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# The characteristics of traditional pathological parameters and emerging molecular subtypes in Chinese women with operable breast cancer

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**Running title:** Pathological characteristics in operable breast cancer

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**Abbreviations:**

Estrogen receptor: ER

Progesterone receptor: PR

Epidermal growth factor receptor 2: HER2

Primary tumor: T

Regional lymph nodes: N

Histologic grade: G

Nottingham prognosis index: NPI

Ductal carcinoma in situ: DCIS

## Abstract

**Aims:** This study investigated the characteristics of traditional pathological parameters and emerging molecular subtypes in Chinese women with operable breast cancer.

**Methods:** 1042 patients with primary operable breast cancer were enrolled in the study, which were collected from Beijing Friendship Hospital between 2008 and 2012. Biopsies or surgical resection specimens were pathologically examined and histological confirmed, and complete pathological records were analyzed.

**Results:** In 1042 patients, the unreported percentages of positive margins, vascular tumor invasion, nerve infiltration and grade were 32.3%, 50.1%, 97.5% and 38.3%. In the population with complete data, the percentages of positive margins, vascular invasion, high histologic grade, N1 + N2, T3 + T4 were 4.0%, 19.4%, 11.8%, 38.2% and 8.1%. The percentages of ER-positive, PR-positive, HER2-positive and Ki-67 high expression were 75.6%, 63.1%, 23.8%, 79.3%. The percentages of Luminal A, Luminal B, HER2-overexpression and Basal-like were 12.4%, 64.5%, 9.7%, 13.4%. Luminal A, luminal B and basal-like were more common in older than 60 years group, 41-60 years group, 20-40 years group, respectively. The 5-year relapse rates according to NPI were 6.4% in low recurrence risk group, 10.9% in moderate recurrence risk group, and 13.3% in high recurrence risk group. The 5-year relapse rates according to molecular subtypes were: luminal A 3.7%, luminal B 7.0%, HER2 overexpression 13.6%, basal-like 14.7%.

**Conclusion:** The reasonable analysis of traditional pathological parameters and emerging molecular subtypes in Chinese women with operable breast cancer may be useful to guide the precise treatment and predict the prognosis.


**Key words:** Breast cancer, histological subtype, molecular subtype, epidermal growth factor receptor 2, basal-like.

**Strengths and limitations of this study**

1. Molecular subtypes helps the precise therapy and prediction of recurrence risk in breast cancer.
2. The distribution percentages were Luminal B (64.5%) > Luminal A (12.4%) > Basal-like (13.4%) > HER2-overexpression (9.7%) in china population.
3. The 5-year relapse rates according to NPI were 6.4%, 10.9% and 13.3% in low, moderate and high recurrence risk groups.
4. The 5-year relapse rates according to molecular subtypes were: luminal A 3.7%, luminal B 7.0%, HER2 overexpression 13.6%, basal-like 14.7%.
5. The comprehensive analysis of traditional pathological parameters and emerging molecular subtypes in Chinese women with breast cancer was useful to predict the prognosis.

## Introduction

Breast cancer is the most common cause of cancer death in women, with approximately 1.67 million cases diagnosed annually worldwide in 2012[1]. Breast cancer is a highly heterogeneous disease. The rational analysis of pathological characteristic is useful for judging the prognosis of patients with breast cancer. Traditional pathological markers including node staging[2,3], positive margin[4,5], vascular tumor invasion[6], differentiation grade[3,7] and lymph vessel tumor embolus grade 3[8] have been verified as independent risk factors for the recurrence and prognosis. Estrogen receptor (ER) and progesterone receptor (PR) have been included in routine pathological practice, and used to predict the patients' course of disease and response to adjuvant hormonal therapy[9-11]. The Nottingham prognosis index (NPI) integrates the size of the lesion, the number of involved lymph nodes and the grade of the tumor; which is often used to determine the prognosis of postoperative breast cancer patients [12-14], although it is sometimes controversial.

In recent years, more and more researches support the detection of multiple genes (21-gene signature, 70-gene signature, TP53 mutation-correlated genes) in breast cancer patients [15-18]. Multi-gene assays could sub-divide patients into high- and low-risk cohorts thereby providing prognostic and predictive decision. However, the cost of these multi-gene assays remains prohibitive for many societies, and it can't be carried by a large scale[19]. So the experts propose that pathology parameters take the place of molecular subtypes. In 2013, the St Gallen Consensus Conference and ESMO Clinical Practice Guidelines recommended surrogate definitions of intrinsic subtypes of breast cancer[20]. According to ER, PR, HER2 and ki67 status, breast cancer is divided into four subtypes: luminal A, luminal B, HER2-overexpression and basal-like. Understanding these molecular subtypes means a big step forward for the individual precise treatment and prediction of recurrence risk[21-23]. Although the immunohistochemical parameters are not as accurate as multi-gene assays, but the simpler detection method and lower cost are easily accepted by most patients.

Although these molecular subtypes have been theoretically accepted, large-scale data on molecular subtype classification and pathological characteristics associated with

different age groups in the Chinese population have not been systematically studied. Therefore, we carried out the present study to investigate traditional pathological markers and emerging molecular subtypes in Chinese women with operable breast cancer.

## Materials and methods

### Study design

1042 patients with primary operable breast cancer from Beijing Friendship Hospital were enrolled in the study between January 2008 and December 2012. Biopsies or surgical resection specimens were pathologically examined and histologically confirmed, and complete pathological records were available. Pathological parameters include tumor location, operation type, distance from the cutting edge, positive margins, vascular tumor invasion, nerve infiltration, histologic grade (G), primary tumor (T), lymph nodes (N), histopathologic type, ER, PR, HER2 and Ki67 status. This study was approved by the Ethics Committee of the Beijing Friendship Hospital, and written informed consent was obtained from all participants.

### Age distribution of patients

In the study, the average age was 55.56 ± 12.37 years (range, 22 to 92 years). Among them, 115 (11.0%) patients were 20 to 40 years, 599 (57.5%) patients were 41 to 60 years, and 328 (31.5%) patients were older than 61 years.

### The diagnosis criterion of traditional pathological markers

T, N, G and histopathologic type were collected and classified according to the American Joint Committee on Cancer TNM Staging System for Breast Cancer (National Comprehensive Cancer Network Guidelines Version 2.2015 for Breast Cancer). G was centrally performed on whole sections according to the recommendations of Nottingham combined with histologic grade (Elston-Ellis modification of Scarff-Bloom-Richardson grading system)[24,25].

Vascular tumor invasion was assessed on hematoxylin-eosin-stained whole sections of primary tumors. Blood / lymph vessels were identified morphologically, which was carefully differentiated from breast ducts / retraction tissue. Tumor cells within vessels mostly formed clusters of various sizes. However, a ≥ 1 single tumor within a

vessel was scored as vascular tumor cell infiltration, if conclusive tumor cell morphology was present.

ER, PR and Ki67 status were determined by immunohistochemical staining. Tumors were considered HER2 positive if they were scored 3+ by immunohistochemical staining or if they were 2+ by immunohistochemical staining and also HER2 amplified (ratio > 2.0) on the basis of fluorescence in situ hybridization.

**Surrogate definitions for molecular subtypes of breast cancer**

Four molecular subtypes (luminal A, luminal B, HER2-overexpression and basal-like) were classified. Table 1 was surrogate definitions of molecular subtypes of breast cancer according to the 2013 St Gallen Consensus Conference and ESMO Clinical Practice Guidelines[20].

**The judgment criterion for the recurrence risk**

For each eligible patient, the Nottingham prognosis index (NPI) was calculated using the formula NPI = (0.2 × S) + N + G. In this formula, S is the tumor size in cm, N is the number of involved lymphatic nodes (>4 = 3, 4–1 = 2, 0 = 1), and G is the degree of malignancy of the tumor (degree 3 = 3, degree 2 = 2, degree 1 = 1). Based on the numerical score obtained from the formula, the patients are located in one of the prognosis groups, good prognostic / low recurrence risk: 2.00 - 3.40, moderate prognostic / moderate recurrence risk: 3.41 - 5.40, poor prognostic / high recurrence risk: >5.41[12-14].

**Definition of study endpoints and Statistical analysis**

The deadline of follow-up was December 31, 2016, or the data of patient death. Disease free survival (DFS) was defined as period from the date of diagnosis to occurrence of any event such as progression, relapse, recurrence or death. All data were analyzed using the SPSS Statistics software (Version 13.0; Chicago, IL, USA). Comparisons were determined using Chi-square test, Fisher's extract test, or independent *t*-test. A *P* value <0.05 was considered statistically significant.

**Result**

**Distribution feathers of pathological parameters**

In 1042 patients, the unreported percentages of positive margins, vascular tumor

invasion, nerve infiltration and grade were 32.3%, 50.1%, 97.5% and 38.3%. In the population with complete data, the percentages of positive margins, vascular invasion, high histologic grade, N1 + N2, T3 + T4 were 4.0%, 19.4%, 11.8%, 38.2% and 8.1%. There were significant differences in neoadjuvant chemotherapy, axillary staging among patients in the three age groups (20-40 years, 41-60 years and ≥61 years, Table 2). In patients with complete data, 171 (16.4%) patients received neoadjuvant chemotherapy and 652 (73.7%) patients received axillary staging. Neoadjuvant chemotherapy was much less in 41-60 years group. Axillary staging was much less in 20-40 years group. There were no significant differences in tumor location, margins, vascular tumor invasion, nerve infiltration, grade, tumor size and lymph nodes (all, $P$>0.05). Features of traditional pathological parameters in patients with operable breast cancer are shown in **Table 2.**

With regard to histopathologic types, 110 (10.6%) patients had ductal carcinoma in situ (DICS) and 932 (89.4%) patients had invasive carcinomas. There were no significant differences in histopathologic types among patients in the three age groups (20-40 years, 41-60 years and ≥61 years). **(Table 3)**

**Distribution feathers of ER/PR/HER2/Ki67 and molecular subtypes**

In the population with complete data, 670 (75.6%) patients were ER-positive, and 196 (23.8%) patients were HER2-positive **(Figure 1**). With a cut-off value of 20%, high expression and low expression of PR were detected in 456 (52.1%) and 105 (12.0%) patients, respectively. With a cut-off value of 14%, high expression and low expression of Ki-67 were detected in 653 (79.3%) and 170 (64.5%) patients, respectively. There was significant difference in Ki67 status among the three age groups (20-0 years, 41-60 years and ≥61 years, $P$=0.025). In HER2-positive tumors, 15.2% of patients were ER-positive and 24% of patients highly expressed Ki-67.

In the population with complete data, 109 (12.4%) patients was luminal A, 565 (64.5%) patients was luminal B, 85 (9.7%) patients was HER2-overexpression and 117 (13.4%) patients was basal-like **(Table 4, Figure 1)**. There was a significant difference in molecular subtypes among the three age groups (20-40 years, 41-60 years and ≥61 years; $P$=0.038). Luminal A was more common in older than 60 years

group, luminal B was more common in 41-60 years group, and basal-like were more common in 20-40 years group **(Figure 2**).

**Distribution of recurrence risk**

Recurrence risk was evaluated based on the NPI. Among the 623 evaluated patients, 263 (42.2%) patients should have good prognostic / low recurrence risk, 312 (50.1%) patients should have moderate prognostic / moderate recurrence risk, and 48 (7.7%) patients should have poor prognostic / high recurrence risk. However, there was no significant difference in recurrence risk among three age groups.

The actual 5-year relapse rates have been recorded in 203 patients. The 5-year relapse rates according to NPI were as follows: 6.4% in low recurrence risk group, 10.9% in moderate recurrence risk group, and 13.3% in high recurrence risk group. The 5-year relapse rates according to molecular subtypes were as follows: luminal A 1 of 27 (3.7%), luminal B 9 of 120 (7.0%), HER2 overexpression 3 of 22 (13.6%), basal-like 5 of 34 (14.7%).

**Discussion**

Several traditional pathological parameters including positive margin, vascular tumor invasion, high histologic grade and lymph node staging have been verified as independent risk factors for recurrence and as markers of prognosis[2-7]. Tumor size has been demonstrated to be the only predictor for distant recurrence-free interval after a pathologic complete response [Hazards ratio = 3.62 (T3 *vs.* T1-2) and Hazards ratio = 2.80 (T4 *vs.* T1-2)][26]. Sarsenov D reported that younger age (< 40year), large tumor size (> 2cm), high grade, triple negative phenotype were identified as independent prognostic factors with a negative impact on overall survival of patients with recurrent breast cancer[27]. In our analysis, the percentages of positive margins, vascular tumor invasion, high histologic grade, N1 + N2, T3 + T4 were 4.0%, 19.4%, 11.8%, 38.2% and 8.1%. These indicators reflect the percentages of patients with poor prognosis from different perspectives. In our study, the unreported percentages of positive margins, vascular tumor invasion, nerve infiltration and grade were 32.3%, 50.1%, 97.5% and 38.3%. These startling data raises the strict demand to the surgeons and pathologist, and that careful operation, strict handling of specimens and accurate

interpretation of results are necessary for treatment and prognosis.

DCIS and invasive ductal cancer were the two main histopathologic types in Chinese breast cancer patients. Julian's study showed that axillary nodal dissection in DCIS is not recommended[28]. In our study, 50% of patients with DCIS received axillary staging. Whether the patients with DCIS should receive axillarily stage is a question worthy of discussion. Although patients with DCIS have a favorable prognosis, recurrence risk was increased in high-grade DCIS (Odds ratio, 4.39)[29]. The DCIS Score (12-gene) assay can provide clinically relevant information on recurrence risk and may facilitate decision making by clinicians[30]. Invasive ductal cancer was found in 89.4% of patients in our study, and Hasebe's study exhibited that type 2 invasive ductal cancer is one of the best factors for accurately predicting locoregional recurrence[8].

ER, PR, Ki67 and HER2 have been routinely applied in the clinical practice. ER and PR are associated with response to hormonal therapy and better clinical outcomes. In our study, ER-positive rate was 75.6%, which was in the range of 70-80% reported by previous studies[31-33]. PR-positive rate was 63.1% in all cases and 81.0% in ER-positive patients, compared with 51.0% in all cases and 67.0% in ER-positive patients reported by Liu *et al*[34]. It has been shown that 5-year adjuvant tamoxifen reduces annual breast cancer death rate by 31% for ER-positive patients[28]. In our study, the high and low expressions of Ki67 were 79.3% and 20.7%, respectively. Ki67 is closely related to cellular proliferation[35], and a larger decrease in Ki67 indicates better responsiveness to chemotherapy[36,37]. Ki67 borderline distribution indicated a significantly more distant bone and liver metastasis and worse disease-specific survival[38]. In our study, the percentage of HER2-positive was 23.8%, which was similar to 25.5% reported by Zhu *et al*[33]. HER2-overexpression is associated with relapse[39,40]. Trastuzumab, a powerful HER2 targeted agent, has dramatically improved the outcomes of patients with HER2-overexpression breast cancer[41-42].

The distribution features in Chinese women were luminal B > basal-like > luminal A > HER2-overexpression. Luminal A, luminal B and basal-like were more common in older than 60 years group, 41-60 years group, 20-40 years group, respectively. The

distribution of molecular subtypes in our study is consistent with that reported by Si *et al* [43]. Molecular subtypes, as emerging pathologic indications, are critical for predicting prognosis and guiding treatment[21,22]. Voduc *et al.* reported that patients with the luminal A subtype have better prognosis than that with HER2-overexpresion and basal-like, as indicated by relatively low rates of local relapse and regional relapse[39]. Luminal A subtype is very sensitive to endocrine therapy, luminal B (HER2-) subtype benefits from endocrine or chemotherapy, luminal B (HER2+) subtype benefits from endocrine or chemotherapy combined wiyh anti-HER2 targeted therapy[43,44], and HER2-overexpression subtype benefits from chemotherapy combined with anti-HER2 targeted therapy[40,42,45]. The target is lacking in basal-like breast cancer, and combined chemotherapy is the standard treatment option.

The NPI is usually used to determine the prognosis of postoperative breast cancer patients. NPI was calculated using tumor size, positive lymphatic nodes and Grade. In our study, the 5-year relapse rates increased with the rise of NPI, the result suggested that the prognosis significance of traditional pathological parameters. The 5-year relapse rates according to molecular subtypes were as follows: basal-like > HER2 overexpression > luminal B > luminal A, and this is consistent with the results reported by Shim H[46]. However, Arvold *et al.* revealed that the 5-year cumulative incidence of local relapse was 0.8% in patients with luminal A, 4.4% in luminal B, 10.8% in HER2-overexpression and 6.7% in basal-like[47], and the patients with HER2-overexpression subtype had the worst prognosis. Both evaluated methods are able to predict the recurrence risk and prognosis, however, the latter shows its unique advantages in guiding specific treatment scheme.

In conclusion, our study has shown the features of traditional pathological parameters and emerging molecular subtypes in Chinese women with operable breast cancer. In-depth understanding of the biological behavior of breast cancer would be beneficial for oncologists to guide treatment, identify recurrence risk and make reasonable follow-ups. However, our study has several limitations. It was a retrospective study conducted in a single institution with a relatively small sample size. We are conducting a study with follow-up data in a larger population in different regions in

China, and the result deserves anticipation.

**Author contributions**

Q.L., B.M.C. designed the study; L.T.Y and L.Z. developed the methodology and performed the analyses; X.Y.J., L.L., T.L. Z.L collected the data; Q.L. and H.G. analyzed the data; and Q.L. wrote the first draft. All the authors contributed to the review and revision of the manuscript, and all authors read and approved the final manuscript.

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**Additional information**

The authors have no conflicts of interest to disclose.

**No additional data available.**

**Figure legends**

**Figure 1** The distribution features of ER/PR/HER2/Ki67 and molecular subtypes in overall patients.

**Figure 2** The distribution features of molecular subtypes in different age groups.

## Tables

**Table 1**. Surrogate definitions of molecular subtypes of breast cancer.

| Molecular Subtype | Luminal A | Luminal B | HER2-overexpression | Basal-like |
|---|---|---|---|---|
| histopathologic surrogate definition | • ER-positive<br>• HER2-negative<br>• Ki67 low<br>• PR high* | HER2-negative<br>• ER-positive<br>• HER2-negative<br>• and either<br>• Ki67 high** or<br>• PR low<br>HER2-positive<br>• ER-positive<br>• HER2-positive<br>• any Ki67<br>• any PR | HER2-positive (non-luminal)<br>• HER2-positive<br>• ER and PR absent | Triple-negative (ductal)<br>• ER and PR absent<br>• HER2-negative |

Notes: *The cut-off value is 20% for PR high expression; **The cut-off value is 14% for Ki67 high expression. Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

**Table 2.** The characteristics of traditional pathological parameters in different age groups

| Pathological parameters | No. of patients (%) | | | | $X^2$ |
|---|---|---|---|---|---|
| | All patients (*N*=1,042) | 20y to 40y (*n*=115) | 41y to 60y (*n*=599) | ≥61y (*n*=328) | |
| **Tumor location** | | | | | 1.60 |
| Left | 536 (51.4) | 54 (47.0) | 306 (51.1) | 176 (53.7) | |
| Right | 506 (48.6) | 61 (53.0) | 293 (48.9) | 152 (46.3) | |
| **Neoadjuvant chemotherapy** | | | | | 18.54 |
| No | 871 (83.6) | 99 (86.1) | 476 (79.5) | 296 (90.2) | |
| Yes | 171 (16.4) | 16 (13.9) | 123 (20.5) | 32 (9.8) | |
| **Axillary staging** | | | | | 18.61 |
| No description | 157 (15.1) | 25 (21.7) | 87 (14.5) | 45 (13.7) | |
| With axillary staging | 652 (62.6) | 51 (44.3) | 389 (64.9) | 212 (64.6) | |
| Without axillary staging | 233 (22.4) | 39 (33.9) | 123 (20.5) | 71 (21.6) | |
| **Margins** | | | | | 7.68 |
| Not detected | 337 (32.3) | 50 (43.5) | 188 (31.4) | 99 (30.2) | |
| Margins no residual cancer | 677 (65.0) | 63 (54.8) | 395 (65.9) | 219 (66.8) | |
| Margins with residual cancer | 28 (2.7) | 2 (1.7) | 16 (2.7) | 10 (3.0) | |
| **Vascular tumor invasion** | | | | | 9.53 |
| Not detected | 522 (50.1) | 67 (58.3) | 286 (47.7) | 169 (51.5) | |
| No | 419 (40.2) | 36 (31.3) | 246 (41.1) | 137 (41.8) | |
| Yes | 101 (9.7) | 12 (10.4) | 67 (11.2) | 22 (6.7) | |
| **Nerve infiltration** | | | | | 4.615 |
| Not detected | 1,016 (97.5) | 112 (97.4) | 586 (97.8) | 318 (97.0) | |
| No | 15 (1.4) | 3 (2.6) | 8 (1.3) | 4 (1.2) | |
| Yes | 11 (1.1) | 0 (0.0) | 5 (0.8) | 6 (1.8) | |
| **Grade** | | | | | 8.37 |
| Not detected | 399 (38.3) | 53 (46.1) | 217 (36.2) | 129 (39.3) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| High histological grade | 76 (7.3) | 9 (7.8) | 49 (8.2) | 18 (5.5) | | |
| Intermediate histological grade | 478 (45.9) | 45 (39.1) | 286 (47.8) | 147 (44.8) | | |
| Low histological grade | 89 (8.5) | 8 (7.0) | 47 (7.8) | 34 (10.4) | | |
| **Tumor size** | | | | | 23.32 | 0.010 |
| TX | 202 (19.4) | 39 (33.9) | 115 (19.2) | 48 (14.6) | | |
| T1 | 352 (33.7) | 34 (29.6) | 203 (33.9) | 114 (34.8) | | |
| T2 | 420 (40.3) | 38 (33.0) | 241 (40.2) | 141 (43.0) | | |
| T3 | 32 (3.1) | 2 (1.7) | 19 (3.2) | 11 (3.3) | | |
| T4 | 36 (3.5) | 2 (1.7) | 21 (3.5) | 13 (4.0) | | |
| **Lymph nodes** | | | | | 22.27 | 0.001 |
| NX | 382 (36.7) | 62 (53.9) | 206 (34.4) | 114 (34.8) | | |
| N0 | 408 (39.2) | 33 (28.7) | 230 (38.4) | 145 (44.2) | | |
| N1 | 250 (24.0) | 20 (17.4) | 162 (27.0) | 68 (20.7) | | |
| N2 | 2 (0.2) | 0 (0.0) | 1 (0.2) | 1 (0.3) | | |

**Table 3.** The characteristics of histopathologic types in different age groups

| Histopathologic types | No. of patients (%) | | | | $X^2$ |
|---|---|---|---|---|---|
| | All patients (*N*=1,042) | 20y to 40y (*n*=115) | 41y to 60y (*n*=599) | ≥61y (*n*=328) | |
| ***In situ* carcinomas** | 110 | 15 | 61 | 34 | 8.47 |
| Intraductal | 106 (96.4) | 14 (93.3) | 59 (96.7) | 33 (97.1) | |
| **Invasive carcinomas** | 932 | 100 | 538 | 294 | 25.97 |
| Not otherwise specified | 112 (12.0) | 22 (22.0) | 63 (11.7) | 27 (9.2) | |
| Ductal | 675 (72.4) | 72 (72.0) | 402 (74.7) | 201 (68.4) | |
| Mucinous | 24 (2.6) | 1 (1.0) | 13 (2.4) | 10 (3.3) | |
| Papillary | 80 (8.6) | 3 (3.0) | 39 (7.2) | 38 (12.9) | |
| Lobular | 27 (2.9) | 1 (1.0) | 14 (2.6) | 12 (4.1) | |
| Tubular | 14 (1.5) | 1 (1.0) | 7 (1.3) | 6 (2.0) | |

**Table 4.** The distribution features of ER/PR/HER2/Ki67 and molecular subtypes in different age groups

| Parameters | No. of patients (%) | | | | $X^2$ | *P*-v |
|---|---|---|---|---|---|---|
| | All patients (*N*=1,042) | 20y to 40y (*n*=115) | 41y to 60y (*n*=599) | ≥61y (*n*=328) | | |
| **ER status** | | | | | **3.293** | **0.5** |
| Positive expression | 670 (64.3) | 68 (59.1) | 384 (64.1) | 218 (66.5) | | |
| Negative expression | 216 (20.7) | 24 (20.9) | 128 (21.4) | 64 (19.5) | | |
| No detected | 156 (15.0) | 23 (20.0) | 87 (14.5) | 46 (14.0) | | |
| **PR status** | | | | | **9.411** | **0.1** |
| High expression | 456 (43.8) | 54 (47.0) | 257 (42.9) | 145 (44.2) | | |
| Low expression | 105 (10.1) | 5 (4.3) | 64 (10.7) | 36 (10.8) | | |
| Negative expression | 314 (30.1) | 30 (26.1) | 189 (31.6) | 95 (29.0) | | |
| No detected | 167 (16.0) | 26 (22.6) | 89 (14.9) | 52 (15.9) | | |
| **HER2 status** | | | | | **10.380** | **0.1** |
| Positive expression | 196 (18.8) | 17 (14.8) | 128 (21.4) | 51 (15.5) | | |
| Negative expression | 627 (60.2) | 65 (56.5) | 351 (58.6) | 211 (64.3) | | |
| No detected | 219 (21.0) | 33 (28.7) | 120 (20.0) | 66 (20.1) | | |
| **Ki67 status** | | | | | **11.302** | **0.0** |
| High expression | 653 (62.7) | 67 (58.3) | 398 (66.4) | 188 (57.3) | | |
| Low expression | 170 (16.3) | 17 (14.8) | 86 (14.4) | 67 (20.4) | | |
| No detected | 219 (21.0) | 31 (27.0) | 115 (19.2) | 73 (22.3) | | |
| **Molecular subtype** | | | | | **16.93** | **0.0** |
| Unclassified | 166 (15.9) | 25 (21.7) | 88 (14.7) | 53 (16.2) | | |
| Luminal A | 109 (10.5) | 12 (10.4) | 48 (8.0) | 49 (14.9) | | |
| Luminal B | 565 (54.2) | 54 (47.0) | 344 (57.4) | 167 (50.9) | | |
| HER2-overexpression | 85 (8.2) | 9 (7.8) | 53 (8.8) | 23 (7.0) | | |
| Basal-like | 117 (11.2) | 15 (13.0) | 66 (11.0) | 36 (11.0) | | |

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

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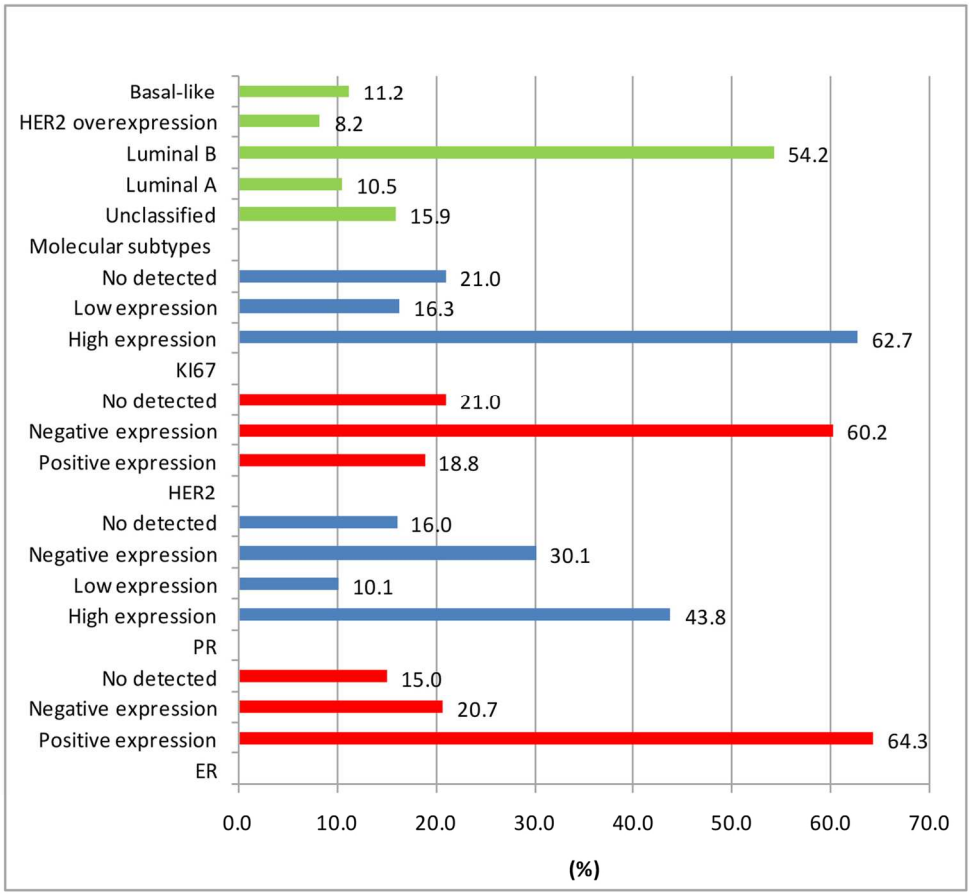


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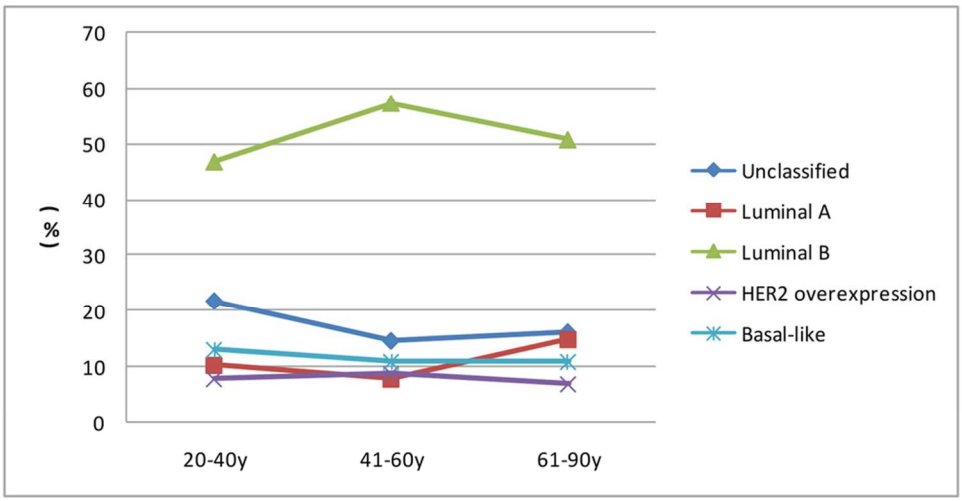


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STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract. Page1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found. Page 3 |
| **Introduction** | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported. Page 5 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses. Page 5-6 |
| **Methods** | | |
| Study design | 4 | Present key elements of study design early in the paper. Page 6 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, follow-up, and data collection. Page 6-7 |
| Participants | 6 | Clearly define all outcomes and diagnostic criteria. Page 6-7 |
| Variables | 7 | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods. Page 6-7 |
| Quantitative variables | 8 | Describe all statistical method. Page 7 |
| Statistical methods | 9 | Describe any methods used to examine subgroups and interactions. Page 7 |
| **Results** | | |
| Participants and Descriptive data | 10 | (a) Distribution feathers of pathological parameters. Page 7-8 |
| | | (b) Distribution feathers of ER/PR/HER2/Ki67 and molecular subtypes. Page 8-9 |
| Outcome data | 11 | Report numbers of outcome events or summary measures over time. Page 8-9 |
| | 12 | Distribution of recurrence risk. Page 9 |
| **Discussion** | | |
| Key results | 13 | Summarise key results with reference to study objectives. Page 9-11 |
| Limitations | 14 | Discuss limitations of the study. Page 11-12 |
| Interpretation | 15 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. Page 9-11 |
| Generalisability | 16 | Discuss the generalisability (external validity) of the study results. Page 9-11 |
| **Other information** | | |
| Funding | 17 | Give the source of funding and the role of the funders for the present study. Page 12 |

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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# The characteristic and prognostic value of traditional pathological parameters and advanced molecular subtypes in Beijing women with operable breast cancer



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# The characteristic and prognostic value of traditional pathological parameters and advanced molecular subtypes in Beijing women with operable breast cancer

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**Running title:** Pathological characteristics of operable breast cancer

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**Abbreviations:**

Estrogen receptor: ER

Progesterone receptor: PR

Epidermal growth factor receptor 2: HER2

Primary tumor: T

Regional lymph nodes: N

Histologic grade: G

Nottingham prognosis index: NPI

Ductal carcinoma in situ: DCIS

## Abstract

**Aims:** This study investigated the characteristics of traditional pathological parameters and advanced molecular subtypes in Beijing women with operable breast cancer.

**Methods:** 1042 patients with primary operable breast cancer were enrolled in the study, which were collected from Beijing Friendship Hospital between 2008 and 2012. Biopsies or surgical resection specimens were pathologically examined and histological confirmed, and complete pathological records were analyzed.

**Results:** In 1042 patients, the percentages of high histological grade, N1 + N2, T2 + T4 were 7.3%, 24.2%, 46.9%. In patients with invasive breast cancer, the percentages of auxiliary staging, positive margins, vascular invasion and nerve infiltration were 65.0%, 2.8%, 10.5% and 1.1%, the missing percentages of auxiliary staging, margins, vascular tumor invasion and nerve infiltration were 14.2%, 31.4%, 46.5% and 97.4%. The percentages of ER-positive, PR-positive, HER2-positive and Ki-67 high expression were 64.3%, 43.8%, 18.8% and 62.7%. The percentages of Luminal A, Luminal B, HER2-overexpression and Basal-like were 10.5%, 54.2%, 8.2% and 11.2%. Luminal A, luminal B and basal-like were more common in older than 60 years group, 41-60 years group, 20-40 years group, respectively. The 5-year relapse rates according to NPI were as follows: 6.2% in low recurrence risk group, 10.4% in moderate recurrence risk group, and 12.9% in high recurrence risk group. The 5-year relapse rates according to molecular subtypes were as follows: luminal A 4.0%, luminal B 7.0%, HER2 overexpression14.2%, basal-like 15.6%.

**Conclusion:** The reasonable analysis of traditional pathological parameters and advanced molecular subtypes in Beijing women with operable breast cancer may be useful to guide precise treatment and predict prognosis.


**Key words:** Breast cancer, histological subtype, molecular subtype, epidermal growth factor receptor 2, basal-like.

**Strengths and limitations of this study**

1. The comprehensive analysis of traditional pathological parameters and advanced molecular subtypes helps the precise therapy and recurrence risk prediction in patients with breast cancer.
2. The distribution percentages were Luminal B (54.2%) > Basal-like (11.2%) > Luminal A (10.5%) > HER2-overexpression (8.2%).
3. The 5-year relapse rates according to NPI were 6.2%, 10.4% and 12.9% in low, moderate and high recurrence risk groups.
4. The 5-year relapse rates according to molecular subtypes were Luminal A 4.0%, uminal B 7.0%, HER2- overexpression 14.2%, Basal-like 15.6%.
5. The pathological and molecular features were analyzed retrospectively, the prognostic significance of both are needed to confirm.

## Introduction

Breast cancer is the most common cause of cancer death in women, with approximately 1.67 million cases diagnosed annually worldwide in 2012[1]. Breast cancer is a highly heterogeneous disease. The rational analysis of pathological characteristic is useful for judging the prognosis of patients with breast cancer. Traditional pathological markers including node staging[2,3], positive margin[4,5], vascular tumor invasion[6], differentiation grade[3,7] and lymph vessel tumor embolus grade 3[8] have been verified as independent risk factors for the recurrence and prognosis. Estrogen receptor (ER) and progesterone receptor (PR) have been included in routine pathological practice, and used to predict the patients' course of disease and response to adjuvant hormonal therapy[9-11]. The Nottingham prognosis index (NPI) integrates the size of the lesion, the number of involved lymph nodes and the grade of the tumor; which is often used to determine the prognosis of postoperative breast cancer patients [12-14], although it is sometimes controversial.

In recent years, more and more researches support the detection of multiple genes (21-gene signature, 70-gene signature, TP53 mutation-correlated genes) in breast cancer patients [15-18]. Multi-gene assays could sub-divide patients into high- and low-risk cohorts thereby providing prognostic and predictive decision. However, the cost of these multi-gene assays remains prohibitive for many societies, and it can't be carried by a large scale[19]. So the experts propose that pathology parameters take the place of molecular subtypes. In 2013, the St Gallen Consensus Conference and ESMO Clinical Practice Guidelines recommended surrogate definitions of intrinsic subtypes of breast cancer[20]. According to ER, PR, HER2 and ki67 status, breast cancer is divided into four subtypes: luminal A, luminal B, HER2-overexpression and basal-like. Understanding these molecular subtypes means a big step forward for the individual precise treatment and prediction of recurrence risk[21-23]. Although the immunohistochemical parameters are not as accurate as multi-gene assays, but the simpler detection method and lower cost are easily accepted by most patients.

Although these molecular subtypes have been theoretically accepted, large-scale data on molecular subtype classification and pathological characteristics associated with different age groups in the Beijing population have not been systematically studied. Therefore, we

carried out the present study to investigate traditional pathological markers and advanced molecular subtypes in Beijing women with operable breast cancer.

## Materials and methods

### Study design

We retrospectively collected all patients (N = 1042) with primary operable breast cancer between January 2008 and December 2012 in Beijing Friendship Hospital. The patients with breast benign diseases or metastatic breast cancer were excluded. Biopsies or surgical resection specimens were pathologically examined and histologically confirmed, and complete clinical and pathological records were available. Pathological parameters include tumor location, operation type, distance from the cutting edge, positive margins, vascular tumor invasion, nerve infiltration, histologic grade (G), primary tumor (T), lymph nodes (N), histopathologic type, ER, PR, HER2 and Ki67 status. This study was approved by the Ethics Committee of the Beijing Friendship Hospital, and written informed consent was obtained from all participants.

### Patient and Public Involvement

All patients with primary operable breast cancer were retrospectively collected in Beijing Friendship Hospital. Informed consents were signed by all patients, and the study was approved by the ethics committee of Beijing Friendship Hospital. All the patients receive the follow-up and 5 year disease free survival (DFS) has been calculated in part patients. Follow-up approach involves hospital medical records and outpatient medical records inquire, contacting the patients / family members for recurrence information. We provide the freely clinical medical supports for all patients in follow-up process, for example, we answer the related medical questions, guide the follow-up paln, and make the next-step therapeutic regimen if recurrence occurs. In the study, the public is not involved.

### The diagnosis criterion of traditional pathological markers

T, N, G and histopathologic type were collected and classified according to the American Joint Committee on Cancer TNM Staging System for Breast Cancer (National Comprehensive Cancer Network Guidelines Version 2.2015 for Breast Cancer). G was centrally performed on whole sections according to the recommendations of Nottingham combined with histologic grade (Elston-Ellis modification of Scarff-Bloom-Richardson

grading system)[24,25].

Vascular tumor invasion was assessed on hematoxylin-eosin-stained whole sections of primary tumors. Blood / lymph vessels were identified morphologically, which was carefully differentiated from breast ducts / retraction tissue. Tumor cells within vessels mostly formed clusters of various sizes. However, a ≥ 1 single tumor within a vessel was scored as vascular tumor cell infiltration, if conclusive tumor cell morphology was present.

ER, PR and Ki67 status were determined by immunohistochemical staining. Tumors were considered HER2 positive if they were scored 3+ by immunohistochemical staining or if they were 2+ by immunohistochemical staining and also HER2 amplified (ratio > 2.0) on the basis of fluorescence in situ hybridization.

**Surrogate definitions for molecular subtypes of breast cancer**

Four molecular subtypes (luminal A, luminal B, HER2-overexpression and basal-like) were classified. Table 1 was surrogate definitions of molecular subtypes of breast cancer according to the 2013 St Gallen Consensus Conference and ESMO Clinical Practice Guidelines[20].

**The judgment criterion for the recurrence risk**

For each eligible patient, the Nottingham prognosis index (NPI) was calculated using the formula NPI = (0.2 × S) + N + G. In this formula, S is the tumor size in cm, N is the number of involved lymphatic nodes (>4 = 3, 4–1 = 2, 0 = 1), and G is the degree of malignancy of the tumor (degree 3 = 3, degree 2 = 2, degree 1 = 1). Based on the numerical score obtained from the formula, the patients are located in one of the prognosis groups, good prognostic / low recurrence risk: 2.00 - 3.40, moderate prognostic / moderate recurrence risk: 3.41 - 5.40, poor prognostic / high recurrence risk: >5.41[12-14].

**Follow-up and Statistical analysis**

The actual 5-year relapse rates have been recorded in 203 patients. The deadline of follow-up was December 31, 2016. DFS was defined as period from the date of diagnosis to occurrence of any event such as progression, recurrence, metastasis or death. Only the patients with invasive breast cancer were included in the prognostic analysis. All data were analyzed using the SPSS Statistics software (Version 13.0; Chicago, IL, USA).

Comparisons were determined using Chi-square test, Fisher's exact test, or independent *t*-test. A *P* value <0.05 was considered statistically significant.

## Results

### Distribution feature of age

In the study, the average age was 55.56 ± 12.37 years (range, 22 to 92 years). Among them, 115 (11.0%) patients were 20 to 40 years, 599 (57.5%) patients were 41 to 60 years, and 328 (31.5%) patients were older than 61 years.

### Distribution feathers of pathological parameters

In 1042 patients, the percentages of high histological grade, N1 + N2, T2 + T4 were 7.3%, 24.2%, 46.9%. In patients with invasive breast cancer, the percentages of without auxiliary staging, positive margins, vascular invasion and nerve infiltration were 20.8%, 2.8%, 10.5% and 1.1%, the missing percentages of auxiliary staging, margins, vascular tumor invasion and nerve infiltration were 14.2%, 31.4%, 46.5% and 97.4%. There were significant differences in neoadjuvant chemotherapy, auxiliary staging, tumor size and lymph nodes in patients among the three age groups (20-40 years, 41-60 years and ≥61 years, Table 2). Neoadjuvant chemotherapy was much less in 41-60 years group. Auxiliary staging, T2 + T4 and N1 + N2 was much less in 20-40 years group. There were no significant differences in tumor location, margins, vascular tumor invasion, nerve infiltration, grade (all, *P*>0.05). Features of traditional pathological parameters in patients with operable breast cancer are shown in **Table 2.** With regard to histopathologic types, 104 (10.0%) patients had ductal carcinoma in situ (DICS) and 938 (90.0%) patients had invasive carcinoma. There were no significant differences in histopathologic types in patients among the three age groups (20-40 years, 41-60 years and ≥61 years).

### Distribution feathers of ER/PR/HER2/Ki67 and molecular subtypes

In 1042 patients, 670 (64.3%) patients were ER-positive, and 196 (18.8%) patients were HER2-positive (Figure 1). With a cut-off value of 20%, high expression and low expression of PR were detected in 456 (43.8%) and 105 (10.1%) patients, respectively. With a cut-off value of 14%, high expression and low expression of Ki-67 were detected in 653 (62.7%) and 170 (16.3%) patients, respectively. There was significant difference of Ki67 status among the three age groups (20-0 years, 41-60 years and ≥61 years, P=0.025).

In HER2-positive tumors, 15.2% of patients were ER-positive and 24% of patients highly expressed Ki-67.

In the population with complete data, 109 (10.5%) patients was luminal A, 565 (54.2%) patients was luminal B, 85 (8.2%) patients was HER2-overexpression and 117 (11.2%) patients was basal-like **(Table 3, Figure 1)**. There was a significant difference in molecular subtypes among the three age groups (20-40 years, 41-60 years and ≥61 years; *P*=0.038). Luminal A was more common in older than 60 years group, luminal B was more common in 41-60 years group, and basal-like were more common in 20-40 years group **(Figure 2**).

**Distribution of recurrence risk**

Recurrence risk was evaluated based on the NPI. Among the 623 evaluated patients, 263 (42.2%) patients should have good prognostic / low recurrence risk, 312 (50.1%) patients should have moderate prognostic / moderate recurrence risk, and 48 (7.7%) patients should have poor prognostic / high recurrence risk. However, there was no significant difference in recurrence risk among three age groups.

The actual 5-year relapse rates of the patients with invasive breast cancers have been recorded in 193 patients. The 5-year relapse rates according to NPI were as follows: 6.2% in low recurrence risk group, 10.4% in moderate recurrence risk group, and 12.9% in high recurrence risk group. The 5-year relapse rates according to molecular subtypes were as follows: luminal A 4.0%, luminal B 7.0%, HER2 overexpression14.2%, basal-like 15.6%.

**Discussion**

Traditional pathological parameters including positive margin, vascular tumor invasion, high histologic grade and lymph node staging have been verified as independent risk factors for recurrence and as markers of prognosis[2-7]. Tumor size has been demonstrated to be closely related to relapse free survivals[26]. Sarsenov D reported that younger age (< 40year), large tumor size (> 2cm), high grade, triple negative phenotype were identified as independent prognostic factors with a negative impact on overall survival of patients with recurrent breast cancer[27]. In our analysis, the percentages of positive margins, vascular tumor invasion, high histologic grade, N1 + N2, T2 + T4 were 2.8%, 10.5%, 7.3%, 24.2% and 46.9%. These indicators reflect the percentages of patients with poor prognosis from

different perspectives. In our study, the missing percentages of positive margins, vascular tumor invasion, nerve infiltration and grade were up to 31.4%, 46.51%, 97.4% and 38.3%. The missing data are at random. Accurate analysis and diagnosis of preoperative staging, standardized surgical operation, standardized pathological slice making and handling, comprehensive and accurate interpretation of pathological findings, and comprehensive detection of prerequisite markers will greatly reduce the missing data. These startling missing data raises the strict demands to the surgeons, physicians and pathologists.

DCIS and invasive ductal cancer were the two main histopathologic types in Beijing breast cancer patients. Julian's study showed that auxiliary nodal dissection in DCIS is not recommended[28]. In our study, 50% of patients with DCIS received auxiliary staging. Whether the patients with DCIS should receive axillarily stage is a question worthy of discussion. Although patients with DCIS have a favorable prognosis, recurrence risk was increased in high-grade DCIS (Odds ratio, 4.39)[29]. The DCIS Score (12-gene) assay provide clinically relevant information on recurrence risk and may facilitate decision making by clinicians[30]. The percentage of invasive ductal cancer was 90.0% in the whole patients, and Hasebe's study exhibited that type 2 invasive ductal cancer was one of the best factors for accurately predicting loco-regional recurrence[8].

ER, PR, Ki67 and HER2 have been routinely applied in the clinical practice. ER and PR are associated with good response to hormonal therapy and better clinical outcomes. In our study, ER-positive rate was 75.6%, which coincided with the results reported by other studies[31-33]. PR-positive rates were 53.9% in all cases and 81.0% in ER-positive patients, which is agreement with the results reported by Liu *et al*[34]. It has been shown that 5-year adjuvant tamoxifen reduces annual breast cancer death rate by 31% for ER-positive patients[28]. In our study, the high and low expressions of Ki67 were 62.7% and 16.3%, respectively. Ki67 is closely related to cellular proliferation[35], and a larger decrease in Ki67 indicates better responsiveness to chemotherapy[36,37]. Ki67 borderline distribution indicated significantly more distant bone and liver metastasis and worse disease-specific survival[38]. In patients with complete data, the percentage of HER2-positive was 23.8%, which was similar to 25.5% reported by Zhu *et al*[33]. HER2-overexpression is associated with more relapse[39,40]. Trastuzumab, a powerful HER2 targeted agent, has dramatically

improved the outcomes of patients with HER2-overexpression breast cancer[41-42].

The distribution features of molecular subtypes were luminal B > basal-like > luminal A > HER2-overexpression. Luminal B, HER2-overexpression and basal-like were more common in 41-60 years group. The distribution of molecular subtypes in our study is consistent with that reported by Si *et al* [43]. Molecular subtypes, as advanced pathologic indications, are critical for predicting prognosis and guiding treatment[21,22]. Voduc *et al.* reported that patients with the luminal A subtype have better prognosis than that with HER2-overexpresion and basal-like, as indicated by relatively low rates of local relapse and regional relapse[39]. Luminal A subtype is very sensitive to endocrine therapy, luminal B (HER2-) subtype benefits from endocrine or chemotherapy, luminal B (HER2+) subtype benefits from endocrine or chemotherapy combined with anti-HER2 targeted therapy[43,44], and HER2-overexpression subtype benefits from chemotherapy combined with anti-HER2 targeted therapy[40,42,45]. The target is lacking in basal-like breast cancer, and combined chemotherapy is the standard treatment option.

The NPI is usually used to determine the prognosis of postoperative breast cancer patients. NPI was calculated using tumor size, positive lymphatic nodes and Grade. In our study, the 5-year relapse rates increased with the rise of NPI, the results suggested that the prognosis significance of traditional pathological parameters. The 5-year relapse rates according to molecular subtypes were as follows: basal-like > HER2 overexpression > luminal B > luminal A, and this is consistent with the results reported by Shim H[46]. However, Arvold *et al.* revealed that the 5-year cumulative incidence of local relapse was 0.8% in patients with luminal A, 4.4% in luminal B, 10.8% in HER2-overexpression and 6.7% in basal-like[47], and the patients with HER2-overexpression subtype had the worst prognosis. Both evaluated methods are able to predict the recurrence risk and prognosis, however, the latter shows its unique advantages in guiding specific treatment scheme.

In conclusion, our study has shown the features of traditional pathological parameters and advanced molecular subtypes in Beijing women with operable breast cancer. In-depth understanding of the biological behavior of breast cancer would be beneficial for oncologists to guide treatment, identify recurrence risk and make reasonable follow-ups. However, our study has several limitations. It was a retrospective study conducted in

single institution with a relatively small sample. At present, we are carrying out a study about molecular subtypes and recurrence risk in a larger population in China, and the result deserves anticipation.

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**Author contributions**

Q.L., B.W.C. designed the study; Q.D. and Y.R.L. developed the methodology and performed the analyses; X.Y.J., L.L., T.L. Q.D. collected the data; Q.L. and H.G. analyzed the data; and Q.L. wrote the first draft. All the authors contributed to the review and revision of the manuscript, and all authors read and approved the final manuscript.

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**Additional information**

The authors have no conflicts of interest to disclose.

**No additional data available.**

**Figure legends**

**Figure 1** The distribution features of ER/PR/HER2/Ki67 and molecular subtypes in overall patients.

**Figure 2** The distribution features of molecular subtypes in different age groups.

## Tables

**Table 1**. Surrogate definitions of molecular subtypes of breast cancer.

| Molecular Subtype | Luminal A | Luminal B | HER2-overexpression | Basal-like |
|---|---|---|---|---|
| histopathologic surrogate definition | • ER-positive<br>• HER2-negative<br>• Ki67 low<br>• PR high* | HER2-negative<br>• ER-positive<br>• HER2-negative<br>• and either<br>• Ki67 high** or<br>• PR low<br>HER2-positive<br>• ER-positive<br>• HER2-positive<br>• any Ki67<br>• any PR | HER2-positive (non-luminal)<br>• HER2-positive<br>• ER and PR absent | Triple-negative (ductal)<br>• ER and PR absent<br>• HER2-negative |

Notes: *The cut-off value is 20% for PR high expression; **The cut-off value is 14% for Ki67 high expression. Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

**Table 2.** The characteristics of traditional pathological parameters in different age groups

| Pathological parameters | No. of patients (%) | | | | $X^2$ | *P*-value |
|---|---|---|---|---|---|---|
| | All patients (*N*=1,042) | 20y to 40y (*n*=115) | 41y to 60y (*n*=599) | ≥61y (*n*=328) | | |
| **In situ and invasive breast cancer** | | | | | | |
| **Tumor size** | | | | | 23.32 | 0.010 |
| TX | 202 (19.4) | 39 (33.9) | 115 (19.2) | 48 (14.6) | | |
| T1 | 352 (33.7) | 34 (29.6) | 203 (33.9) | 114 (34.8) | | |
| T2 | 420 (40.3) | 38 (33.0) | 241 (40.2) | 141 (43.0) | | |
| T3 | 32 (3.1) | 2 (1.7) | 19 (3.2) | 11 (3.3) | | |
| T4 | 36 (3.5) | 2 (1.7) | 21 (3.5) | 13 (4.0) | | |
| **Lymph nodes** | | | | | 22.27 | 0.001 |
| NX | 382 (36.7) | 62 (53.9) | 206 (34.4) | 114 (34.8) | | |
| N0 | 408 (39.2) | 33 (28.7) | 230 (38.4) | 145 (44.2) | | |
| N1 | 250 (24.0) | 20 (17.4) | 162 (27.0) | 68 (20.7) | | |
| N2 | 2 (0.2) | 0 (0.0) | 1 (0.2) | 1 (0.3) | | |
| **Grade** | | | | | 8.37 | 0.212 |
| Not detected | 399 (38.3) | 53 (46.1) | 217 (36.2) | 129 (39.3) | | |
| High histological grade | 76 (7.3) | 9 (7.8) | 49 (8.2) | 18 (5.5) | | |
| Intermediate histological grade | 478 (45.9) | 45 (39.1) | 286 (47.8) | 147 (44.8) | | |
| Low histological grade | 89 (8.5) | 8 (7.0) | 47 (7.8) | 34 (10.4) | | |
| **Invasive breast cancer** | | | | | | |
| **Auxillary staging** | | | | | 15.12 | 0.004 |
| No description | 133(14.2) | 20(19.8) | 78(14.4) | 35(11.9) | | |
| With auxiliary staging | 610(65.0) | 49(48.5) | 363(67.0) | 198(67.1) | | |
| Without auxiliary staging | 195(20.8) | 32(31.7) | 101(18.6) | 62(21.0) | | |
| **Margins** | | | | | 9.63 | 0.055 |
| Not detected | 294 (31.4) | 44 (43.6) | 168 (31.1) | 82 (27.8) | | |
| No residual cancer | 617 (65.8) | 56 (55.4) | 358 (66.2) | 203 (68.8) | | |
| With residual cancer | 26 (2.8) | 1 (1.0) | 15 (2.8) | 10 (3.4) | | |
| **Vascular tumor invasion** | | | | | 7.47 | 0.102 |
| Not detected | 436(46.5) | 54(53.5) | 239(44.2) | 143(48.5) | | |
| No | 403(43.0) | 35(34.7) | 238(44.0) | 130(44.1) | | |
| Yes | 98(10.5) | 12(11.9) | 64(11.8) | 22(7.5) | | |
| **Nerve infiltration** | | | | | 4.19 | 0.380 |
| Not detected | 913(97.4) | 98(97.0)) | 528(97.6) | 287(97.3) | | |
| No | 14(1.5) | 3(3.0) | 8(1.5) | 1(1.0) | | |
| Yes | 10(1.1) | 0(0.0) | 5(0.9) | 5(1.7) | | |

**Table 3.** The distribution features of ER/PR/HER2/Ki67 and molecular subtypes in different age groups

| Parameters | No. of patients (%) | | | | $X^2$ | *P*-value |
|---|---|---|---|---|---|---|
| | All patients (*N*=1,042) | 20y to 40y (*n*=115) | 41y to 60y (*n*=599) | ≥61y (*n*=328) | | |
| **ER status** | | | | | **3.293** | **0.510** |
| Positive expression | 670 (64.3) | 68 (59.1) | 384 (64.1) | 218 (66.5) | | |
| Negative expression | 216 (20.7) | 24 (20.9) | 128 (21.4) | 64 (19.5) | | |
| No detected | 156 (15.0) | 23 (20.0) | 87 (14.5) | 46 (14.0) | | |
| **PR status** | | | | | **9.411** | **0.152** |
| High expression | 456 (43.8) | 54 (47.0) | 257 (42.9) | 145 (44.2) | | |
| Low expression | 105 (10.1) | 5 (4.3) | 64 (10.7) | 36 (10.8) | | |
| Negative expression | 314 (30.1) | 30 (26.1) | 189 (31.6) | 95 (29.0) | | |
| No detected | 167 (16.0) | 26 (22.6) | 89 (14.9) | 52 (15.9) | | |
| **HER2 status** | | | | | **10.380** | **0.110** |
| Positive expression | 196 (18.8) | 17 (14.8) | 128 (21.4) | 51 (15.5) | | |
| Negative expression | 627 (60.2) | 65 (56.5) | 351 (58.6) | 211 (64.3) | | |
| No detected | 219 (21.0) | 33 (28.7) | 120 (20.0) | 66 (20.1) | | |
| **Ki67 status** | | | | | **11.302** | **0.023** |
| High expression | 653 (62.7) | 67 (58.3) | 398 (66.4) | 188 (57.3) | | |
| Low expression | 170 (16.3) | 17 (14.8) | 86 (14.4) | 67 (20.4) | | |
| No detected | 219 (21.0) | 31 (27.0) | 115 (19.2) | 73 (22.3) | | |
| **Molecular subtype** | | | | | **16.93** | **0.031** |
| Unclassified | 166 (15.9) | 25 (21.7) | 88 (14.7) | 53 (16.2) | | |
| Luminal A | 109 (10.5) | 12 (10.4) | 48 (8.0) | 49 (14.9) | | |
| Luminal B | 565 (54.2) | 54 (47.0) | 344 (57.4) | 167 (50.9) | | |
| HER2-overexpression | 85 (8.2) | 9 (7.8) | 53 (8.8) | 23 (7.0) | | |
| Basal-like | 117 (11.2) | 15 (13.0) | 66 (11.0) | 36 (11.0) | | |

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

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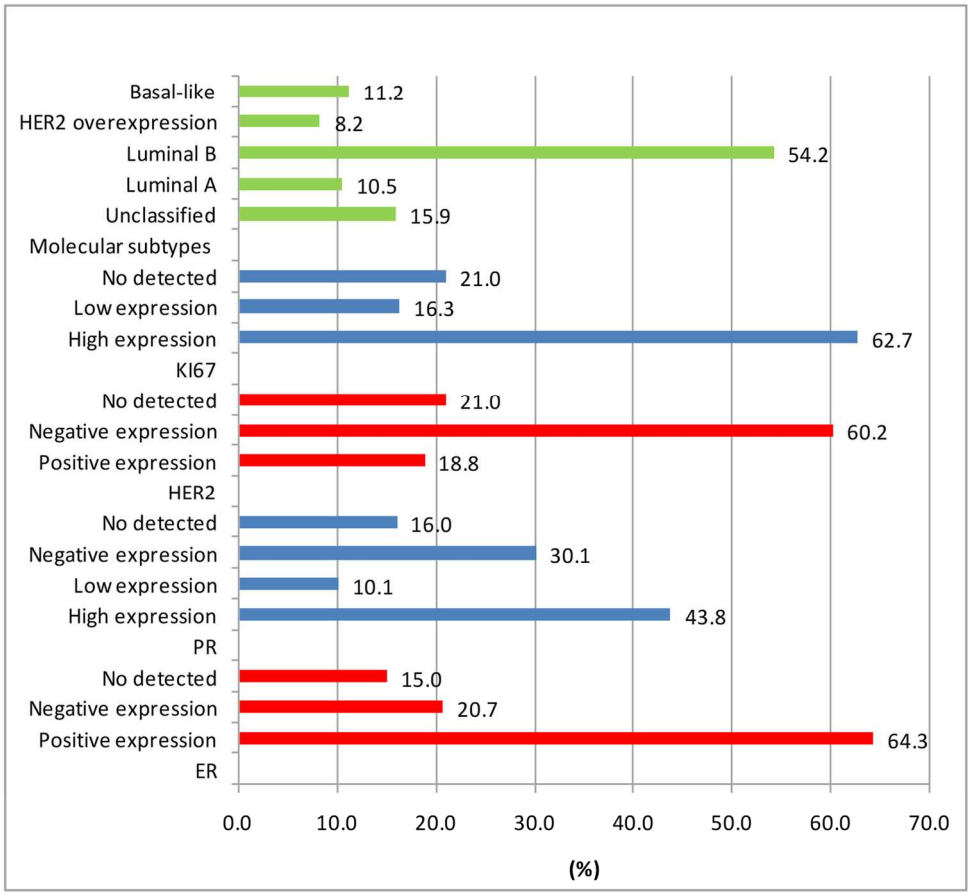


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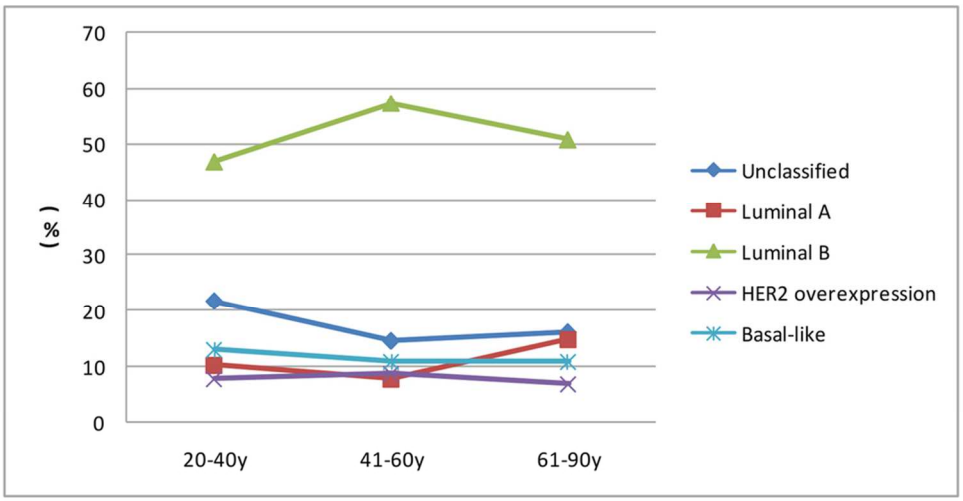


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STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract. Page1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found. Page 3 |
| **Introduction** | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported. Page 5 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses. Page 5-6 |
| **Methods** | | |
| Study design | 4 | Present key elements of study design early in the paper. Page 6 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, follow-up, and data collection. Page 6-7 |
| Participants | 6 | Clearly define all outcomes and diagnostic criteria. Page 6-7 |
| Variables | 7 | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods. Page 6-7 |
| Quantitative variables | 8 | Describe all statistical method. Page 7 |
| Statistical methods | 9 | Describe any methods used to examine subgroups and interactions. Page 7 |
| **Results** | | |
| Participants and Descriptive data | 10 | (a) Distribution feathers of pathological parameters. Page 7-8 |
| | | (b) Distribution feathers of ER/PR/HER2/Ki67 and molecular subtypes. Page 8-9 |
| Outcome data | 11 | Report numbers of outcome events or summary measures over time. Page 8-9 |
| | 12 | Distribution of recurrence risk. Page 9 |
| **Discussion** | | |
| Key results | 13 | Summarise key results with reference to study objectives. Page 9-11 |
| Limitations | 14 | Discuss limitations of the study. Page 11-12 |
| Interpretation | 15 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. Page 9-11 |
| Generalisability | 16 | Discuss the generalisability (external validity) of the study results. Page 9-11 |
| **Other information** | | |
| Funding | 17 | Give the source of funding and the role of the funders for the present study. Page 12 |

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

# The retrospective analysis about characteristic and prognostic value of traditional pathological parameters and advanced molecular subtypes in Beijing women with operable breast cancer



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| | |

# The retrospective analysis about characteristic and prognostic value of traditional pathological parameters and advanced molecular subtypes in Beijing women with operable breast cancer

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**Running title:** Pathological characteristics of operable breast cancer

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**Abbreviations:**

Estrogen receptor: ER

Progesterone receptor: PR

Epidermal growth factor receptor 2: HER2

Primary tumor: T

Regional lymph nodes: N

Histologic grade: G

Nottingham prognosis index: NPI

Ductal carcinoma in situ: DCIS

## Abstract

**Objectives** This study investigated the characteristics and prognostic value of traditional pathological parameters and advanced molecular subtypes in Beijing women with operable breast cancer.

**Design** A retrospective study through case information enquiry or telephone follow-up.

**Setting** Beijing Friendship Hospital.

**Participants** 1042 patients with primary operable breast cancer between 2008 and 2012 were enrolled in the study.

**Measures** The characteristic and 5-year relapse rates according to NPI and molecular subtypes were analyzed.

**Results** In 1042 patients, the percentages of high histological grade, N1 + N2, T2 + T4 were 7.3%, 24.2%, 46.9%. In patients with invasive breast cancer, the percentages of auxiliary staging, positive margins, vascular invasion and nerve infiltration were 65.0%, 2.8%, 10.5% and 1.1%, the missing percentages of auxiliary staging, margins, vascular tumor invasion and nerve infiltration were 14.2%, 31.4%, 46.5% and 97.4%. The percentages of ER-positive, PR-positive, HER2-positive and Ki-67 high expression were 64.3%, 43.8%, 18.8% and 62.7%. The percentages of Luminal A, Luminal B, HER2-overexpression and Basal-like were 10.5%, 54.2%, 8.2% and 11.2%. Luminal A, luminal B and basal-like were more common in older than 60 years group, 41-60 years group, 20-40 years group, respectively. The 5-year relapse rates according to NPI were as follows: 6.2% in low recurrence risk group, 10.4% in moderate recurrence risk group, and 12.9% in high recurrence risk group. The 5-year relapse rates according to molecular subtypes were as follows: luminal A 4.0%, luminal B 7.0%, HER2 overexpression14.2%, basal-like 15.6%.

**Conclusions** The reasonable analysis of traditional pathological parameters and advanced molecular subtypes in Beijing women with operable breast cancer may be useful to guide precise treatment and predict prognosis.


**Key words:** Breast cancer, histological subtype, molecular subtype, epidermal growth factor receptor 2, basal-like.

**Strengths and limitations of this study**

1. The characteristic of traditional pathological parameters and advanced molecular subtypes were contrasted.
2. The 5-year relapse rates according to NPI were reported.
3. The 5-year relapse rates according to molecular subtypes were reported.
4. The study was retrospective, and perspective study is expected.
5. It was conducted in single institution, and the results of multi-center are ongoing.

## Introduction

Breast cancer is the most common cause of cancer death in women, with approximately 1.67 million cases diagnosed annually worldwide in 2012[1]. Breast cancer is a highly heterogeneous disease. The rational analysis of pathological characteristic is useful for judging the prognosis of patients with breast cancer. Traditional pathological markers including node staging[2,3], positive margin[4,5], vascular tumor invasion[6], differentiation grade[3,7] and lymph vessel tumor embolus grade 3[8] have been verified as independent risk factors for the recurrence and prognosis. Estrogen receptor (ER) and progesterone receptor (PR) have been included in routine pathological practice, and used to predict the patients' course of disease and response to adjuvant hormonal therapy[9-11]. The Nottingham prognosis index (NPI) integrates the size of the lesion, the number of involved lymph nodes and the grade of the tumor; which is often used to determine the prognosis of postoperative breast cancer patients [12-14], although it is sometimes controversial.

In recent years, more and more researches support the detection of multiple genes (21-gene signature, 70-gene signature, TP53 mutation-correlated genes) in breast cancer patients [15-18]. Multi-gene assays could sub-divide patients into high- and low-risk cohorts thereby providing prognostic and predictive decision. However, the cost of these multi-gene assays remains prohibitive for many societies, and it can't be carried by a large scale[19]. So the experts propose that pathology parameters take the place of molecular subtypes. In 2013, the St Gallen Consensus Conference and ESMO Clinical Practice Guidelines recommended surrogate definitions of intrinsic subtypes of breast cancer[20]. According to ER, PR, HER2 and ki67 status, breast cancer is divided into four subtypes: luminal A, luminal B, HER2-overexpression and basal-like. Understanding these molecular subtypes means a big step forward for the individual precise treatment and prediction of recurrence risk[21-23]. Although the immunohistochemical parameters are not as accurate as multi-gene assays, but the simpler detection method and lower cost are easily accepted by most patients.

Although these molecular subtypes have been theoretically accepted, large-scale data on molecular subtype classification and pathological characteristics associated with different age groups in the Beijing population have not been systematically studied. Therefore, we

carried out the present study to investigate traditional pathological markers and advanced molecular subtypes in Beijing women with operable breast cancer.

## Materials and methods

### Study design

We retrospectively collected all patients (N = 1042) with primary operable breast cancer between January 2008 and December 2012 in Beijing Friendship Hospital. The patients with breast benign diseases or metastatic breast cancer were excluded. Biopsies or surgical resection specimens were pathologically examined and histologically confirmed, and complete clinical and pathological records were available. Pathological parameters include tumor location, operation type, distance from the cutting edge, positive margins, vascular tumor invasion, nerve infiltration, histologic grade (G), primary tumor (T), lymph nodes (N), histopathologic type, ER, PR, HER2 and Ki67 status. This study was approved by the Ethics Committee of the Beijing Friendship Hospital, and written informed consent was obtained from all participants.

### The observation endpoints

All the patients receive the follow-up and the 5-year relapse rates have been calculated in part patients. Follow-up approach involves hospital medical records and outpatient medical records inquire, contacting the patients / family members for recurrence information. All patients with primary operable breast cancer were retrospectively collected in Beijing Friendship Hospital. Informed consents were signed by all patients, and the study was approved by the ethics committee of Beijing Friendship Hospital.

### Patient and Public Involvement

The patients and or public were not involved in study design or conduct of the study. We provided the freely clinical medical supports for all patients in follow-up process, for example, we answered the related medical questions, guided the follow-up plan, and made the next-step therapeutic regimen if recurrence occurred.

### The diagnosis criterion of traditional pathological markers

T, N, G and histopathologic type were collected and classified according to the American Joint Committee on Cancer TNM Staging System for Breast Cancer (National Comprehensive Cancer Network Guidelines Version 2.2015 for Breast Cancer). G was

centrally performed on whole sections according to the recommendations of Nottingham combined with histologic grade (Elston-Ellis modification of Scarff-Bloom-Richardson grading system)[24,25].

Vascular tumor invasion was assessed on hematoxylin-eosin-stained whole sections of primary tumors. Blood / lymph vessels were identified morphologically, which was carefully differentiated from breast ducts / retraction tissue. Tumor cells within vessels mostly formed clusters of various sizes. However, a ≥ 1 single tumor within a vessel was scored as vascular tumor cell infiltration, if conclusive tumor cell morphology was present.

ER, PR and Ki67 status were determined by immunohistochemical staining. Tumors were considered HER2 positive if they were scored 3+ by immunohistochemical staining or if they were 2+ by immunohistochemical staining and also HER2 amplified (ratio > 2.0) on the basis of fluorescence in situ hybridization.

**Surrogate definitions for molecular subtypes of breast cancer**

Four molecular subtypes (luminal A, luminal B, HER2-overexpression and basal-like) were classified. Table 1 was surrogate definitions of molecular subtypes of breast cancer according to the 2013 St Gallen Consensus Conference and ESMO Clinical Practice Guidelines[20].

**The judgment criterion for the recurrence risk**

For each eligible patient, the Nottingham prognosis index (NPI) was calculated using the formula NPI = (0.2 × S) + N + G. In this formula, S is the tumor size in cm, N is the number of involved lymphatic nodes (>4 = 3, 4–1 = 2, 0 = 1), and G is the degree of malignancy of the tumor (degree 3 = 3, degree 2 = 2, degree 1 = 1). Based on the numerical score obtained from the formula, the patients are located in one of the prognosis groups, good prognostic / low recurrence risk: 2.00 - 3.40, moderate prognostic / moderate recurrence risk: 3.41 - 5.40, poor prognostic / high recurrence risk: >5.41[12-14].

**Follow-up and Statistical analysis**

The actual 5-year relapse rates have been recorded in 203 patients. The deadline of follow-up was December 31, 2016. DFS was defined as period from the date of diagnosis to occurrence of any event such as progression, recurrence, metastasis or death. Only the

patients with invasive breast cancer were included in the prognostic analysis. All data were analyzed using the SPSS Statistics software (Version 13.0; Chicago, IL, USA). Comparisons were determined using Chi-square test, Fisher's exact test, or independent *t*-test. A *P* value <0.05 was considered statistically significant.

## Results

### Distribution feature of age

In the study, the average age was 55.56 ± 12.37 years (range, 22 to 92 years). Among them, 115 (11.0%) patients were 20 to 40 years, 599 (57.5%) patients were 41 to 60 years, and 328 (31.5%) patients were older than 61 years.

### Distribution feathers of pathological parameters

In 1042 patients, the percentages of high histological grade, N1 + N2, T2 + T4 were 7.3%, 24.2%, 46.9%. In patients with invasive breast cancer, the percentages of without auxiliary staging, positive margins, vascular invasion and nerve infiltration were 20.8%, 2.8%, 10.5% and 1.1%, the missing percentages of auxiliary staging, margins, vascular tumor invasion and nerve infiltration were 14.2%, 31.4%, 46.5% and 97.4%. There were significant differences in neoadjuvant chemotherapy, auxiliary staging, tumor size and lymph nodes in patients among the three age groups (20-40 years, 41-60 years and ≥61 years, Table 2). Neoadjuvant chemotherapy was much less in 41-60 years group. Auxiliary staging, T2 + T4 and N1 + N2 was much less in 20-40 years group. There were no significant differences in tumor location, margins, vascular tumor invasion, nerve infiltration, grade (all, *P*>0.05). Features of traditional pathological parameters in patients with operable breast cancer are shown in **Table 2.** With regard to histopathologic types, 104 (10.0%) patients had ductal carcinoma in situ (DICS) and 938 (90.0%) patients had invasive carcinoma. There were no significant differences in histopathologic types in patients among the three age groups (20-40 years, 41-60 years and ≥61 years).

### Distribution feathers of ER/PR/HER2/Ki67 and molecular subtypes

In 1042 patients, 670 (64.3%) patients were ER-positive, and 196 (18.8%) patients were HER2-positive (Figure 1). With a cut-off value of 20%, high expression and low expression of PR were detected in 456 (43.8%) and 105 (10.1%) patients, respectively. With a cut-off value of 14%, high expression and low expression of Ki-67 were detected

in 653 (62.7%) and 170 (16.3%) patients, respectively. There was significant difference of Ki67 status among the three age groups (20-0 years, 41-60 years and ≥61 years, P=0.025). In HER2-positive tumors, 15.2% of patients were ER-positive and 24% of patients highly expressed Ki-67.

In the population with complete data, 109 (10.5%) patients was luminal A, 565 (54.2%) patients was luminal B, 85 (8.2%) patients was HER2-overexpression and 117 (11.2%) patients was basal-like **(Table 3, Figure 1)**. There was a significant difference in molecular subtypes among the three age groups (20-40 years, 41-60 years and ≥61 years; *P*=0.038). Luminal A was more common in older than 60 years group, luminal B was more common in 41-60 years group, and basal-like were more common in 20-40 years group **(Figure 2**).

**Distribution of recurrence risk**

Recurrence risk was evaluated based on the NPI. Among the 623 evaluated patients, 263 (42.2%) patients should have good prognostic / low recurrence risk, 312 (50.1%) patients should have moderate prognostic / moderate recurrence risk, and 48 (7.7%) patients should have poor prognostic / high recurrence risk. However, there was no significant difference in recurrence risk among three age groups.

The actual 5-year relapse rates of the patients with invasive breast cancers have been recorded in 193 patients. The 5-year relapse rates according to NPI were as follows: 6.2% in low recurrence risk group, 10.4% in moderate recurrence risk group, and 12.9% in high recurrence risk group. The 5-year relapse rates according to molecular subtypes were as follows: luminal A 4.0%, luminal B 7.0%, HER2 overexpression14.2%, basal-like 15.6%.

**Discussion**

Traditional pathological parameters including positive margin, vascular tumor invasion, high histologic grade and lymph node staging have been verified as independent risk factors for recurrence and as markers of prognosis[2-7]. Tumor size has been demonstrated to be closely related to relapse free survivals[26]. Sarsenov D reported that younger age (< 40year), large tumor size (> 2cm), high grade, triple negative phenotype were identified as independent prognostic factors with a negative impact on overall survival of patients with recurrent breast cancer[27]. In our analysis, the percentages of positive margins, vascular

tumor invasion, high histologic grade, N1 + N2, T2 + T4 were 2.8%, 10.5%, 7.3%, 24.2% and 46.9%. These indicators reflect the percentages of patients with poor prognosis from different perspectives. In our study, the missing percentages of positive margins, vascular tumor invasion, nerve infiltration and grade were up to 31.4%, 46.51%, 97.4% and 38.3%. The missing data are at random. Accurate analysis and diagnosis of preoperative staging, standardized surgical operation, standardized pathological slice making and handling, comprehensive and accurate interpretation of pathological findings, and comprehensive detection of prerequisite markers will greatly reduce the missing data. These startling missing data raises the strict demands to the surgeons, physicians and pathologists.

DCIS and invasive ductal cancer were the two main histopathologic types in Beijing breast cancer patients. Julian's study showed that auxiliary nodal dissection in DCIS is not recommended[28]. In our study, 50% of patients with DCIS received auxiliary staging. Whether the patients with DCIS should receive axillarily stage is a question worthy of discussion. Although patients with DCIS have a favorable prognosis, recurrence risk was increased in high-grade DCIS (Odds ratio, 4.39)[29]. The DCIS Score (12-gene) assay provide clinically relevant information on recurrence risk and may facilitate decision making by clinicians[30]. The percentage of invasive ductal cancer was 90.0% in the whole patients, and Hasebe's study exhibited that type 2 invasive ductal cancer was one of the best factors for accurately predicting loco-regional recurrence[8].

ER, PR, Ki67 and HER2 have been routinely applied in the clinical practice. ER and PR are associated with good response to hormonal therapy and better clinical outcomes. In our study, ER-positive rate was 75.6%, which coincided with the results reported by other studies[31-33]. PR-positive rates were 53.9% in all cases and 81.0% in ER-positive patients, which is agreement with the results reported by Liu *et al*[34]. It has been shown that 5-year adjuvant tamoxifen reduces annual breast cancer death rate by 31% for ER-positive patients[28]. In our study, the high and low expressions of Ki67 were 62.7% and 16.3%, respectively. Ki67 is closely related to cellular proliferation[35], and a larger decrease in Ki67 indicates better responsiveness to chemotherapy[36,37]. Ki67 borderline distribution indicated significantly more distant bone and liver metastasis and worse disease-specific survival[38]. In patients with complete data, the percentage of HER2-positive was 23.8%,

which was similar to 25.5% reported by Zhu *et al*[33]. HER2-overexpression is associated with more relapse[39,40]. Trastuzumab, a powerful HER2 targeted agent, has dramatically improved the outcomes of patients with HER2-overexpression breast cancer[41-42].

The distribution features of molecular subtypes were luminal B > basal-like > luminal A > HER2-overexpression. Luminal B, HER2-overexpression and basal-like were more common in 41-60 years group. The distribution of molecular subtypes in our study is consistent with that reported by Si *et al* [43]. Molecular subtypes, as advanced pathologic indications, are critical for predicting prognosis and guiding treatment[21,22]. Voduc *et al.* reported that patients with the luminal A subtype have better prognosis than that with HER2-overexpresion and basal-like, as indicated by relatively low rates of local relapse and regional relapse[39]. Luminal A subtype is very sensitive to endocrine therapy, luminal B (HER2-) subtype benefits from endocrine or chemotherapy, luminal B (HER2+) subtype benefits from endocrine or chemotherapy combined with anti-HER2 targeted therapy[43,44], and HER2-overexpression subtype benefits from chemotherapy combined with anti-HER2 targeted therapy[40,42,45]. The target is lacking in basal-like breast cancer, and combined chemotherapy is the standard treatment option.

The NPI is usually used to determine the prognosis of postoperative breast cancer patients. NPI was calculated using tumor size, positive lymphatic nodes and Grade. In our study, the 5-year relapse rates increased with the rise of NPI, the results suggested that the prognosis significance of traditional pathological parameters. The 5-year relapse rates according to molecular subtypes were as follows: basal-like > HER2 overexpression > luminal B > luminal A, and this is consistent with the results reported by Shim H[46]. However, Arvold *et al.* revealed that the 5-year cumulative incidence of local relapse was 0.8% in patients with luminal A, 4.4% in luminal B, 10.8% in HER2-overexpression and 6.7% in basal-like[47], and the patients with HER2-overexpression subtype had the worst prognosis. Both evaluated methods are able to predict the recurrence risk and prognosis, however, the latter shows its unique advantages in guiding specific treatment scheme.

In conclusion, our study has shown the features of traditional pathological parameters and advanced molecular subtypes in Beijing women with operable breast cancer. In-depth understanding of the biological behavior of breast cancer would be beneficial for

oncologists to guide treatment, identify recurrence risk and make reasonable follow-ups. However, our study has several limitations. It was a retrospective study conducted in single institution with a relatively small sample. At present, we are carrying out a study about molecular subtypes and recurrence risk in a larger population in China, and the result deserves anticipation.

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**Author contributions**

Q.L., B.W.C. designed the study; Q.D. and Y.R.L. developed the methodology and performed the analyses; X.Y.J., L.L., T.L. Q.D. collected the data; Q.L. and H.G. analyzed the data; and Q.L. wrote the first draft. All the authors contributed to the review and revision of the manuscript, and all authors read and approved the final manuscript.

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**Conflict of interest**

None declared.

**Data sharing statement**

All data are available from the author QL.

**Figure legends**

**Figure 1** The distribution features of ER/PR/HER2/Ki67 and molecular subtypes in overall patients.

**Figure 2** The distribution features of molecular subtypes in different age groups.

## Tables

**Table 1**. Surrogate definitions of molecular subtypes of breast cancer.

| Molecular Subtype | Luminal A | Luminal B | HER2-overexpression | Basal-like |
|---|---|---|---|---|
| histopathologic surrogate definition | • ER-positive<br>• HER2-negative<br>• Ki67 low<br>• PR high* | HER2-negative<br>• ER-positive<br>• HER2-negative<br>• and either<br>• Ki67 high** or<br>• PR low<br>HER2-positive<br>• ER-positive<br>• HER2-positive<br>• any Ki67<br>• any PR | HER2-positive (non-luminal)<br>• HER2-positive<br>• ER and PR absent | Triple-negative (ductal)<br>• ER and PR absent<br>• HER2-negative |

Notes: *The cut-off value is 20% for PR high expression; **The cut-off value is 14% for Ki67 high expression. Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

**Table 2.** The characteristics of traditional pathological parameters in different age groups

| Pathological parameters | No. of patients (%) | | | | $X^2$ | *P*-value |
|---|---|---|---|---|---|---|
| | All patients (*N*=1,042) | 20y to 40y (*n*=115) | 41y to 60y (*n*=599) | ≥61y (*n*=328) | | |
| **In situ and invasive breast cancer** | | | | | | |
| **Tumor size** | | | | | 23.32 | 0.010 |
| TX | 202 (19.4) | 39 (33.9) | 115 (19.2) | 48 (14.6) | | |
| T1 | 352 (33.7) | 34 (29.6) | 203 (33.9) | 114 (34.8) | | |
| T2 | 420 (40.3) | 38 (33.0) | 241 (40.2) | 141 (43.0) | | |
| T3 | 32 (3.1) | 2 (1.7) | 19 (3.2) | 11 (3.3) | | |
| T4 | 36 (3.5) | 2 (1.7) | 21 (3.5) | 13 (4.0) | | |
| **Lymph nodes** | | | | | 22.27 | 0.001 |
| NX | 382 (36.7) | 62 (53.9) | 206 (34.4) | 114 (34.8) | | |
| N0 | 408 (39.2) | 33 (28.7) | 230 (38.4) | 145 (44.2) | | |
| N1 | 250 (24.0) | 20 (17.4) | 162 (27.0) | 68 (20.7) | | |
| N2 | 2 (0.2) | 0 (0.0) | 1 (0.2) | 1 (0.3) | | |
| **Grade** | | | | | 8.37 | 0.212 |
| Not detected | 399 (38.3) | 53 (46.1) | 217 (36.2) | 129 (39.3) | | |
| High histological grade | 76 (7.3) | 9 (7.8) | 49 (8.2) | 18 (5.5) | | |
| Intermediate histological grade | 478 (45.9) | 45 (39.1) | 286 (47.8) | 147 (44.8) | | |
| Low histological grade | 89 (8.5) | 8 (7.0) | 47 (7.8) | 34 (10.4) | | |
| **Invasive breast cancer** | | | | | | |
| **Auxillary staging** | | | | | 15.12 | 0.004 |
| No description | 133(14.2) | 20(19.8) | 78(14.4) | 35(11.9) | | |
| With auxiliary staging | 610(65.0) | 49(48.5) | 363(67.0) | 198(67.1) | | |
| Without auxiliary staging | 195(20.8) | 32(31.7) | 101(18.6) | 62(21.0) | | |
| **Margins** | | | | | 9.63 | 0.055 |
| Not detected | 294 (31.4) | 44 (43.6) | 168 (31.1) | 82 (27.8) | | |
| No residual cancer | 617 (65.8) | 56 (55.4) | 358 (66.2) | 203 (68.8) | | |
| With residual cancer | 26 (2.8) | 1 (1.0) | 15 (2.8) | 10 (3.4) | | |
| **Vascular tumor invasion** | | | | | 7.47 | 0.102 |
| Not detected | 436(46.5) | 54(53.5) | 239(44.2) | 143(48.5) | | |
| No | 403(43.0) | 35(34.7) | 238(44.0) | 130(44.1) | | |
| Yes | 98(10.5) | 12(11.9) | 64(11.8) | 22(7.5) | | |
| **Nerve infiltration** | | | | | 4.19 | 0.380 |
| Not detected | 913(97.4) | 98(97.0)) | 528(97.6) | 287(97.3) | | |
| No | 14(1.5) | 3(3.0) | 8(1.5) | 1(1.0) | | |
| Yes | 10(1.1) | 0(0.0) | 5(0.9) | 5(1.7) | | |

**Table 3.** The distribution features of ER/PR/HER2/Ki67 and molecular subtypes in different age groups

| Parameters | No. of patients (%) | | | | $X^2$ | *P*-value |
|---|---|---|---|---|---|---|
| | All patients (*N*=1,042) | 20y to 40y (*n*=115) | 41y to 60y (*n*=599) | ≥61y (*n*=328) | | |
| **ER status** | | | | | **3.293** | **0.510** |
| Positive expression | 670 (64.3) | 68 (59.1) | 384 (64.1) | 218 (66.5) | | |
| Negative expression | 216 (20.7) | 24 (20.9) | 128 (21.4) | 64 (19.5) | | |
| No detected | 156 (15.0) | 23 (20.0) | 87 (14.5) | 46 (14.0) | | |
| **PR status** | | | | | **9.411** | **0.152** |
| High expression | 456 (43.8) | 54 (47.0) | 257 (42.9) | 145 (44.2) | | |
| Low expression | 105 (10.1) | 5 (4.3) | 64 (10.7) | 36 (10.8) | | |
| Negative expression | 314 (30.1) | 30 (26.1) | 189 (31.6) | 95 (29.0) | | |
| No detected | 167 (16.0) | 26 (22.6) | 89 (14.9) | 52 (15.9) | | |
| **HER2 status** | | | | | **10.380** | **0.110** |
| Positive expression | 196 (18.8) | 17 (14.8) | 128 (21.4) | 51 (15.5) | | |
| Negative expression | 627 (60.2) | 65 (56.5) | 351 (58.6) | 211 (64.3) | | |
| No detected | 219 (21.0) | 33 (28.7) | 120 (20.0) | 66 (20.1) | | |
| **Ki67 status** | | | | | **11.302** | **0.023** |
| High expression | 653 (62.7) | 67 (58.3) | 398 (66.4) | 188 (57.3) | | |
| Low expression | 170 (16.3) | 17 (14.8) | 86 (14.4) | 67 (20.4) | | |
| No detected | 219 (21.0) | 31 (27.0) | 115 (19.2) | 73 (22.3) | | |
| **Molecular subtype** | | | | | **16.93** | **0.031** |
| Unclassified | 166 (15.9) | 25 (21.7) | 88 (14.7) | 53 (16.2) | | |
| Luminal A | 109 (10.5) | 12 (10.4) | 48 (8.0) | 49 (14.9) | | |
| Luminal B | 565 (54.2) | 54 (47.0) | 344 (57.4) | 167 (50.9) | | |
| HER2-overexpression | 85 (8.2) | 9 (7.8) | 53 (8.8) | 23 (7.0) | | |
| Basal-like | 117 (11.2) | 15 (13.0) | 66 (11.0) | 36 (11.0) | | |

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

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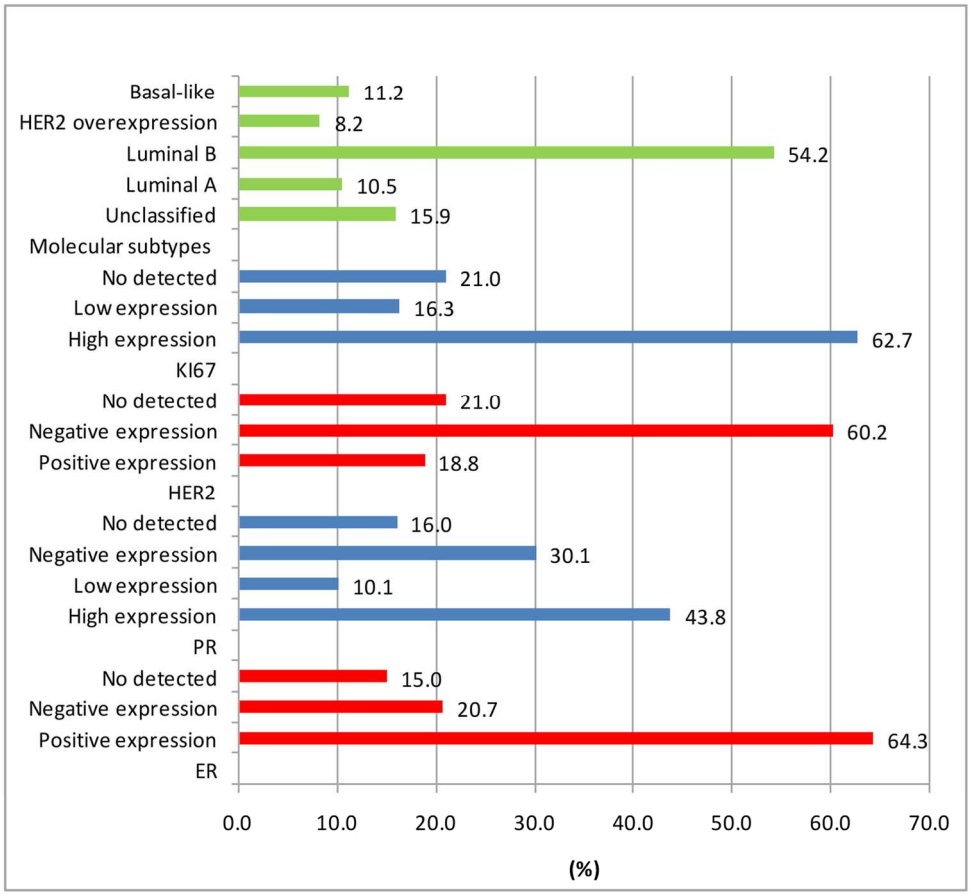


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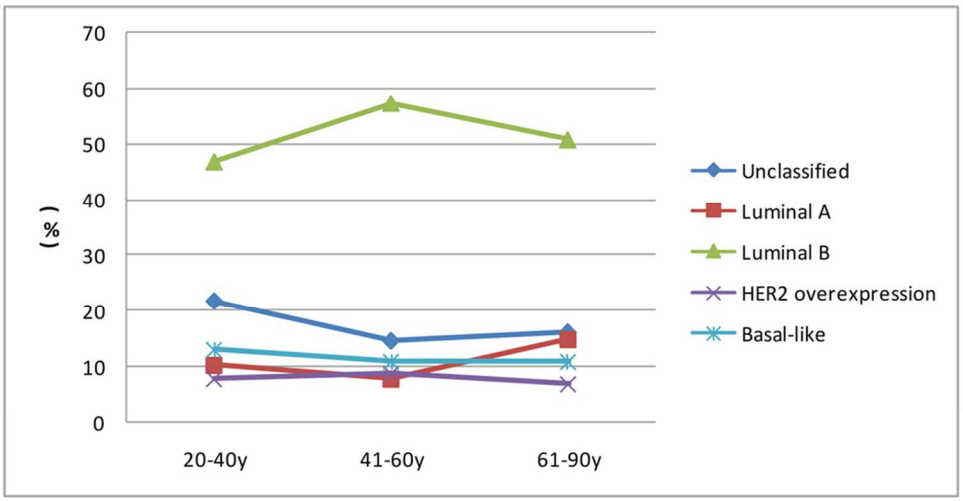


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STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract. Page1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found. Page 3 |
| **Introduction** | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported. Page 5 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses. Page 5-6 |
| **Methods** | | |
| Study design | 4 | Present key elements of study design early in the paper. Page 6 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, follow-up, and data collection. Page 6-7 |
| Participants | 6 | Clearly define all outcomes and diagnostic criteria. Page 6-7 |
| Variables | 7 | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods. Page 6-7 |
| Quantitative variables | 8 | Describe all statistical method. Page 7 |
| Statistical methods | 9 | Describe any methods used to examine subgroups and interactions. Page 7 |
| **Results** | | |
| Participants and Descriptive data | 10 | (a) Distribution feathers of pathological parameters. Page 7-8 |
| | | (b) Distribution feathers of ER/PR/HER2/Ki67 and molecular subtypes. Page 8-9 |
| Outcome data | 11 | Report numbers of outcome events or summary measures over time. Page 8-9 |
| | 12 | Distribution of recurrence risk. Page 9 |
| **Discussion** | | |
| Key results | 13 | Summarise key results with reference to study objectives. Page 9-11 |
| Limitations | 14 | Discuss limitations of the study. Page 11-12 |
| Interpretation | 15 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. Page 9-11 |
| Generalisability | 16 | Discuss the generalisability (external validity) of the study results. Page 9-11 |
| **Other information** | | |
| Funding | 17 | Give the source of funding and the role of the funders for the present study. Page 12 |

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.