patients. **(A)** The expression of five key genes is in the TCGA database. **(B)** The expression of five key genes in the HPA platform. HPA, Human Protein Atlas; TCGA, The Cancer Genome Atlas.

death (22). Many pro-apoptotic signals could activate caspase-9, which is an initiating protease. It was an initiator and does not need to be cleaved, but only needed to be activated to activate caspase-3 and downstream caspase to initiate cell death. Caspase-9 had an important effect on the apoptosis of a variety of cancer cell types, and its positive and negative regulators were reported in the previous literature (23). The expression of GSDMA was significantly increased in the skin and gut, however, it was depleted in gastric cancer (24). But GSDMA polymorphism was associated with childhood asthma, inflammatory bowel disease and systemic sclerosis (25). NLR pyrin domain-containing protein 6 (originally named PYPAF5) belonged to the NOD-like receptor family, and together with NLRP4, NLRP7, NLRP3, and NLRC1 constituted the ability to construct a fully operational inflammasome (26). The inflammasome NLRP6 played a vital part in adjusting inflammation and hosted resistance to gut microbiomes. Water shortage stress could cause NLRP6 inhibition and alterations in the composition of the gut microbiome, and mice were more likely to suffer from intestinal inflammation (27). Colitis and colorectal tumors were significantly increased in mice lacking NLRP6 (28). The cytosolic lipoteichoic acid bound to and activated NLRP6, which aggravated systemic Gram-positive pathogen infection by producing IL-18, which was a new innate immune pathway (29). In this experiment, we found that the expression of NLRP6 was different in BC and paracarcinoma tissues, with low expression in BC tissues and relatively high expression in paracarcinoma tissues. The coefficient was negative when the risk score model was subsequently established, which was consistent with the previous experimental results.

This study also had many limitations. Firstly, this was a retrospective analysis which cannot draw a causal relationship between abnormal gene expression and BC, so future prospective research is needed. In addition, additional *in vitro* and *in vivo* functional analyses were required to validate and extend these results. The results obtained by pure bioinformatics analysis can only be used to predict conclusions and are not sufficient for obtaining accurate conclusions. Finally, we only explored the possible mechanism of pyroptosis in BC at the level of bioinformatics, and the specific mechanisms of related molecules in BC were still to be studied.

## CONCLUSION

Our research confirmed that the expression of pyroptosis-related genes was different in BC and normal breast tissues and clarified the close relationship between changes in the expression of pyroptosis-related genes and the malignant progression of BC. The characteristics of pyroptosis-related genes were identified and verified, which could accurately predict the prognosis of BC patients. These results provided evidence for the biomarkers of BC to judge the progress and prognosis and provided a new potential therapeutic target for targeted therapy. In the future, editing of genes related to pyrolysis may become an effective option for BC treatment through gene therapy.

## DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: The datasets generated for this study can be found in TCGA ($n$ = 1,222, 1,109 BC samples and 113 normal breast samples) and separate GEO microarray datasets (GSE42568, 104 BC samples and 17 normal breast samples).

## ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

ZL and XG designed the study and revised the manuscript. TR and JZ analyzed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2022.788437/full#supplementary-material

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# The Prognoses of Young Women With Breast Cancer (≤35 years) With Different Surgical Options: A Propensity Score Matching Retrospective Cohort Study

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**Background:** Compared with older patients, young women with breast cancer (YWBCs) have a poorer prognosis and a higher risk of recurrence. Ages ≤35 years are independent risk factors for local recurrence of breast cancer. Surgery is the most important local treatment for YWBC, and there is still a lack of prospective studies comparing surgical options for recurrence and survival. We retrospectively compared the effects of surgical options on disease-free survival (DFS) and overall survival (OS) of YWBC at Fudan University Shanghai Cancer Center (FUSCC).

**Methods:** YWBCs (age ≤35 years) who underwent surgery at FUSCC between 2008 and 2016 were retrospectively analyzed and divided into three groups according to surgical options: 1) breast-conserving surgery (BCS), 2) mastectomy alone (M), and 3) mastectomy with reconstruction (RECON). The DFS and OS outcome rates from the three surgical options were compared using the Kaplan–Meier method and Cox regression model. Propensity score matching (PSM) was also used to balance the baseline characteristics to eliminate selection bias.

**Results:** A total of 1,520 YWBCs were enrolled with a median follow-up of 5.1 years, including 524 patients (34.5%) who underwent BCS, 676 patients (44.5%) who underwent M, and 320 patients (21.1%) who underwent RECON. The 5-year DFS rates were 96%, 87%, and 93%, respectively ($P < 0.001$); the 5-year OS rates were 98%, 94%, and 97%, respectively ($P = 0.002$). Multivariate Cox analysis showed that DFS and OS were significantly improved in patients undergoing BCS compared with those undergoing M, with hazard ratios (HR) of 0.448 (95% CI 0.276–0.728; $P = 0.001$) and 0.405 (95% CI 0.206–0.797, $P = 0.009$), respectively. After PSM, DFS and OS rates were significantly improved in patients undergoing BCS compared to patients undergoing M (DFS, $P = 0.001$; OS, $P = 0.009$); RECON was also improved compared to patients undergoing M in

terms of DFS and OS, but the difference was not statistically significant (DFS, $P = 0.164$; OS, $P = 0.130$).

**Conclusions:** The surgical options were independent factors affecting DFS and OS in YWBC, and the DFS and OS rates were significantly improved in the BCS group compared to those in the M group. BCS is preferred for early YWBC, and RECON is the best option for remodeling the body images of YWBC who do not have breast-conserving conditions.

**Keywords: young breast cancer, survival, propensity score matching, surgical options, breast-conserving surgery**

## 1 INTRODUCTION

Breast cancer is the most common malignancy among young women, accounting for 22% of cancer fatalities in 2017 (1). The controversiality of the cutoff age for defining young women with breast cancer (YWBCs) is different between China and Western countries. For instance, the European Society for Medical Oncology (ESMO) uses a cutoff of <40 years old, while the consensus and guidelines in China define the cutoff as age 35 or younger. There is a significant age difference in the worldwide incidence of breast cancer: the average age of breast cancer diagnosis is 45–55 years in China (2), which is 10 years younger than that in Western countries. Moreover, breast cancer patients under the age of 40 account for less than 7% of all breast cancer patients in developed countries. YWBCs account for more than 10% of all breast cancer patients in China (3). To certain the reasonable cutoff value for defining YWBC, The Korean Breast Cancer Society analyzed 9,885 breast cancer patients and found that the risk of death from breast cancer rises dramatically among women under the age of 35 (4). There is no consensus on a cutoff age value for defining YWBC by Eastern and Western scholars, although some researchers regard 35 years as a reasonable age value. However, the stratification of age has been widely accepted by doctors for decision-making regarding diagnosis and treatment.

There are three surgical options for breast cancer treatment: 1) breast-conserving surgery (BCS), 2) mastectomy alone (M), and 3) mastectomy with reconstruction (RECON). M is the most important local treatment for breast cancer; randomized controlled studies, such as the NSABP B-06 (5) and Milan (6) trials, demonstrated that survival outcomes after BCS combined with radiotherapy are equivalent to those after M for early breast cancer. Moreover, some studies have shown that BCS compared to M not only improved esthetic outcomes but also may be associated with survival benefits in recent years (7, 8). A large cohort study was published in *Lancet Oncology* in 2016, which found that BCS combined with radiation resulted in improved 10-year overall survival (OS) as compared to mastectomy (9). However, several retrospective studies have found that age is an independent risk factor for local recurrence in patients who underwent BCS (10–12). A Japanese study found that age was an independent factor for predicting ipsilateral breast tumor recurrence (IBTR) ($P = 0.047$); when patients were aged 40 years or younger, the 10-year IBTR rate was 15.7%; this was 3.8% in those aged 41–50 and 2% in those aged over 50 (10). Previous studies reported that age 35 years or younger was an independent risk factor for local recurrence in patients who underwent BCS (11, 13). A recent large cohort study demonstrated that the survival outcomes of BCS were better than those of M, and BCS should not be regarded as equal to M (14). However, the study did not focus on YWBC. There is still a lack of prospective studies to explore whether BCS could improve YWBC's survival outcomes compared to other surgical options (15). Based on the demographic characteristics of Chinese patients with breast cancer, patients who were 35 years old or younger were included in our study. We retrospectively compared the effects of the three surgical options on the disease-free survival (DFS) and OS rates of YWBCs at Fudan University Shanghai Cancer Center (FUSCC). Therefore, our research may provide evidence-based data on surgical options for YWBC and explore the potential factors of these surgical options in terms of differences in cancer survival.

## 2 METHODS

The FUSCC Ethics Committee approved this study (050432). Written informed consent for the study was waived due to the retrospective nature of our study.

### 2.1 Patient Screening

We retrospectively analyzed breast cancer patients who were inpatients at FUSCC for treatment between 2008 and 2016. The detailed inclusion criteria included: 1) primary and untreated breast cancer; 2) ages ≤35 years old; 3) patients who underwent surgery in our hospital and had no distant metastasis; 4) patients with Tis–T3 tumors according to the American Joint Committee on Cancer (AJCC) TNM stage system. The exclusion criteria included: 1) follow-up times shorter than 1 month; 2) patients with bilateral breast cancer or occult breast cancer; 3) patients who underwent neoadjuvant chemotherapy; and 4) lack of clinical data or follow-up data. A flowchart is shown in **Figure 1**.

### 2.2 Clinical Data Collection

YWBCs were identified from the FUSCC Breast Cancer Database. Two writers double-checked all of the information from the patients' medical records (LP, LL). Prognostic data and follow-up information were provided by our breast cancer database.

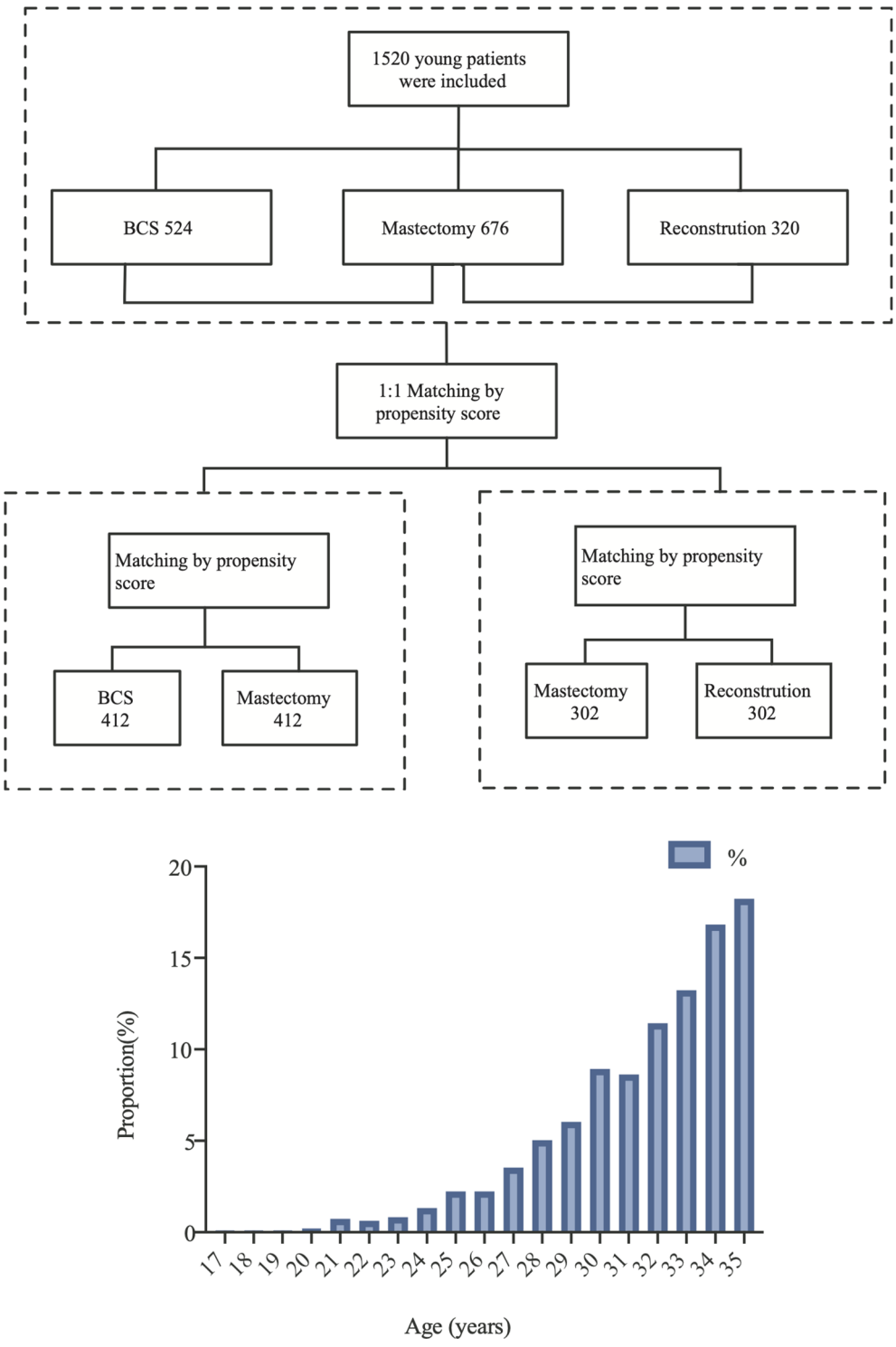


**FIGURE 1** | Flowchart and age composition ratio of included patients.

Study variables included patient age, body mass index (BMI), histological type, tumor grade, postoperative tumor size, lymph node metastasis status, estrogen receptor (ER) status, progesterone receptor (PR) status, and human epidermal growth factor receptor-2 (HER-2) status. The proportion of the patients included in this study is shown in **Figure 2**. BMI values were classified according to the criteria of the guidelines for the prevention and treatment of overweight and obesity in Chinese

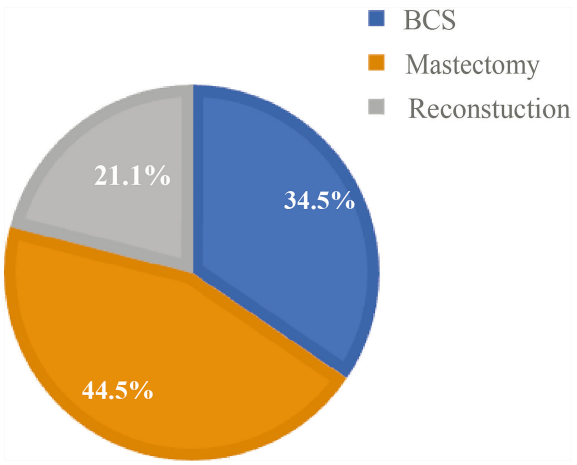


**FIGURE 2** | Percentage of surgical options in young patients at Fudan University Shanghai Cancer Center (FUSCC).

adults: normal, 18.5 ≤ BMI < 24; underweight, BMI < 18.5; and overweight, BMI ≥ 24. Oncological characteristics included histological type, such as ductal carcinoma *in situ* (DCIS), invasive ductal carcinoma (IDC), and others, as well as tumor grades classified as I, II, or III.

The clinical and pathological staging system of AJCC version 8 was used to evaluate patients' T- and N-stage status (16). Hormone receptor-positive (HR, ER, or PR status) was defined as 1% expression by immunohistochemistry (IHC). HER2-positive breast cancer was defined as IHC staining 3+ or *ERBB2* gene amplification by fluorescence *in situ* hybridization (FISH). HER2-negative was defined as IHC staining 0 or 1+ or HER2 IHC staining 2+ and no gene amplification by FISH; triple-negative breast cancer (TNBC) was defined when ER status, PR status, and HER2 status were all negative. OS was calculated as the time from the initial pathological diagnosis to death from any cause as the clinical outcome assessment. DFS was defined as the time from the initial pathological diagnosis to the appearance of recurrence, metastasis, or breast cancer-related death. All patients were followed until the date of death or December 19, 2019. Patients lacking follow-up data were excluded from the study.

## 2.3 Propensity Score Matching

The R (version 4.0.4, https://www.r-project.org/) software was used for PSM using the "MatchIt" R packages. YWBCs who underwent surgery were divided into three groups according to surgical options: (1) BCS, (2) M, and (3) RECON. Survival outcomes were compared among the three surgical options using PSM to minimize the impact of selection bias and confounding variables. The variables included BMI, histological type, tumor grade, T stage, N stage, ER status, PR status, and HER2 status, as well as molecular subtypes. Patients were 1:1 matched using a caliper value of 0.5. The BCS vs. the M group had 412 patients after matching, and the M group had 302 patients (**Figure 1**).

## 2.4 Statistical Analysis

The baseline characteristics of the subgroup of surgical options were compared using Pearson's chi-square test. DFS and OS were determined by Kaplan–Meier analysis and Cox regression model, and the survival outcomes of the three surgical options were compared using the log-rank test. A *P*-value <0.05 (95% confidence level) was considered statistically significant. All statistical analyses were conducted using SPSS (version 25.0; IBM Corporation, Armonk, NY, USA), and all survival curves were plotted using GraphPad Prism (Version 8.0; GraphPad Software, Inc., La Jolla, CA, USA).

# 3 RESULTS

## 3.1 Characteristics of Patients

A total of 1,520 YWBCs were included in the study. The age composition is shown in **Figure 1**. The median follow-up duration was 5.1 years. A total of 524 patients (34.5%) underwent BCS, 676 patients (44.5%) underwent M, and 320 patients (21.1%) underwent RECON [**Figure 2**; ages, 31.02 (17–35), 32.23 (21–35), and 30.91 (19–35) years, respectively; **Table 1**].

Before PSM, there were significant differences in BMI, histological subtype, T stage, N stage, and molecular subtypes among the three subgroups (**Table 1**). Analysis of the molecular subtypes showed that a larger proportion of TNBC patients underwent BCS as opposed to M and RECON (22% vs. 14% vs. 13%), while HER2-positive patients underwent BCS less frequently than M and RECON (16% vs. 29% vs. 27%) (**Table 1**). Compared with those who underwent BCS and RECON, a high proportion of patients who underwent M were overweight (21%), had T2 or T3 tumors (41%) and lymph node involvement (pN+, 48%), and were HER2-positive (29%). Compared with the other surgical options, patients who received RECON were mostly underweight (RECON vs. M vs. BCS, 17% vs. 10% vs. 11%, respectively), had ductal carcinoma *in situ* (RECON vs. M vs. BCS, 14% vs. 6% vs. 8%), and had negative lymph node involvement (RECON vs. M vs. BCS, 71% vs. 51% vs. 70%).

Our results demonstrated that the surgical options could be affected by the patients' baseline characteristics. Therefore, patients were 1:1 matched to adjust for selective bias after PSM, with well-balanced BCS (n = 412) and M (n = 412) groups and with well-balanced RECON (n = 302) and M (n = 302) groups. After PSM, there were no differences between the matched groups in terms of their baseline matching variables (i.e., age, BMI, histology type and grade, T and N stages, ER status, PR status, and HER2 status) (**Tables 2**, **3**).

## 3.2 Kaplan–Meier and Cox Analysis

### 3.2.1 Disease-Free Survival

The 5-year DFS rates were 96%, 87%, and 93% after BCS, M, and RECON, respectively; the 10-year DFS rates were 93%, 82%, and 87%, respectively, and the log-rank test showed a significant difference ($P < 0.001$) (**Figure 3**).

**TABLE 1 |** Baseline characteristics of young breast cancer patients with different surgical methods before propensity score matching.

| Characteristic | | Before PSM No. (%) | | | *P-value* |
|---|---|---|---|---|---|
| | | BCS | Mastectomy | Reconstruction | |
| | | N = 524 | N = 676 | N = 320 | |
| Age | (average range) | 31.02 (17~35) | 32.23 (21~35) | 30.91 (19~35) | |
| BMI | | | | | *P < 0.001* |
| | Normal (healthy weight) | 382 (74.2) | 460 (69.3) | 221 (69.9) | |
| | Underweight | 55 (10.7) | 63 (9.5) | 53 (16.8) | |
| | Overweight | 78 (15.1) | 141 (21.2) | 42 (13.3) | |
| Histology type | | | | | *P < 0.001* |
| | DCIS | 42 (8) | 41 (6.1) | 45 (14.1) | |
| | IDC | 428 (81.7) | 593 (87.7) | 252 (78.8) | |
| | Other | 54 (10.3) | 42 (6.2) | 23 (7.2) | |
| Grade | | | | | *P = 0.560* |
| | I, II | 206 (51.4) | 290 (54.2) | 116 (55.5) | |
| | III | 195 (48.6) | 245 (45.8) | 93 (44.5) | |
| pT | | | | | |
| | Tis | 42 (8) | 41 (6.1) | 44 (13.8) | *P < 0.001* |
| | T1 | 239 (45.6) | 260 (38.5) | 145 (45.3) | |
| | T2 | 104 (19.8) | 255 (37.7) | 74 (23.1) | |
| | T3 | 0 | 22 (3.3) | 10 (3.1) | |
| | NA | 139 (26.5) | 98 (14.5) | 47 (14.7) | |
| pN | | | | | *P < 0.001* |
| | N0 | 366 (69.8) | 346 (51.2) | 227 (70.9) | |
| | N1 | 114 (21.8) | 191 (28.3) | 59 (18.4) | |
| | N2 | 20 (3.8) | 88 (13) | 20 (6.3) | |
| | N3 | 11 (2.1) | 47 (7) | 8 (2.5) | |
| | NA | 13 (2.5) | 4 (0.6) | 6 (1.9) | |
| ER | | | | | *P = 0.660* |
| | Negative | 145 (27.7) | 185 (27.4) | 80 (25) | |
| | Positive | 378 (72.3) | 490 (72.6) | 240 (75) | |
| PR | | | | | *P = 0.660* |
| | Negative | 162 (31) | 224 (33.2) | 107 (33.4) | |
| | Positive | 361 (69) | 451 (66.8) | 213 (66.6) | |
| HER2 | | | | | *P < 0.001* |
| | Negative | 439 (83.8) | 478 (70.7) | 234 (73.1) | |
| | Positive | 85 (16.2) | 198 (29.3) | 86 (26.8) | |
| Molecular subtypes | | | | | *P < 0.001* |
| | HR-/HER2+ | 20 (3.8) | 82 (12.1) | 33 (10.3) | |
| | HR+/HER2- | 323 (61.6) | 381 (56.4) | 191 (59.7) | |
| | HR+/HER2+ | 65 (12.4) | 116 (17.2) | 53 (16.6) | |
| | TNBC | 116 (22.1) | 97 (14.3) | 43 (13.4) | |

*BMI, body mass index; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; NA, not available; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TNBC, Triple-Negative Breast Cancer.*

The multivariate Cox analysis showed that patients who underwent BCS had a significantly lower hazard of disease recurrences compared with those who underwent M [hazard ratio (HR) 0.441, 95% CI 0.274–0.709, $P = 0.001$], which could be an independent prognostic indicator for DFS. Compared to patients without lymph node metastasis, our results also showed that axillary lymph node involvement was an independent prognostic indicator of DFS (HR 1.661; 95% CI, 1.155–2.390; $P = 0.006$). BMI status, tumor size, histological type, grade, ER status, PR status, and HER2 status were not independent prognostic factors of DFS.

### 3.2.2 Overall Survival

The 5-year OS rates after BCS, M, and RECON were 98%, 94%, and 97%, respectively; the 10-year OS rates were 97%, 87%, and 91%, respectively, and the log-rank test indicated a significant difference ($P = 0.002$) (**Figure 3**).

The multivariate Cox analysis showed that patients who underwent BCS had a significantly lower risk of death compared to those who underwent M (HR 0.461; 95% CI, 0.238–0.895; $P = 0.022$), which could be an independent prognostic indicator of OS. BMI status, tumor size, axillary lymph node status, histological type, grade, ER status, PR status, and HER2 status were not independent prognostic factors of OS.

### 3.2.3 After Propensity Score Matching

After PSM, our results based on the Kaplan–Meier and Cox analyses were consistent with those of the prematched results (**Figures 3**, **4**). The matching variables were BMI, histological

**TABLE 2 |** After propensity score matching, the baseline characteristics of breast-conserving surgery vs. mastectomy alone.

| Characteristic | | Before PSM No. (%) | | *P-value* | After PSM No. (%) | | *P-value* |
|---|---|---|---|---|---|---|---|
| | | BCS | Mastectomy | | BCS | Mastectomy | |
| | | N = 524 | N = 676 | | N = 412 | N = 412 | |
| Age | (average range) | 31.02 (17~35) | 32.23 (21~35) | | 31.19 (18~35) | 32.25 (21~35) | |
| BMI | | | | *P < 0.001* | | | *P = 0.851* |
| | Normal (healthy weight) | 382 (74.2) | 460 (69.3) | | 291 (72.2) | 299 (73.8) | |
| | Underweight | 55 (10.7) | 63 (9.5) | | 44 (10.9) | 40 (9.9) | |
| | Overweight | 78 (15.1) | 141 (21.2) | | 68 (16.9) | 66 (16.3) | |
| Histology type | | | | *P < 0.001* | | | *P = 0.611* |
| | DCIS | 42 (8) | 41 (6.1) | | 41 (10) | 33 (8) | |
| | IDC | 428 (81.7) | 593 (87.7) | | 342 (83) | 348 (84.5) | |
| | Other | 54 (10.3) | 42 (6.2) | | 29 (7) | 31 (7.5) | |
| Grade | | | | *P = 0.560* | | | *P = 0.340* |
| | I, II | 206 (51.4) | 290 (54.2) | | 148 (35.9) | 168 (40.8) | |
| | III | 195 (48.6) | 245 (45.8) | | 166 (40.3) | 150 (36.4) | |
| pT | | | | *P < 0.001* | | | *P = 0.706* |
| | Tis | 42 (8) | 41 (6.1) | | 41 (10) | 34 (8.3) | |
| | T1 | 239 (45.6) | 260 (38.5) | | 188 (45.6) | 202 (49) | |
| | T2 | 104 (19.8) | 255 (37.7) | | 104 (25.2) | 97 (23.5) | |
| | T3 | 0 | 22 (3.3) | | – | – | |
| | NA | 139 (26.5) | 98 (14.5) | | 79 (19.2) | 79 (19.2) | |
| pN | | | | *P < 0.001* | | | *P = 0.312* |
| | N0 | 366 (69.8) | 346 (51.2) | | 267 (64.8) | 278 (67.5) | |
| | N1 | 114 (21.8) | 191 (28.3) | | 105 (25.5) | 103 (25) | |
| | N2 | 20 (3.8) | 88 (13) | | 20 (4.9) | 19 (4.6) | |
| | N3 | 11 (2.1) | 47 (7) | | 11 (2.7) | 10 (2.4) | |
| | NA | 13 (2.5) | 4 (0.6) | | 9 (2.2) | 2 (0.5) | |
| ER | | | | *P = 0.660* | | | *P = 0.404* |
| | Negative | 145 (27.7) | 185 (27.4) | | 110 (26.7) | 99 (24) | |
| | Positive | 378 (72.3) | 490 (72.6) | | 301 (73.1) | 313 (76) | |
| PR | | | | *P = 0.660* | | | *P = 0.398* |
| | Negative | 162 (31) | 224 (33.2) | | 125 (30.4) | 114 (27.7) | |
| | Positive | 361 (69) | 451 (66.8) | | 286 (69.6) | 297 (72.3) | |
| HER2 | | | | *P < 0.001* | | | *P = 1.000* |
| | Negative | 439 (83.8) | 478 (70.7) | | 333 (80.8) | 333 (80.8) | |
| | Positive | 85 (16.2) | 198 (29.3) | | 79 (19.2) | 79 (19.2) | |
| Molecular subtypes | | | | *P < 0.001* | | | *P = 0.939* |
| | HR-/HER2+ | 20 (3.8) | 82 (12.1) | | 20 (4.9) | 20 (4.9) | |
| | HR+/HER2- | 323 (61.6) | 381 (56.4) | | 250 (60.7) | 257 (62.4) | |
| | HR+/HER2+ | 65 (12.4) | 116 (17.2) | | 59 (14.3) | 59 (14.3) | |
| | TNBC | 116 (22.1) | 97 (14.3) | | 83 (20.1) | 76 (18.4) | |

*PSM, propensity score matching; BCS, breast-conserving surgery; N, number; BMI, body mass index; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; NA, not available; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TNBC, Triple-Negative Breast Cancer.*

type, tumor grade, postoperative pathological T stage, axillary N stage, ER status, PR status, HER2 receptor status, and molecular subtype. After PSM, DFS and OS rates were significantly improved in patients undergoing BCS compared with those undergoing M (DFS, $P$ = 0.001; OS, $P$ = 0.009; **Figure 4**), and the Cox analysis showed that BCS could improve DFS and OS [DFS: HR 0.378 (95% CI 0.227~0.630), $P$ < 0.001; OS: HR 0.357 (95% CI 0.181~0.700), $P$ = 0.003], which was consistent with the unmatched results. Patients who underwent RECON also showed improved DFS and OS rates compared with those who underwent M, but this difference was not statistically significant (DFS, $P$ = 0.164; OS, $P$ = 0.130; **Figure 5**).

# 4 DISCUSSION

## 4.1 Study Findings

In our study, we compared the survival outcomes of different surgical options for YWBC; we found that the DFS and OS rates in the BCS group improved significantly in comparison to those in the M group, results that were similar to those seen in non-young patients. However, these results should be considered cautiously because the baseline characteristics and tumor burden of the patients between these two surgical options were significantly different. Some previous studies have shown that the selective bias of surgical options varies significantly depending on the institutions and surgeons (17, 18). In clinical

**TABLE 3 |** After propensity score matching, the baseline characteristics of reconstruction after total mastectomy vs. mastectomy alone.

| Characteristic | | Before PSM No. (%) | | *P-value* | After PSM No. (%) | | *P-value* |
|---|---|---|---|---|---|---|---|
| | | Mastectomy | Reconstruction | | Mastectomy | Reconstruction | |
| | | N = 676 | N = 320 | | N = 302 | N = 302 | |
| Age | (average range) | 32.23 (21~35) | 30.91 (19~35) | | 32.5 (21~35) | 30.99 (19~35) | |
| BMI | | | | *P < 0.001* | | | *P = 0.904* |
| | Normal (healthy weight) | 460 (69.3) | 221 (69.9) | | 216 (72.2) | 218 (73.2) | |
| | Underweight | 63 (9.5) | 53 (16.8) | | 37 (12.4) | 38 (12.8) | |
| | Overweight | 141 (21.2) | 42 (13.3) | | 46 (15.4) | 42 (14.1) | |
| Histology type | | | | *P < 0.001* | | | *P = 0.647* |
| | DCIS | 41 (6.1) | 45 (14.1) | | 35 (11.6) | 42 (13.9) | |
| | IDC | 593 (87.7) | 252 (78.8) | | 243 (80.5) | 239 (79.1) | |
| | Other | 42 (6.2) | 23 (7.2) | | 24 (7.9) | 21 (7) | |
| Grade | | | | *P = 0.560* | | | *P = 0.938* |
| | I, II | 290 (54.2) | 116 (55.5) | | 118 (39.1) | 115 (38.1) | |
| | III | 245 (45.8) | 93 (44.5) | | 91 (30.1) | 90 (29.8) | |
| pT | | | | *P < 0.001* | | | *P = 0.510* |
| | Tis | 41 (6.1) | 44 (13.8) | | 34 (11.3) | 41 (13.6) | |
| | T1 | 260 (38.5) | 145 (45.3) | | 142 (47) | 137 (45.4) | |
| | T2 | 255 (37.7) | 74 (23.1) | | 69 (22.8) | 71 (23.5) | |
| | T3 | 22 (3.3) | 10 (3.1) | | 5 (1.7) | 10 (3.3) | |
| | NA | 98 (14.5) | 47 (14.7) | | 52 (17.2) | 43 (14.2) | |
| pN | | | | *P < 0.001* | | | *P = 0.730* |
| | N0 | 346 (51.2) | 227 (70.9) | | 222 (73.5) | 210 (69.5) | |
| | N1 | 191 (28.3) | 59 (18.4) | | 55 (18.2) | 59 (19.5) | |
| | N2 | 88 (13) | 20 (6.3) | | 14 (4.6) | 20 (6.6) | |
| | N3 | 47 (7) | 8 (2.5) | | 8 (2.6) | 8 (2.6) | |
| | NA | 4 (0.6) | 6 (1.9) | | 3 (1) | 5 (1.7) | |
| ER | | | | *P = 0.660* | | | *P = 0.580* |
| | Negative | 185 (27.4) | 80 (25) | | 83 (27.5) | 77 (25.5) | |
| | Positive | 490 (72.6) | 240 (75) | | 219 (72.5) | 225 (74.5) | |
| PR | | | | *P = 0.660* | | | *P = 1.000* |
| | Negative | 224 (33.2) | 107 (33.4) | | 102 (33.8) | 102 (33.8) | |
| | Positive | 451 (66.8) | 213 (66.6) | | 200 (66.2) | 200 (66.2) | |
| HER2 | | | | *P < 0.001* | | | *P = 0.645* |
| | Negative | 478 (70.7) | 234 (73.1) | | 224 (74.2) | 219 (72.5) | |
| | Positive | 198 (29.3) | 86 (26.8) | | 78 (25.8) | 83 (27.5) | |
| Molecular subtypes | | | | *P < 0.001* | | | *P = 0.866* |
| | HR-/HER2+ | 82 (12.1) | 33 (10.3) | | 31 (10.3) | 32 (10.6) | |
| | HR+/HER2- | 381 (56.4) | 191 (59.7) | | 175 (57.9) | 177 (58.6) | |
| | HR+/HER2+ | 116 (17.2) | 53 (16.6) | | 47 (15.6) | 51 (16.9) | |
| | TNBC | 97 (14.3) | 43 (13.4) | | 49 (16.2) | 42 (13.9) | |

*PSM, propensity score matching; N, number; BMI, body mass index; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; NA, not available; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TNBC, Triple-Negative Breast Cancer.*

settings, surgical decision-making for patients needs to incorporate age, family history, BMI, histological type and grade, TNM stage, molecular subtypes, and other special conditions. Thus, selective bias was unavoidable. Our results also demonstrated that the surgical options may be affected by the patients' baseline characteristics. The patients who underwent M or RECON had a HER2 positive status, large tumor size (≥T2 stage), or more lymph node involvement (≥N1 stage) compared with those who underwent BCS (**Table 1**). Thus, PSM was used to adjust for confounding factors. After PSM, DFS and OS rates were significantly improved in the BCS group compared to those in the M group, and the RECON group also had improved rates compared to the M group; however, the improvements were not statistically significant (**Figures 4**, **5**).

## 4.2 Surgical Options and Systemic Therapy by Molecular Subtype

Several retrospective studies have demonstrated that age is an independent risk factor for tumor recurrence after BCS (10–12). The local recurrence of YWBC who underwent BCS could be reduced by systemic treatment in earlier studies, and the oncological outcomes of BCS combined with radiotherapy were regarded as being equal to M. With the advancement of systemic treatment, a recent large cohort suggested that BCS could improve survival outcomes compared to M, and these two surgical options should not be regarded as equal. A study in 2013 found that systemic therapy was associated with a nearly 60% lower incidence of local recurrence (HR 0.42; 95% CI 0.28–0.60; $P < 0.0001$) in YWBCs (aged ≤40 years) in the Netherlands, and

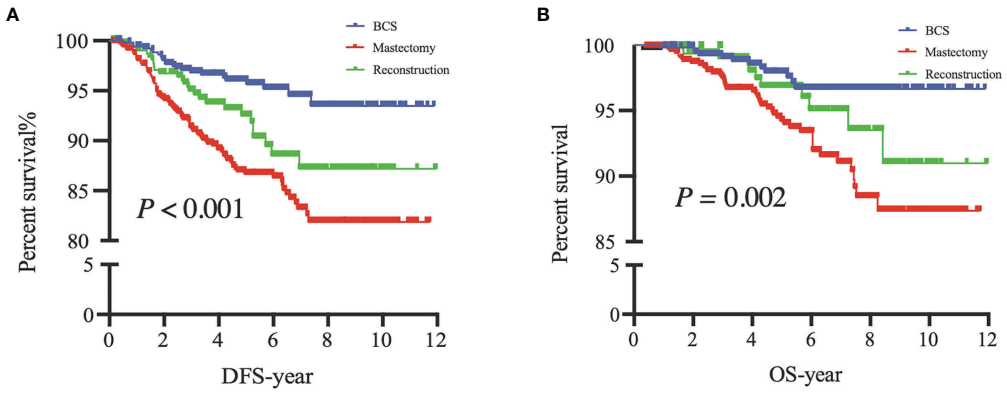


**FIGURE 3** | Disease-free survival (DFS) of patients among the three surgical options **(A)** and overall survival (OS) of patients among the three surgical options **(B)**. BCS, breast-conserving surgery; Mastectomy, mastectomy alone; Reconstruction, mastectomy with reconstruction.

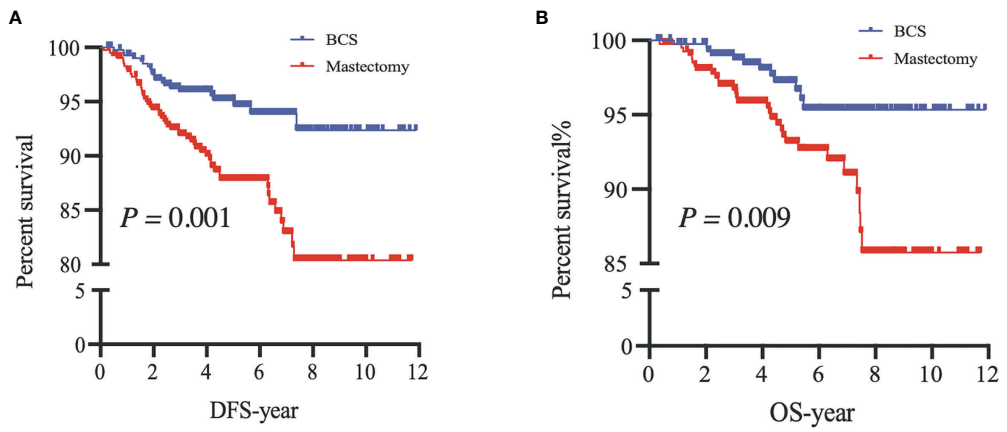


**FIGURE 4** | After propensity score matching, disease-free survival (DFS) **(A)** and overall survival (OS) **(B)** of patients between breast-conserving surgery (BCS) and mastectomy alone (Mastectomy).

distant relapse-free survival was not affected by late local recurrences (HR 1.24; 95% CI 0.74–2.08; $P$ = 0.407) (19). A meta-analysis in 2015 summarized six studies that included 22,598 patients and showed that it appears unlikely that mastectomy provides a better OS than BCS in YWBC (≤40 years) (20). The rates of local and regional recurrence in YWBC (<35 years) were not affected by the surgical options. However, the recurrence varied by biomarker subtype, and when examined over the full study period ($P$ = 0.056 and $P$ = 0.014, respectively), these differences were borderline significant but leveled off after the introduction of trastuzumab after 2005 ($P$ = 0.24 and $P$ = 0.42, respectively) (21). However, it has been more than 10 years since these studies were conducted, and systemic therapy for breast cancer has developed rapidly in the past 10 years, especially in terms of precision treatment of molecular subtypes. Our results showed that the molecular subtypes were significantly different between the patients who underwent different surgical options (**Table 1**). Patients who underwent BCS were mainly the HR+/HER2- (62%) subtype that required adjuvant endocrine therapy (**Table 1**). The TEXT and SOFT trials found that ovarian function suppression plus tamoxifen or exemestane, instead of tamoxifen alone, significantly improved the 5-year breast cancer-free interval of YWBCs (<35 years) with HR-positive breast cancer (22). Our study included YWBCs (≤35 years old) between 2008 and 2016 and similarly found that patients who underwent BCS had improved DFS and OS outcomes compared to those who underwent BCS. This may be related to the advancement of precision treatment of molecular subtypes in recent years. Earlier studies on anti-HER2-targeted therapy have not been widely performed, and the times and intensities of endocrine therapy are different from those in the recent past. Molecular subtype markers have been transformed from prognostic markers to a therapeutic basis. Therefore, systemic therapy may play an essential role in reducing the recurrence and metastasis of BCS, thereby increasing the DFS and OS rates of YWBCs.

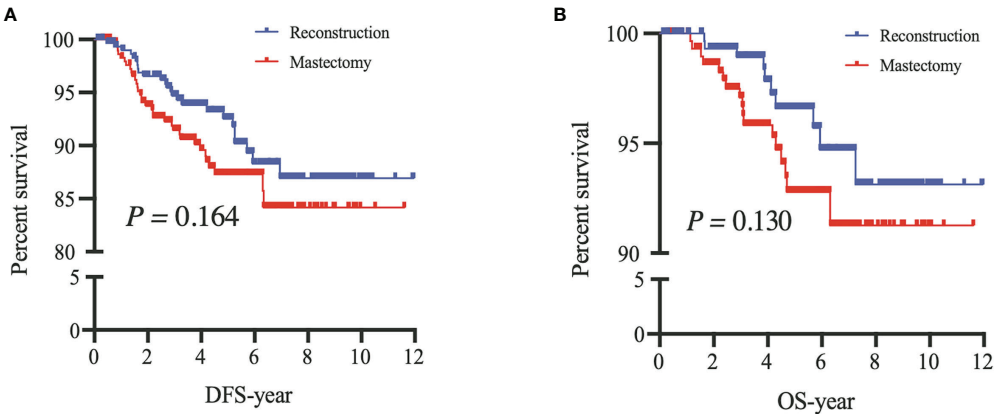


**FIGURE 5** | After propensity score matching, disease-free survival (DFS) **(A)** and overall survival (OS) **(B)** of patients between mastectomy with reconstruction (Reconstruction) and mastectomy alone (Mastectomy).

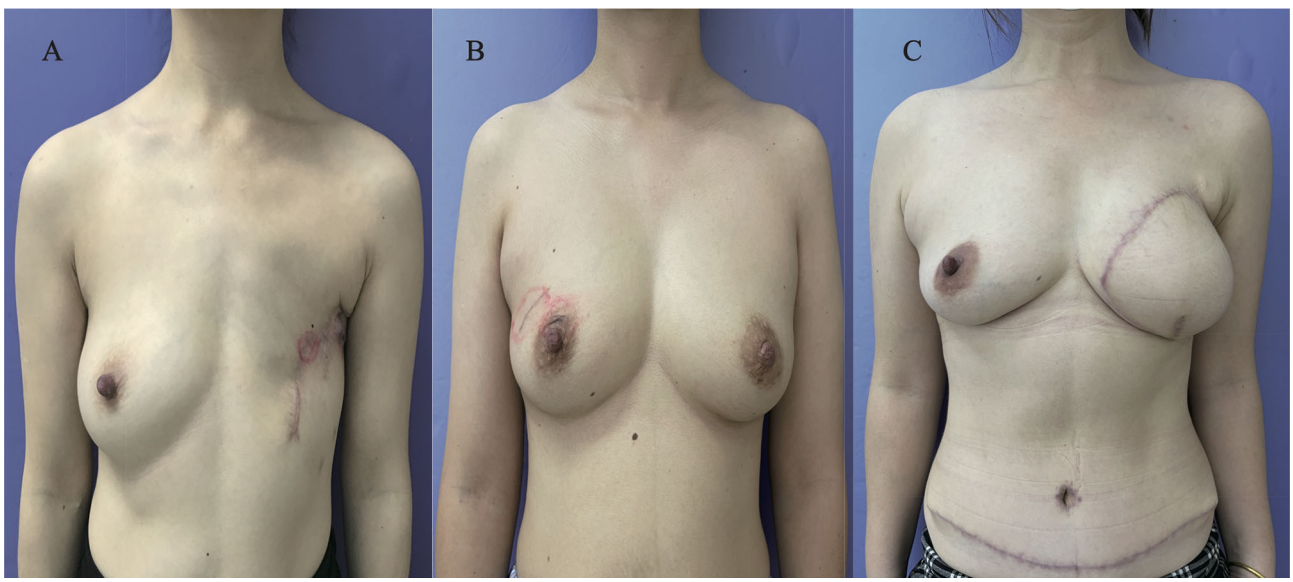


**FIGURE 6** | Postoperative esthetic results of patients from surgical options. **(A)** The esthetic images of patients who underwent mastectomy alone after 1 year; **(B)** the esthetic images of patients who had breast-conservation surgery after 6 months; **(C)** the esthetic images of patients who underwent mastectomy with reconstruction [deep inferior epigastric perforator flap (DIEP)] after 3 months.

## 4.3 Surgical Options and Radiation Therapy as Well as Other Factors

The DFS and OS of YWBCs who underwent BCS were better than those who underwent M. All of these findings were based on adjuvant radiotherapy followed BCS. Moreover, the improved irradiation techniques for YWBCs play an important role in local recurrence. A randomized phase 3 trial (23) showed that the absolute probability of ipsilateral breast tumor recurrence was highly linked with the age of the patients. For individuals 35 years or younger, the 20-year cumulative incidence was 34.5%. However, a radiation boost followed by whole-breast irradiation (WBI) enhanced local control. The recurrences without or with boost irradiation were 13% and 9%, respectively, with the greatest absolute benefit in young patients. A review (24) summarized five randomized studies over a 10-year period to determine whether to receive a tumor bed boost or not after WBI and found that providing a boost resulted in a decrease in local recurrences while having no significant influence on other oncological outcomes. Therefore, tumor bed boost after WBI may be an effective factor for improvement of the DFS.

The oncological safety of BCS is likely due to advances in systemic therapy, and optimal esthetics were achieved using BCS

as opposed to M. RECON was the main method chosen to reshape the esthetics of the breasts in those who had contraindications to BCS. Local treatment of YWBCs, particularly those who underwent mastectomy, may have a long-term impact on breast satisfaction and psychosocial and sexual outcomes (25, 26). The DFS and OS rates of YWBCs who underwent BCS were better than those who underwent M, which may be due to improvements in systemic therapy and psychosocial factors; these findings warrant further investigation. Several studies found that the quality of life of the patients in the BCS and RECON groups was superior to that in the mastectomy group (27, 28). For instance, the YWBCs who underwent mastectomy had worse body images, sexual health, and anxiety than women who underwent less extensive surgery (24). Our patients' esthetic results of three surgical options were consistent with those of other studies (**Figure 6**), BCS had greater breast satisfaction and quality-of-life ratings than RECON (29). However, there is no substantial evidence that BCS or RECON is superior. There were also other potential reasons for the improvements in DFS and OS results seen in patients who underwent BCS: the higher rates seen in BCS patients were linked to higher socioeconomic levels (14, 30), indicating that those patients were well educated and had higher incomes and health insurance. To summarize, the DFS and OS rates were significantly improved in patients who underwent BCS compared to those who underwent M, which may be a result of the patients' quality of life or socioeconomic level.

## 5 CONCLUSION

The surgical options were independent factors that affected DFS and OS in YWBCs, and the DFS and OS rates were significantly improved in patients who underwent BCS compared to those who underwent M. This may be related to the development of systemic therapy and adjuvant radiotherapy to reduce the local recurrence of BCS. In addition, a complete body image could allow patients to return to their families and to society, as well as ensure a good quality of life. These findings warrant further investigation. Therefore, BCS is preferred for early YWBCs, and RECON is the best option for remodeling the body images of YWBCs who do not have breast-conserving conditions.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Requests to access these datasets should be directed to 19111230031@fudan.edu.cn.

## ETHICS STATEMENT

The Fudan University Shanghai Cancer Center Ethics Committee approved this study (050432). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and institutional requirements.

## AUTHOR CONTRIBUTIONS

PL, BY, and LZ collected the data. PL and LL planned and analyzed the study and wrote the paper. YC and BX assisted in the study. JX and JW revised the article. The final article was read and accepted by all contributors.

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