Establish a Prediction Model for the Recurrence Time of Hormone Receptor Positive /human Epidermal Growth Factor Receptor 2-negative Breast Cancer

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Abstract

Objectives

This study aimed to develop a model to predict recurrence time of hormone receptor-positive (HR+) /human epidermal growth factor receptor 2 negative (HER2-) breast cancer patients.

Methods

We included HR+ HER2- metastatic breast cancer (MBC) female patients who arrived at the Department of Breast Oncology in Peking University Cancer Hospital. Data were collected through consulting medical records. Patients were divided into early recurrence group and late recurrence group according to disease-free survival (DFS). Predictors of recurrence time were identified and a nomogram was developed and validated using concordance index (C-index), AUC (area under the curve), and calibration plots.

Results

A total of 639 patients coming to the hospital between April 2007 and October 2019 were included. Median DFS was 50.0 months. 382 patients (59.8%) were presented with early recurrence (DFS $\leq$ 5 years), and 257 patients (40.2%) were presented with late recurrence (DFS >5 years). A nomogram based on potentially associated clinicopathological factors was developed and validation results showed the nomogram was well-calibrated to predict recurrence time (AUC=0.703, C-index=0.697).

Conclusions

We have established a well-calibrated nomogram through 639 patients. HR+/HER2- BC patients with premenopausal status at initial diagnosis, less positive lymph nodes, lower level of Ki-67 were more common in late recurrence. The nomogram can predict the risk of late recurrence. Prospectively designed studies are needed to further validate our model.

Introduction

Breast cancer increased rapidly in China, the paper published in "Chinese journal of cancer research" showed that the age-standardized incidence rate was 36.1/100,000 in 2018.⁽¹⁾ Approximately 30 percent of early breast cancer (BC) would appear recurrence^{(2, (3))}. Clinicopathological characteristics such as hormone receptor (HR) status and number of metastatic lymph nodes, are used to predict recurrence risk as well as recurrence time. Among those characteristics, HR status is widely thought to be able to stratify early recurrence ($\leq$ 5 years) and late recurrence ($>$ 5 years)^{(4, (5))}. For HR negative BC, recurrence rate reaches the highest level during the first two years after initial diagnosis, and drops rapidly to a low level afterwards. Most of the recurrence happens during the five years after initial diagnosis. However, for HR positive (HR+) BC, about 50 percent of recurrence happens after five-year adjuvant

endocrine therapy^{(6, (7), (8)}, and the recurrence risk tends to be stable at a low level after at least twenty years^{(3), (9)}.

Estrogen receptor (ER), progesterone receptor (PR) or both are found in about 70 percent of all BC, and such tumors are considered HR+ BC^{(10), (11)}. Adjuvant therapy of early BC is important in improving prognosis, and adjuvant chemotherapy has been proved to significantly reduce recurrence rate of the first five years after diagnosis^{(12), (13)}. However, HR+/ human epidermal growth factor receptor 2 - negative (HER2-) BC patients still suffer from high risk of late recurrence^{(14), (15)}. Previous studies have shown that five-year endocrine therapy could reduce a third of late recurrence (15-year recurrence)⁽¹⁶⁾, and extending the time of adjuvant endocrine therapy to 10 years could reduce risk of late recurrence in the second decade after initial diagnosis^{(17), (18), (19), (20), (21)}. Therefore, reliable prediction of recurrence time for HR+/ HER2- BC patients is necessary for appropriate treatment.

In foreign studies, researchers have explored the factors affecting recurrence time in HR+/ HER2- BC patients. For example, Yamashita et al found larger tumor size, more number of positive lymph nodes and higher histological grade were associated with early recurrence. After stratified by age or hormonal status, besides the factor mentioned before, PR status and Ki-67 expression level were associated with recurrence time as well⁽¹⁸⁾. Due to heterogeneity, prognosis of BC is influenced by race, so study results abroad may not apply to Chinese. Therefore, this study is aimed to explore factors predicting recurrence time and construct a predictive model, providing reference for treatment of patients with HR+ /HER2- BC in China.

Methods

Study population and design

Patients diagnosed with histologically confirmed metastatic recurrence of HR+/HER2- BC who arrived at the Department of Breast Oncology in Peking University Cancer Hospital between April 2007 and October 2019 were included. Patients who were stage $\geq$ at initial diagnosis or had other malignant tumors were excluded. The primary outcome was disease-free survival (DFS), which was defined as the time from initial diagnosis to first recurrence. Patients were stratified into two groups-early recurrence group and late recurrence group, according to DFS. DFS >5 years was defined as late recurrence and DFS $\leq$ 5 years was defined as early recurrence. Metastatic sites were identified by imaging examinations or rebiopsy. Multiple metastasis was defined as metastatic lesions involved more than one organ. Through consulting medical records, data about clinicopathological factors, adjuvant therapies and metastatic characteristics were collected. This study was conformed to guidelines of the 1996 Declaration of Helsinki and approved by the Ethics Committee of Peking University Cancer Hospital (No.2017KT40).

IHC evaluation

Expression of ER, PR, Ki-67 was evaluated by immunohistochemistry (IHC). HR was considered positive if there was $\geq 1\%$ positive nuclear staining for either ER and/or PR. HER2 was considered positive as IHC score of 3+, or 2+ with fluorescent in situ hybridization showing gene amplification. HER2-positive patients were excluded from this study. According to expression level of ER and PR, HR was stratified into four groups. Group 1 was defined as 1+ or $\leq 25\%$. Group 2 was defined as 2+ or 25%~50%. Group 3 was defined as 3+ or 50%~75%. Group 4 was defined as $\geq 75\%$. Similarly, Ki-67 was stratified into four groups according to the expression level as well.

Statistical methods

Statistics analysis was performed using SPSS 23.0 and R 4.0.0 software. DFS was evaluated using the Kaplan-Meier log-rank test. Chi-square test was used to compare clinicopathological characteristics between early and late recurrence group. Multivariate analysis was performed using logistic regression model to identify independent factors influencing recurrence time. On the basis of potentially associated prognostic factors, a nomogram was constructed. The discrimination of the nomogram was evaluated by receiver operating characteristic (ROC) curve and Concordance-index (C-index). Calibration was performed by bootstrapping with 1000 resamples. *P*-value < 0.05 was considered as statistically significant.

Results

Patient characteristics

A total of 639 female patients who presented a metastatic recurrence of BC were included in the study. Most patients (91.1%) were diagnosed after 2000/1/1. A description of patients in the study was given in Table 1. Median age at initial diagnosis of primary tumor was 47 years old with 263 patients (41.2%) aged ≥ 50 years old. A majority of patients (61.0%) were premenopausal at initial diagnosis. Most patients (528/639, 83.0%) received adjuvant therapy after surgery, while (108/639, 17.0%) of them received neoadjuvant therapy before surgery. All patients received surgery and most of the surgery (93.6%) was mastectomy including radical and simple mastectomy. 40 patients (6.4%) had breast conserving surgery. Most common type of primary tumor (513/539, 84.1%) was invasive ductal carcinoma. Most of the patients received adjuvant/neoadjuvant chemotherapy (591/639, 92.5%) and endocrine therapy (505/639, 79.0%), while 273 patients (273/639, 42.7%) received adjuvant radiotherapy. 382 patients (59.8%) were presented with early recurrence, and 257 patients (40.2%) were presented with late recurrence. Most common site of first metastasis was bone (297/639, 46.5%). Second common site was lymph node (253/639, 39.6%). Lung and liver takes 24.7% (158/639) and 16.1% (103/639) of all metastatic sites respectively. Brain metastasis took 1.9% (12/639) of all (Figure 1). Median DFS (Disease-free survival) was 50.0 months (range 3.0-324.0 months). Proportion of recurrence over time is depicted in Figure 2. Approximately half of the patients (335/639, 52.4%) appeared recurrence at the fourth year. During the follow-up of first five-year, number of patients who appearing recurrence grew rapidly. At the

fifth year of follow-up, 59.8% (382/639) of patients appeared relapse. Most (557/639, 87.2%) metastasis took place within ten years of follow-up.

Univariate analysis

Univariate survival analysis for DFS showed the following parameters as significant factors (Figure 3): age at diagnosis, hormonal status at diagnosis, treatment mode, histology of primary tumor, Scarff-Bloom-Richardson (SBR) grade, Ki-67, number of positive lymph nodes. Older age (≥ 50), postmenopausal, higher SBR grade, higher level of Ki-67, more positive lymph nodes were associated with shorter DFS. In addition, patients receiving neoadjuvant therapy (most of those patients was presented as later stage) relapsed earlier than not receiving therapy before surgery ($P=0.003$) in this study. Level of hormonal receptor and tumor size did not show significant impact on recurrence time. Even if tumor size did not show impact on DFS ($P=0.157$), patients diagnosed with larger tumor size, tended to relapse earlier. Some variables (neoadjuvant/adjuvant chemo-/radio-therapy) need to be described taking possible confusing factors into account to understand their impact. Neoadjuvant/adjuvant chemo-/radio-therapy seems to have no impact on recurrence time. Patients treated with chemotherapy or radiotherapy had more positive lymph nodes ($P<0.001$ for both) than patients not treated with this type of treatment. Metastasis did not appear later in this group of patients even if they received treatment to delay recurrence.

Results comparing early recurrence and late recurrence group were shown in Table 2 and Figure 4. Patients with early recurrence took 59.8% (382/639) of all patients, which was more than patients with late recurrence (257/639). Compared with late recurrence, there were more patients who were postmenopausal at initial diagnosis (42.6% vs 33.6%), received neoadjuvant therapy (20.6% vs 11.7%), had higher level of Ki-67, had more positive lymph nodes, did not receive radiotherapy (46.6% vs 37.0%) in early recurrent patients. There was no significant difference in proportion of patients receiving chemotherapy between two groups ($P=0.480$), which was probably due to more positive lymph nodes in patients receiving chemotherapy as was mentioned before. Referring to first metastatic sites (Table 3), bone was most common in both groups. There were more liver metastasis (19.4% vs 11.3%, $P=0.006$), chest/skin/soft tissue metastasis (28.8% vs 21.4%, $P=0.036$) in patients with early recurrence than late recurrence, while there were more pleura metastasis (5.0% vs 12.8%, $P<0.001$) in patients with late recurrence than with early recurrence. There was no significant difference in bone metastasis, lymph node metastasis, lung metastasis and brain metastasis between two groups, and so was the number of first metastatic site.

Multivariate analysis

Referring to results of univariate analysis and clinical experience, logistic regression analysis was performed. Age at initial diagnosis, hormonal status at initial diagnosis, treatment mode, type of surgery, expression level of Ki-67, N stage, neo-/adjuvant chemotherapy, neo-/adjuvant endocrine therapy and adjuvant radiotherapy were included in logistic model, and the results were shown in Table 4. Postmenopausal status, higher expression of Ki-67, more positive lymph nodes was more common in

patients with early recurrence than late recurrence ($P=0.046, 0.003, 0.021$ respectively). Age of menopause in Chinese female is usually around 50 years old. To clarify whether there was interaction between age and hormonal status, interaction analysis was performed. The result indicated that there was no synergistic effect on recurrence time when combining age and hormonal status ($P=0.662$).

Construction and validation of the predictive model

Logistic regression-based nomogram was constructed on the basis of potentially associated factors. Only patients with complete data of associated factors could be included to develop the nomogram. Because many patients lacked data on Ki-67 (Table 1), the factors mentioned before except expression level of Ki-67 were adopted to construct Nomogram 1 at first (Figure 5). There were 563 patients in the development setting. C-index for Nomogram 1 was 0.637, and AUC was 0.635 (95% CI: 0.589-0.681). Internal validation was performed using bootstrap resampling method, and calibration curve was depicted in Figure 6. To optimize the model, considering the important role of Ki-67 in recurrence, Nomogram 2 was constructed with expression level of Ki-67 adopted at last (Figure 7). There were 357 patients in the development setting of Nomogram 2. C-index for Nomogram 2 was 0.697, and AUC was 0.703 (95% CI: 0.643-0.763) (Figure 8). Calibration curve was depicted in Figure 9. The results in calibration curve showed absolute error and squared error were smaller in Nomogram 2 than that in Nomogram 1, and the calibration curve in Nomogram 2 showed the prediction (solid line) was more closely approximates the 45-degree line than that in Nomogram 1. Moreover, the ROC curve showed Nomogram 2 exhibited better discrimination to predict the probability of late recurrence than Nomogram 1. From the nomogram, probability of late recurrence of every patients could be easily obtained through calculating the total scores of clinicopathological factors.

Discussion

Late recurrence is not only observed in HR+ BC, but also frequently found in many other solid tumors, such as thyroid, prostate cancer and melanoma. The mechanism of late recurrence is poorly understood yet. Some researchers think this is partly due to change of immune microenvironment in primary tumor and tumor dormancy^{(22, (23, (24, (25, (26, (27)}. Although late recurrence remains a tough problem for HR+ BC patients, more and more evidence shows that extending time of endocrine therapy could reduce the risk of late recurrence⁽⁹⁾. Therefore, identifying patients at high risk of late recurrence and proposing a more appropriate treatment plan for them become a critical task. In this study, we found the features of late recurrence and some possible factors that might influence the recurrence time in HR+/HER2- patients, and constructed a model to predict the risk of late recurrence.

It is controversial whether hormonal status has impact on recurrence risk and recurrence time. Ditsatham et al found patients in premenopausal status had higher risk of recurrence than those in postmenopausal status⁽²⁸⁾. In other studies, there was no difference in DFS between patients with late recurrence in premenopausal and postmenopausal status⁽²⁹⁾, and there was no difference in hormonal status between patients with early and late recurrence⁽³⁰⁾. In this study, hormonal status was an important factor

affecting recurrence time in BC patients both in univariate and multivariate analysis. Patients in premenopausal status appeared relapse later than postmenopausal patients (OR=0.477, $P=0.046$). This phenomenon is probably due to comorbid condition, tumor burden and immune microenvironment⁽²⁴⁾. As postmenopausal patients are older, the possibilities of having serious comorbid conditions are higher than younger premenopausal patients. And there might be a big difference in immune microenvironment between premenopausal and postmenopausal patients as well. As a result, although premenopausal patients has higher level of estrogen, they might appear recurrence later than postmenopausal patients. Thus the time of endocrine therapy for premenopausal patients should be extended.

In previous studies, some researchers found lymph node status could provide predictive information on late recurrence of BC^{(31, (32, (33)}. Risk of late recurrence especially distant recurrence increased when there were positive lymph nodes⁽³⁴⁾. Some results revealed risk of late recurrence increased significantly when there were more than three positive lymph nodes^{(29, (35)}. In this study, >3 positive lymph nodes was more frequently seen in patients with early recurrence than late recurrence. Meanwhile, even if there were less than 3 positive lymph nodes, quite a number of (67.6%) patients appeared late recurrence. The results of this study testifies the influence of positive lymph nodes on recurrence time. This indicates that in future clinical work, patients with more than 3 positive lymph nodes should receive adjuvant radiotherapy and extended time of endocrine therapy to delay recurrence. On the other hand, for patients with small number of or without positive lymph nodes, the risk of late recurrence may not be that low, and other factors should be taken into consideration.

In biological studies, Ki-67 is supposed to be an important prognostic factor. Ki-67 is a monoclonal antibody, existing in all cell cycles except G0. It is a marker of cell proliferation, reflecting the aggressiveness of tumor cells. It is still under debated whether Ki-67 is an independent prognostic factor. Some studies reported its role in predicting prognosis of BC^{(36, (37, (38, (39)}, while other studies did not⁽⁴⁰⁾. In a research on late recurrence of BC, high level of Ki-67 was associated with short DFS in univariate analysis, while only histological grade and lymph node metastasis were independent prognostic factors in multiple analysis⁽²⁹⁾. In this study, there was significant statistical difference in the level of Ki-67 between early and late recurrence group, and high level of Ki-67 was more frequently seen in patients with early recurrence ($P=0.005$). After excluding potential influence of other factors in multivariate analysis, Ki-67 was an independent factor affecting recurrence time of BC.

Influence of treatment mode on recurrence time was rarely discussed in previous studies. Akrami et al found chemotherapy before surgery was not related to recurrence time⁽⁴¹⁾. In this study, treatment mode, including neoadjuvant therapy and adjuvant therapy, was defined according to whether patients received treatment before surgery. Patients received neoadjuvant therapy to reduce tumor for surgery, and these patients were usually in poor condition at initial diagnosis. Our data testified this condition with the result that neoadjuvant therapy was more frequently seen in patients with early recurrence in univariate analysis (20.6% vs 11.7%, $P=0.003$). Patients with later disease stage are more likely to undergo neoadjuvant therapy, which is why earlier recurrence occurred more in patients receiving neoadjuvant

therapy. This phenomenon reflects the fact that characteristics of primary tumor greatly affect recurrence time, and even if patients receive treatment before surgery, the recurrence could not be delayed.

Akrami et al found adjuvant radiotherapy is a predictive factor of late recurrence⁽⁴¹⁾. In another study, recurrence time was not associated with adjuvant radiotherapy⁽³⁰⁾. In univariate analysis of this study, adjuvant radiotherapy was more frequently seen in late recurrence group than in early recurrence group (63.0% vs 53.4%). However, in multivariate analysis, there was no significant difference in radiotherapy between the two groups ($P=0.719$). Because adjuvant radiotherapy is related to some clinical factors, such as breast-conserving surgery and positive lymph nodes. We further analyzed the relationship between type of surgery, lymph node metastasis and adjuvant radiotherapy. Adjuvant radiotherapy was not associated with type of surgery ($P=0.126$), but was associated with positive lymph nodes ($P<0.001$). Patients treated with adjuvant radiotherapy had more positive lymph nodes. Recurrence was not delayed even if they receive radiotherapy.

It has been demonstrated that breast tumor molecular subtypes are associated with site of metastasis. Bone is the most common metastatic site in HR+ BC^{(42, (43, (44, (45)}. This phenomenon is probably due to the important role of bone marrow in hematopoiesis. Dormant BC micrometastasis reside in specific bone marrow niches could transit from bone to circulation, thus causing metastasis and proliferation for tumor cells^{(46, (47)}. In this study, no matter in early recurrence or late recurrence group, bone was the most common first metastatic site (44.0% and 50.2% respectively). On the other hand, liver and chest/skin/soft tissue metastasis were more frequently seen in early recurrence group (19.4% vs 11.3%, 28.8% vs 21.4%). Pleura metastasis was more frequently seen in late recurrence group (12.8% vs 5.0%, $P<0.001$). There was no difference in lung metastasis between the two groups ($P=0.405$). In some other studies, the relationship between recurrence time and metastatic site was a little different. For example, in a Japanese study, lung metastasis was more common in HR+/HER2- patients with late recurrence⁽⁴⁸⁾. In another Japanese study, compared with early recurrence within 10 years, patients with late recurrence had less chest and liver metastasis and more lung and pleura metastasis⁽³²⁾. Metastatic site is not only associated with primary tumor, but also has relationship with microenvironment and organ characteristics.

According to the results of univariate and multivariate analysis, we constructed a nomogram using R software and optimize it for better prediction value with a higher AUC value of 0.703 and a better calibration curve. Age, hormonal status, positive lymph nodes, Ki-67, treatment mode, type of surgery, radiotherapy, chemotherapy and endocrine therapy were included in this model. Doctors could easily identify patients with higher risk of late recurrence with the help of this model and give them more intensive treatment.

This study has some advantages. First, the sample size was large relatively thus ensuring reliability of the results. Second, this study included patients diagnosed in recent 20 years with a relatively long follow-up time and made a comprehensive review of the medical records allowing to clarify clinicopathological factors influencing recurrence time. Meanwhile, this study proposed a visual model helping better

understand and calculate risk of late recurrence. This study has a few limitations as well. For example, this is a retrospective research with selection bias and incomplete data. Histological grade, an important prognostic factor, was not available in nearly half of the patients. The result on histological grade should be interpreted more carefully. Furthermore, we developed and validated a nomogram using data from the same center. We still need another population from different center to externally validate this nomogram.

Currently, more and more researches are focused on the role of genes and biomarkers in predicting late recurrence risk besides clinicopathological factors⁽⁴⁹⁾, such as Oncotype DX, MammaPrint, BC Index and circulating tumor cell, which has the potential to optimize the candidates for extended endocrine therapy^{(31, (50, (51, (52, (53, (54, (55, (56, (57, (58)}. Combination of clinicopathological factors, genes and biomarkers may add significant prognostic information for late recurrence of BC, and provide more useful reference for future work.

Conclusion

Our study showed that HR+/HER2- BC patients with premenopausal status at initial diagnosis, less positive lymph nodes, lower level of Ki-67 were more common in late recurrence. We developed a well-calibrated nomogram predicting recurrence time of HR+/HER2- BC patients. The nomogram can predict the risk of late recurrence. Prospectively designed studies are needed to further validate our model.

Declarations

Ethics approval and consent to participate This study was conformed to guidelines of the 1996 Declaration of Helsinki and approved by the Ethics Committee of Peking University Cancer Hospital (No.2017KT40).

Consent for publication Not applicable.

Availability of data and materials Data in this article is available upon request.

Competing Interest The authors declare that they have no competing interests.

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Authors' contributions Huiping Li and Mengyu Hu contribute to conception and design of the study. Mengyu Hu and Huajie Xing analyzed and interpreted the patient data. Mengyu Hu was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Patient characteristics (n=639)

Characteristics	Number of patients	%	(No. of data available, %)
Age at diagnosis			
Median(range)	47(24,80)	-	639,100%
<50 years old	376	58.8	
≥50 years old	263	41.2	
Diagnosis period			639,100%
>1985/1/1 and <2000/1/1	57	8.9	
≥2000/1/1 and ≤2011/1/1	320	50.1	
≥2011/1/1 and ≤2020/1/1	262	41.0	
Hormonal status			620,97.0%
Premenopausal	378	61.0	
Postmenopausal	242	39.0	
Unknown	19	-	
Body mass index			626,98.0%
Median(range)	24.7(15.6,44.7)	-	
Underweight	17	2.7	
Normal	253	40.4	
Overweight	250	39.9	
Obese	106	16.9	
Unknown	13	-	
ECOG grade			609,95.3%
0	468	76.8	
1	107	17.6	
2	22	3.6	
≥3	12	2.0	
Unknown	30	-	
Treatment mode			636,99.5%
Adjuvant therapy	528	83.0	
Neoadjuvant therapy	108	17.0	
Unknown	3	-	

Type of surgery		622,97.3%
Mastectomy	582	93.6
Breast conserving surgery	40	6.4
Unknown	17	-
Histology		610,95.5%
Invasive ductal	513	84.1
Invasive lobular	44	7.2
Other (mucoid, simple carcinoma, etc)	53	8.7
Unknown	29	-
Histological SBR grade		339,53.1%
G1	12	3.5
G2	244	72.0
G3	83	24.5
Unknown	300	-
Hormonal receptor category		576,90.1%
1+ or 25%	152	26.4
2+ or 25%~50%	96	16.7
3+ or 50%~75%	155	26.9
≥75%	173	23.0
Unknown	63	-
Ki-67 category		387,60.4%
1+ or <25%	181	46.8
2+ or 25%~50%	134	34.6
3+ or 50%~75%	54	14.0
≥75%	18	4.7
Unknown	252	-
Tumor size		516,80.8%
≤2cm	178	34.5
2~5cm	279	54.1
≥5cm	42	8.1

T4	17	3.3	
Unknown	123	-	
Lymph node			587,91.9%
0	173	29.5	
1~3	180	30.7	
4~9	124	21.1	
≥10	110	18.7	
Unknown	52	-	
Adjuvant/neoadjuvant chemotherapy			639,100%
Yes	591	92.5	
No	48	7.5	
Adjuvant/neoadjuvant endocrine therapy			639,100%
Yes	505	79.0	
No	134	21.0	
Adjuvant radiotherapy			639,100%
Yes	273	42.7	
No	366	57.3	
Recurrence time			639,100%
Early recurrence (DFS ≤5 years)	382	59.8	
Late recurrence (DFS >5 years)	257	40.2	
First metastatic sites			639,100%
Bone	297	46.5	
Lymph nodes	253	39.6	
Chest wall and skin	165	25.8	
Lung	158	24.7	
Liver	103	16.1	
Pleura	52	8.1	
Brain	12	1.9	
Other	71	11.1	
Disease-free survival (months)			639,100%

Median (range)	50.0(3.0,324.0)	-
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ECOG, Eastern Cooperative Oncology Group; SBR, Scarff Bloom Richardson.

Table 2. Univariate analysis

Variable	Early recurrence: DFS ≤5 years (N=382)	Late recurrence: DFS >5 years (N=257)	<i>P</i> value
Age at diagnosis			0.150
<50 years	216 56.5%	160 62.3%	
≥50 years	166 43.5%	97 37.7%	
Hormonal status			0.024
Premenopausal	214 57.4%	164 66.4%	
Postmenopausal	159 42.6%	83 33.6%	
Unknown	9	10	
Body mass index			0.165
Underweight	6 1.6%	11 4.4%	
Normal	152 40.3%	101 40.6%	
Overweight	151 40.1%	99 39.8%	
Obese	68 18.0%	38 15.3%	
Unknown	5	8	
ECOG (performance status)			0.128
0	276 75.2%	192 79.3%	
1	64 17.4%	43 17.8%	
2	18 4.9%	4 1.7%	
≥3	9 2.5%	3 1.2%	
Unknown	15	15	
Treatment mode			0.003
Adjuvant therapy	301 79.4%	227 88.3%	
Neoadjuvant therapy	78 20.6%	30 11.7%	
Unknown	3	0	
Type of surgery			0.168
Mastectomy	343 92.5%	239 95.2%	
Breast conserving surgery	28 7.5%	12 4.8%	
Unknown	11	6	
Histology			0.107

Invasive ductal	32486.4%	18980.4%	
Invasive lobular	256.7%	198.1%	
Other	266.9%	2711.5%	
Unknown	7	22	
Histological SBR grade			0.170
G1	62.5%	66.2%	
G2	17371.5%	7173.2%	
G3	6326.0%	2020.6%	
Unknown	140	160	
Hormonal receptor category			0.127
1+ or <25%	8724.2%	6530.1%	
2+ or 25%~50%	5715.8%	3918.1%	
3+ or 50%~75%	9626.7%	5927.3%	
≥75%	12033.3%	539.2%	
Unknown	22	41	
Ki-67 category			0.005
1+ or <25%	11941.9%	6260.2%	
2+ or 25%~50%	10336.3%	3130.1%	
3+ or 50%~75%	4515.8%	98.7%	
≥75%	176.0%	11.0%	
Unknown	98	154	
Tumor size			0.337
≤2cm	10833.2%	7036.6%	
2~5cm	18155.7%	9851.3%	
>5cm	237.1%	199.9%	
T4	134.0%	42.1%	
Unknown	57	66	
Lymph node			0.003
0	9025.1%	8336.2%	
1~3	10830.2%	7231.4%	

4~9	7922.1%	4519.7%	
≥10	8122.6%	2912.7%	
Unknown	24	28	
Neo-/adjuvant chemotherapy			0.480
Yes	318.1%	176.6%	
No	35191.9%	24093.4%	
Neo-/adjuvant endocrine therapy			0.172
Yes	8722.8%	4718.3%	
No	29577.2%	21081.7%	
Adjuvant radiotherapy			0.016
Yes	20453.4%	16263.0%	
No	17846.6%	9537.0%	

ECOG, Eastern Cooperative Oncology Group; SBR, Scarff Bloom Richardson; CI, confidence interval; HR, hazard ratio

Table 3. First metastatic sites of the patients

Variable	Early recurrence DFS ≤5 years (N=382)	Late recurrence DFS >5 years (N=257)	<i>P</i> value
First metastatic site*			
Bone	16844.0%	12950.2%	0.122
Lymph node	14838.7%	10540.9%	0.592
Chest/skin/soft tissue	11028.8%	5521.4%	0.036
Lung	9023.6%	6826.5%	0.405
Liver	7419.4%	2911.3%	0.006
Brain	92.4%	31.2%	0.278
Pleura	195.0%	3312.8%	0.001
Number of first metastatic sites			0.222
Single	21255.5%	13050.6%	
Multiple	17044.5%	12749.4%	

*There might be more than one site.

Table 4. Multivariate logistic regression analysis

variable	OR	95% CI	<i>P</i> value
Age at diagnosis			
<50 years	1	1	
≥50 years	1.769	0.874-3.581	0.113
Hormonal status			
Premenopausal	1	1	
Postmenopausal	0.477	0.230-0.987	0.046
Treatment mode			
Adjuvant therapy	1	1	
Neoadjuvant therapy	0.639	0.330-1.237	0.184
Type of surgery			
Mastectomy	1	1	
Breast conserving surgery	0.348	0.093-1.298	0.116
Ki-67 category			0.003
1+ or <25%	1	1	
2+ or 25%~50%	0.483	0.278-0.840	0.010
3+ or 50%~75%	0.343	0.145-0.809	0.015
≥75%	0.077	0.009-0.617	0.016
Lymph node			0.021
0	1	1	
1~3	0.742	0.384-1.434	0.375
4~9	0.479	0.216-1.063	0.070
≥10	0.235	0.091-0.607	0.003
Adjuvant radiotherapy			
Yes	1	1	
No	0.892	0.479-1.663	0.719
Neo-/adjuvant chemotherapy			
Yes	1	1	
No	0.546	0.181-1.652	0.284
Neo-/adjuvant endocrine therapy			

Yes	1	1	
No	0.601	0.279-1.295	0.194

OR <1 suggests there was more risk in early recurrence (DFS ≤5 years) than late recurrence (DFS >5 years). Otherwise there was less risk in early recurrence than late recurrence.

Figures

Figure 1

First metastatic sites of patients. Most common site of first metastasis was bone. Second common site was lymph node. There were more live metastasis (19.4% vs 11.3%, P=0.006), chest/skin/soft tissue metastasis (28.8% vs 21.4%, P=0.036) in patients with early recurrence than late recurrence, while there were more pleura metastasis (5.0% vs 12.8%, P<0.001) in patients with late recurrence than with early recurrence.

Figure 2

Proportion of recurrence over time of all patients. Half of the patients appeared recurrence at the fourth year. At the fifth year of follow-up, 59.8% of patients appeared replase. 87.2% of all recurrence took place in ten years of follow-up.

Figure 3

Kaplan-Meier curves for recurrence-free survival. Univariate survival analysis for DFS showed the following parameters as significant factors: age at diagnosis, hormonal status at diagnosis, treatment mode, histology of primary tumor, SBR grade, Ki-67, number of positive lymph nodes.

Figure 4

Forrest plot in univariate analysis. Premenopausal, adjuvant therapy, less positive lymph nodes, lower level of Ki-67, not receiving radiotherapy were more common in late recurrence group.

Figure 5

Nomogram . Age at initial diagnosis, hormonal status at initial diagnosis, treatment mode, type of surgery, positive lymph nodes, neo-/adjuvant chemotherapy, neo-/adjuvant endocrine therapy and adjuvant radiotherapy were used to construct logistic regression-based nomogram .

Figure 6

Calibration curve of Nomogram 1. B stands for bootstrap. Internal validation was performed for Nomogram 1 using bootstrap resampling method, and calibration curve was depicted as above. In internal validation, 1000 repetitions of resampling were carried out. Based on the results of internal validation, calibration curve was depicted. The closer the curve is to the diagonal line, the more reliable the nomogram is. Absolute error and squared error were used to describe difference between predictive value and actual value. The smaller the error is, the more reliable the nomogram is.

Figure 7

Nomogram 7. Age at initial diagnosis, hormonal status at initial diagnosis, treatment mode, type of surgery, positive lymph nodes, level of Ki-67, neo-/adjuvant chemotherapy, neo-/adjuvant endocrine therapy and adjuvant radiotherapy were used to construct logistic regression-based nomogram 7.

Figure 8

ROC curve of Nomogram 1. ROC curve was depicted to examine the reliability of Nomogram 1. Area under ROC curve (AUC) was 0.703 which showed good discrimination ability of the model.

Figure 9

Calibration curve of Nomogram 1. B stands for bootstrap. Internal validation was performed for Nomogram 1 using bootstrap resampling method, and calibration curve was depicted as above. In internal validation, 1000 repetitions of resampling were carried out. Based on the results of internal validation, calibration curve was depicted. The closer the curve is to the diagonal line, the more reliable the nomogram is. Absolute error and squared error were used to describe difference between predictive value and actual value. The smaller the error is, the more reliable the nomogram is.