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Characteristics and prognostic values of traditional pathological parameters and advanced molecular subtypes in women in Beijing with operable breast cancer: a retrospective analysis

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ABSTRACT

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

Design A retrospective study through case information enquiry or telephonic 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 characteristics and 5-year relapse rates according to the Nottingham Prognosis Index (NPI) and molecular subtypes were analysed.

Results In 1042 patients, the percentages of high histological grade, N1+N2, T2+T4 were 7.3%, 24.2%, 46.9%, respectively. 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%, respectively. The missing percentages of auxiliary staging, margins, vascular tumour invasion and nerve infiltration were 14.2%, 31.4%, 46.5% and 97.4%, respectively. The percentages of ER-positive, PR-positive, HER2-positive and Ki-67 high expression were 64.3%, 43.8%, 18.8% and 62.7%, respectively. The percentages of luminal A, luminal B, HER2-overexpression and basal-like breast cancers were 10.5%, 54.2%, 8.2% and 11.2%, respectively. Luminal A, luminal B and basal-like breast cancer subtypes were more common in the >60 years group, the 41–60 years group and the 20–40 years group, respectively. The 5-year relapse rates according to NPI were as follows: 6.2% in the low recurrence risk group, 10.4% in the moderate recurrence risk group and 12.9% in the 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 Reasonable analysis of traditional pathological parameters and advanced molecular subtypes in women with operable breast cancer in Beijing may be useful to guide precise treatment and predict prognosis.

Strengths and limitations of this study

► The characteristics of traditional pathological parameters and advanced molecular subtypes of operable breast cancer in women in Beijing were compared.
► The 5-year relapse rates according to the Nottingham Prognosis Index were reported.
► The 5-year relapse rates according to molecular subtypes were reported.
► The study was retrospective, and a perspective study is expected.
► It was conducted in a single institution, and multicentre studies are ongoing.

INTRODUCTION

Breast cancer is the most common cause of cancer death in women, with approximately 1.67 million cases diagnosed worldwide in 2012.[1] Breast cancer is a highly heterogeneous disease. Rational analysis of pathological characteristics is useful for judging the prognosis of patients with breast cancer. Traditional pathological markers including node staging,[2 3] positive margin,[4 5] vascular tumour invasion,[6] differentiation grade[3 7] and lymph vessel tumour embolus grade 3[8] have been verified as independent risk factors for the recurrence and prognosis. Oestrogen receptor (ER) and progesterone receptor (PR) have been included in routine pathological practice, and are 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 tumour, which is often used to determine the prognosis of postoperative patients with breast cancer,[12–14] although it is sometimes controversial.

In recent years, more and more research supports the detection of multiple genes (21-gene signature, 70-gene signature, TP53 mutation-correlated genes) in patients with breast cancer.[5–18] Multigene assays could subdivide patients into high-risk and low-risk cohorts thereby providing prognostic and predictive decisions. However, the cost of these multigene assays remains prohibitive for many societies, and it can't be carried out on a large scale.[19] So experts propose that molecular subtypes can be replaced by pathology parameters. In 2013, the St Gallen Consensus Conference and European Society for Medical Oncology (ESMO) Clinical Practice Guidelines recommended surrogate definitions of intrinsic subtypes of breast cancer.[20] According to the 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 multigene assays, 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 population of Beijing have not been systematically studied. Therefore, the present study was carried out to investigate traditional pathological markers and advanced molecular subtypes in women in Beijing with operable breast cancer.

MATERIALS AND METHODS

Study design
We retrospectively collected data on all patients (n=1042) with primary operable breast cancer between January 2008 and December 2012 at the Beijing Friendship Hospital. Patients with benign diseases of the breast 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 included tumour location, operation type, distance from the cutting edge, positive margins, vascular tumour invasion, nerve infiltration, histological grade (G), primary tumour (T), lymph nodes (N), histopathological type, and ER, PR, HER2 and Ki67 status. Written informed consent was obtained from all participants.

The observation end points
All the patients were followed up and 5-year relapse rates were calculated in some of the patients. The follow-up approach involved checking of hospital medical records and outpatient medical records, and contacting the patients/family members for recurrence information. All the patients with primary operable breast cancer were retrospectively collected at Beijing Friendship Hospital. Informed consent was signed by all patients.

Patient and public involvement
The patients and/or public were not involved in the study design or conduct of the study. Free clinical medical support was provided to all the patients during the follow-up process, for example, the related medical questions were answered, the patients were given guidance on the follow-up plan, and were advised on the next-step therapeutic regimen if recurrence occurred.

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

Vascular tumour invasion was assessed on H&E-stained whole sections of primary tumours. Blood/lymph vessels were identified morphologically and were carefully differentiated from breast ducts/retraction tissue. Tumour cells within vessels mostly formed clusters of various sizes. However, at least one single tumour within a vessel was scored as vascular tumour cell infiltration, if conclusive tumour cell morphology was present.

ER, PR and Ki67 statuses were determined by immunohistochemical staining. Tumours 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 hybridisation.

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

The judgement criterion for the recurrence risk
For each eligible patient, NPI was calculated using the formula NPI = (0.2×S)+N + G. In this formula, S is the tumour size in cm, N is the number of lymphatic nodes involved (>4 = 3, 4–1=2, 0=1) and G is the degree of malignancy of the tumour (degree 3=3, degree 2=2, degree 1=1). Based on the numerical score obtained from the formula, patients are placed 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 for 203 patients. The deadline of follow-up was 31 December 2016. Disease free survival (DFS) was defined as the period from the date of diagnosis to occurrence of

Table 1 Surrogate definitions of molecular subtypes of breast cancer

Molecular subtypes	Luminal A	Luminal B	HER2-overexpression	Basal-like

Histopathological surrogate definition	► ER-positive
	► HER2-negative
	► Ki67 low
	► PR high*
		HER2-negative
		► ER-positive
		► HER2-negative
		► and either
		  – Ki67 high† or
		  – PR low
		► HER2-positive
		► ER-positive
		► HER2-positive
		► any Ki67
		► any PR
			HER2-positive (non-luminal)
			► HER2-positive
			► ER and PR absent
				Triple-negative (ductal)
				► ER and PR absent
				► HER2-negative

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

any event such as progression, recurrence, metastasis or death. Only patients with invasive breast cancer were included in the prognostic analysis. All data were analysed using the SPSS Statistics software (V.13.0; Chicago, Illinois, USA). Comparisons were determined using the χ^2 test, Fisher's exact test or independent t-test. A p value <0.05 was considered statistically significant.

RESULTS
Distribution features of age
In the study, the average age of the patients was 55.56±12.37 years (range 22 to 92 years). Of these patients 115 (11.0%) were 20–40 years, 599 (57.5%) patients were 41–60 years and 328 (31.5%) patients were older than 61 years.

Distribution features of pathological parameters
In 1042 patients the percentages of high histological grade, N1+N2, T2+T4 were 7.3%, 24.2%, 46.9%, respectively. In patients with invasive breast cancer, the percentages of auxiliary staging, positive margins, vascular invasion and nerve infiltration were 20.8%, 2.8%, 10.5% and 1.1%, respectively. The missing percentages of auxiliary staging, margins, vascular tumour invasion and nerve infiltration were 14.2%, 31.4%, 46.5% and 97.4%, respectively. There were significant differences in neoadjuvant chemotherapy, auxiliary staging, tumour 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 the 41–60 years group. Auxiliary staging, T2+T4 and N1+N2 was much less in the 20–40 years group. There were no statistically significant differences in tumour location, margins, vascular tumour 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 histopathological types, 104 (10.0%) patients had ductal carcinoma in situ (DCIS) and 938 (90.0%) patients had invasive carcinoma. There were no significant differences in histopathological types in patients among the three age groups (20–40 years, 41–60 years and ≥61 years).

Distribution features of ER/PR/HER2/Ki67 and molecular subtypes
Of 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 tumours, 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 had luminal A, 565 (54.2%) patients had luminal B, 85 (8.2%) patients had HER2-overexpression and 117 (11.2%) patients has basal-like (table 3, figure 1) molecular subtype. There was a statistically significant difference in the 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 the >60 years age group, luminal B was more common in the 41–60 years age group and basal-like was more common in the 20–40 years age group (figure 2).

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

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

| Pathological parameters	No. of patients (%)				X^2	P values
	All patients (n=1042)	20 –40 years (n=115)	41–60 years (n=599)	≥61 years (n=328)		
In situ and invasive breast cancer						
Tumour 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 auxillary staging	610 (65.0)	49 (48.5)	363 (67.0)	198 (67.1)		
Without auxillary 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 tumour 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)		

follows: 6.2% in the low recurrence risk group, 10.4% in the 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 tumour invasion, high histological grade and lymph node staging have been verified as independent risk factors for recurrence and as markers of prognosis.[2–7] Tumour size has been demonstrated to be closely related to relapse-free survivals.[26] Sarsenov et al reported that younger age (<40 years), large tumour size (>2 cm), high grade and 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 tumour invasion, high histological
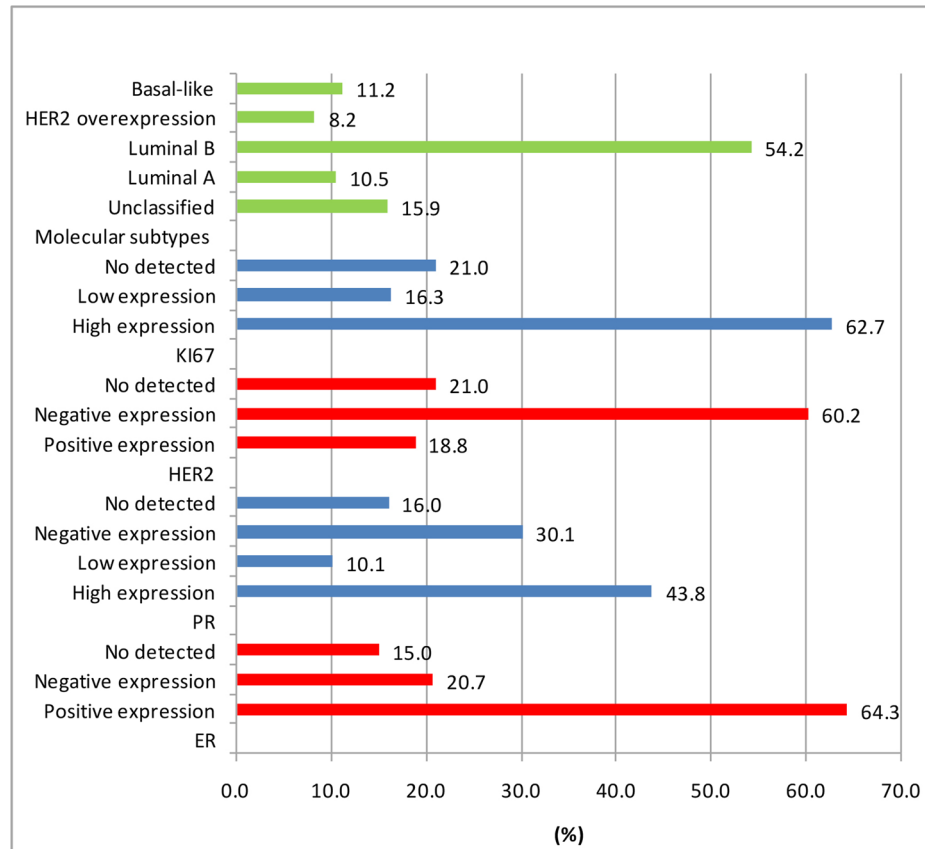
Figure 1 The overall distribution features of ER/PR/HER2/Ki67 and molecular subtypes in all patients. ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

grade, N1+N2 and T2+T4 were 2.8%, 10.5%, 7.3%, 24.2% and 46.9%, respectively. These indicators reflect the percentages of patients with poor prognosis from different perspectives. In our study, the missing percentages of positive margins, vascular tumour invasion, nerve infiltration and grade were up to 31.4%, 46.51%, 97.4% and 38.3%, respectively. The missing data are at random. Accurate analysis and diagnosis of preoperative staging, standardised surgical operation, standardised 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 raise the strict demands to the surgeons, physicians and pathologists.

DCIS and invasive ductal cancer were the two main histopathological types of breast cancer in patients in Beijing. Julian et al'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 patients with DCIS should receive auxiliary staging is a question worthy of discussion. Although patients with DCIS have a favourable prognosis, recurrence risk was increased in high-grade DCIS (OR, 4.39).[29] The DCIS score (12-gene) assay provides clinically relevant information on recurrence risk and may facilitate decision making by clinicians.[30] The percentage of invasive ductal cancers was 90.0% in the entire patient sample, and Hasebe et al's

study showed that type 2 invasive ductal cancer was one of the best factors for accurately predicting locoregional recurrence.[8]

ER, PR, Ki67 and HER2 have been routinely applied in clinical practice. ER and PR are associated with good response to hormonal therapy and better clinical outcomes. In our study, the 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 in 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] Borderline distribution of Ki67 indicated significantly more distant bone and liver metastases and worse disease-specific survival.[38] In patients with complete data, 23.8% were HER2-positive, which is similar to 25.5% reported by Zhu et al.[33] HER2 overexpression is directly proportional to 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
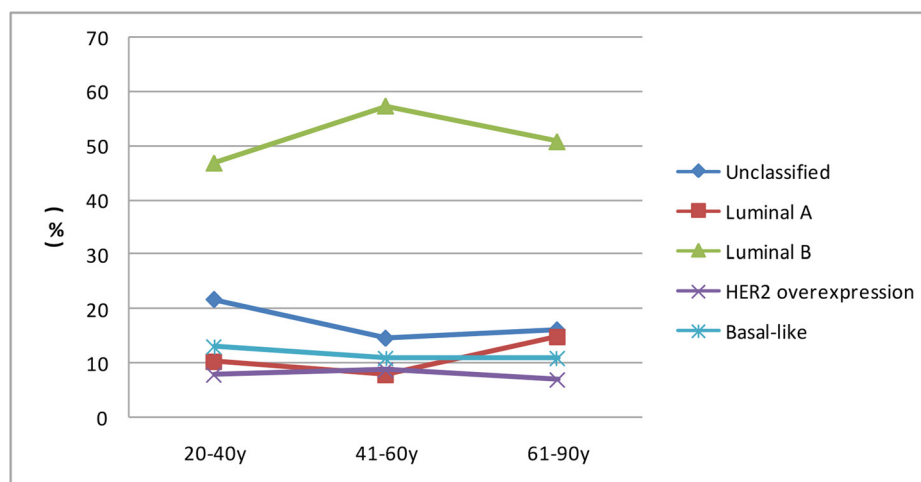
Table 3 The distribution features of ER/PR/HER2/Ki67 and molecular subtypes in the different age groups

Parameters	No. of patients (%)				X²	P values
	All patients (n=1042)	20–40 years (n=115)	41–60 years (n=599)	≥61 years (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)		
Not 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)		
Not 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)		
Not 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)		
Not 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)		

ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

subtypes were more common in the 41–60 years age group. The distribution of molecular subtypes in our study is consistent with that reported by Si *et al*.[43] Molecular subtypes, as advanced pathological 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 those with HER2-overexpression and basal-like subtypes, as indicated by the relatively

Figure 2 The distribution features of molecular subtypes in the different age groups. HER2, human epidermal growth factor receptor 2.

low rates of local and regional relapse.[39] The luminal A subtype is very sensitive to endocrine therapy, the luminal B (HER2−) subtype benefits from endocrine therapy or chemotherapy, the luminal B (HER2+) subtype benefits from endocrine therapy or chemotherapy combined with anti-HER2-targeted therapy[43 44] and the 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.

NPI is usually used to determine the prognosis of postoperative patients with breast cancer. NPI was calculated using tumour size, positive lymphatic nodes and grade. In our study, the 5-year relapse rates increased with the rise of NPI, suggesting significance of traditional pathological parameters in prognosis. 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 et al.[46] However, Arvold et al showed that the 5-year cumulative incidence of local relapse was 0.8% in patients with luminal A, 4.4% in those with luminal B, 10.8% in those with HER2-overexpression and 6.7% in those with basal-like subtypes,[47] and the patients with the HER2-overexpression subtype had the worst prognosis. Both the evaluated methods are able to predict recurrence risk and prognosis, however, the latter shows its unique advantages in guiding specific treatment schemes.

In conclusion, our study has shown the features of traditional pathological parameters and advanced molecular subtypes in women in Beijing with operable breast cancer. In-depth understanding of the biological behaviour 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. At present, we are carrying out a study on molecular subtypes and recurrence risk in a larger population in China, and the results are awaited.

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