Combination of ultrasonic elastography and Ki-67 index as a novel predictive modality for the prognosis in clinic stage III breast cancer

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Research article

Keywords: Ki-67; ultrasonic elastography; breast cancer; survival analysis; prognosis

Posted Date: September 6th, 2019

DOI: https://doi.org/10.21203/rs.2.13995/v1

Abstract

Background To investigate the feasibility of combination of ultrasonic elastography (UE) and Ki-67 index as a novel predictive modality for the prognosis of clinic stage Ⅱ breast cancer.

Methods Of 112 patients, Multi-parameter of UE were obtained after analyzing elastograms of 112 lesions which were recorded classification of Ki-67 by histopathologic diagnosis. Multivariate Survival analysis was performed by using multivariate Cox model. The nomograms was established to predict the probability of clinic stage Ⅱ breast cancer according to the selected independently significant variables in the multivariate Cox model. The model was internally validated using Harrell's concordance index.

Results ⅡC had significant difference in all ultrasonic elastography (UE) parameters ($P < 0.05$) and were statistically different from ⅡA and ⅡB ($P < 0.01$). The Cox model was RR (t)= Exp(1.104* X1+0.901*X4+0.972*X5) (X1: classification of Ki-67, X4: classification of strain ratio, X5: classification of Young's modulus). The nomogram showed that Ki-67 had the greatest influence on survival prediction, followed by Young's modulus and strain ratio. Internal validation revealed a concordance index of 0.76.

Conclusions Our study explored a novel modality in predicting the prognosis of clinic stage Ⅱ breast cancer combining a tumor marker with breast UE imaging. A nomogram was developed to prove that the model was helpful to estimate the 5-year survival of the patients.

Background

The clinic stage Ⅱ (ⅡA, ⅡB, ⅡC) breast cancer was usually considered as advanced breast cancer with a 5-year survival rate range from 30% to 40% and the treatments mainly rely on systemic chemotherapy, local radiotherapy and surgical excision[1, 2]. In recent years, the prognostic stage group was first included into the cancer staging system of American Joint Committee Cancer (AJCC) 8th in 2016[3] because the effect of treatment often determines the prognosis of patients. Therefore, a reliable predictive modality was crucial for surveillance of the disease and evaluation of prognosis.

Previous studies have reported that ultrasonic elastography (UE) which includes compression elastography (CE) and shear wave elastography (SWE) was useful in differentiating benign from malignant breast lesions because of that qualitative and quantitative information on tissue stiffness could be obtained by it additional to that provided by conventional ultrasound[4–6], especially to identify breast cancers with different grades which may appear the same sonographic features of conventional ultrasound. It was a radiation-free and easy-to-operate method compare to mammography and magnetic resonance imaging and its repeatability and low price also made it a rare advantage. Researchers

suggested that ultrasonic elastography also played an important role in evaluating the prognosis of breast cancer forwhich with higher clinic stage has more aggressive pathological properties tend to have higher stiffness readings and worse prognosis[7, 8]. Thus, UE would be helpful to determine optimal individualised treatments for patients with clinic stage breast cancer if it was confirmed to be a reliable prognostic modality.

The Ki–67 antigen, as an important tumor marker associated with cell proliferation, was thought to affect survival of breast cancer[9, 10]. A higher (≥25%) or lower (12 %) Ki–67 index is a significant predictive factor for the response or resistance to the treatment and the over expression of Ki–67 is also an important risk factor for local recurrence of breast cancer. A recent research proposed that a predictive modality combined radiological and pathological biomarkers could provide individualised treatment regimens which could improve the pathological complete response (pCR) rates and prognosis for patients with advanced breast cancer[7]. Hence, it remains to be observed whether the combination of elastography and other tumor markers can improve the diagnostic performance of prediction through a single marker[11].

In summary, our purpose of the study was to access predictive diagnostic performance of combining UE parameters and Ki–67 index in clinic stage breast cancer using survival analysis and nomogram in order to develop it a novel predictive modality for the prognosis of breast cancer.

Materials

Patients selection

This study was approved by our institutional ethics committee and informed consent was gained from each patient before the UE examination. From January 2013 to February 2019, 122 lesions in 118 patients of breast cancer in clinic stage underwent conventional ultrasound and UE before surgical excision or ultrasound guided biopsy (UGB). Inclusion criteria: Primary breast cancer, not metastatic cancer and distant metastasis. Single lesion. No radiotherapy or chemotherapy. No severe cardiovascular disease. Finally, 112 lesions in 112 patients of breast cancer in clinic stage (mean age 46.92 ± 8.45 years old, range 23–58 years old) were included in the final analysis. 10 lesions in 6 patients were excluded later for the following reasons: 6 lesions in 4 patients were received radiotherapy or chemotherapy before the study. 2 lesions in 2 patients suffered from severe cardiovascular disease. 2 patients were found to have more than one lump.

Instruments and methods

US imaging and UE examinations were performed using an Voluson E10 US scanner (GE, Connecticut, USA) equipped with a 9–15 MHz ML6–15-D linear probe which had a function of real-time CE imaging and an Aprio 500 US scanner (TOSHIBA Tokyo, Japan) equipped with a 7–14 MHz PLT–805AT linear probe which had a function of real-time SWE imaging were used in this research.

All the lesions were first examined in the model of two-dimensional gray-scale to locate the position and measure the size. Then the function of CE were activated and the images were displayed in a mode of split-screen with the US images in the left and the same images with color-coded elasticity features superimposed in the right. Using a free-hand technique, the breast gland was vertically compressed by the probe under slight and constant pressure. The real-time pressure elastic indicator bar, displayed on the right screen, was used to help the operator to obtain accurate elastograms. One reproducible elastogram was selected and digitally recorded by the operator when the real-time pressure elastic indicator bar turned green and lasted for at least 3 or 5 consecutive frames[12].

SWE images were obtained during standard gray-scale US imaging. The SWE probe was applied as slightly as possible to the lesions and kept for a few seconds to allow the high-quality SWE images to be frozen and saved. An optionally sized region of interest (ROI) box was set in the lesion area, including the lesion, surrounding normal breast gland and adjacent fatty tissue[13]. The combined gray-scale US and SWE examination was between 5–10 min, of which 1–2 min were for SWE image acquisition. The elasticity of lesion was depicted by a color-coded map representing Young's modules (kPa) and shear wave speed (m/s) at each pixel with a color range from blue (soft) to red (hard). The average of the three measurements from three images was used for further analysis.

The blue area percentage was obtained by analyzing the elastograms of lesions, from which the elastic score was determined by the Tsukuba score system. The strain ratio was obtained after the strain rate of the lesion and the soft tissue adjacent to it at the same depth was measured. Young's modulus and shear wave speed were automatically calculated from SWE images by the machine's software system[12, 13]. All the elastic imaging operations were performed by two performers who had more than 3 years' experience in breast ultrasonic examination and evaluated by two observers who had more than 6 years' experience in breast ultrasonic diagnosis.

Immunohistochemical analysis

All the specimens collected after surgical excision and UGB were evaluated by the same pathologist. Positive status for ER (estrogen receptor) and PR (progesterone receptor) was defined as nuclear staining in≥1% of the tumour cells. Immunohistochemical staining for HER2 (human epidermal growth factor receptor–2) was scored according to standard criteria as 0, 1+, 2+, or 3+. Scores of 0 and 1+ were considered as negative. Cells with Ki–67 nuclear staining were considered to be positive (cancerous). The proliferation index of Ki–67 was determined by the percentage of Ki–67 positive cells among at least 500 cancerous cells in hot spots per slide: negative (Ki–67 ▯), ▯10%; weakly positive (Ki–67 ▯), 11%–25%; positive result (Ki–67 ▯), 26%–50%; strong positive (Ki–67 ▯), ▯51%. All cases were assigned to four major molecular subtypes according to the St. Gallen International Expert Panel consensus: luminal A (ER positive, PR≥20%, HER2 negative, and Ki–67▯14%); luminal B (ER positive and PR▯20% or HER2 positive or Ki–67≥14%); triple negative (ER negative, PR negative, and HER2 negative); and HER2 (ER negative, PR

negative, and HER2 positive) [9] [9]. All samples were evaluated pathologically according to the World Health Organization classification standards[3].

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 software (IBM Inc, Chicago, USA). Numerical variables were expressed as means±standard deviations. Variance of analysis (ANOVA) was used to analyse the differences in clinicopathological characteristics among the three subgroups (A, B, C) and (Ki−67, and). Kaplan-Meier method was performed to analyze the survival difference in all cases. Log-rank method was used to compare the survival rates between groups. Survival analysis was performed by using multivariate Cox model. The nomograms were established with the selected independently significant variables in the multivariate Cox model. The predictive accuracy of the model was quantified by calculating the concordance index (c-index). A c-index of 0.5 indicates that outcomes are completely random, whereas close to 1 indicates that the accuracy of the model is better.

Results

Patient population and baseline characteristics

20 patients underwent conservative breast surgery and 92 patients underwent mastectomy. Histopathology and molecular subtypes of the 112 lesions were confirmed by UGB, which revealed that 89 were invasive ductal carcinomas (79.5%), 14 were ductal carcinoma in situ (12.5%) and 9 were invasive lobular carcinomas (8.0%) (Table1). Other immunohistochemical and molecular factors such as ER positive status, and PR positive status were significantly different among the three clinic stage groups ($P < 0.01$), suggesting that these factors may have predictive value. In addition, the Ki−67 index significantly differed for pairwise comparisons among the three subgroups, which was consistent with previous works (Figure1)[10].

UE evaluation

Acquisition of elastography parameters and process of UGB are shown in Figure 2. UE stiffness readings at three subgroup of clinic stage breast cancer were summarised in Table 2. The elastic score of all elastograms evaluated by all the observers were 3 points and above. ANOVA indicted that C was statistically different to other two groups in all the parameters ($P<0.05$) and B had no statistical difference except elastic score and Young's modulus compared to the A (Table 2). Above findings validated the feasibility of UE for monitoring stiffness of lesions during different subgroup of clinic stage breast cancer.

COX regression analysis

At the deadline of the follow-up date, of 20 patients with ⅠA, 11 died and 9 had no definite outcome. For 41 patients with ⅠB, 32 died and 9 had no final outcome. For 51 patients with ⅠC, 31 died and 20 had no final outcome. The survival time of our study ranged from 8 months to 75 months and the median survival time was 61 months. Ki–67 and all UE parameters were grouped and assigned according to grade (Ki–67 and elastic score) and median (blue area percentage, strain ratio, Young's modulus and shear wave speed). (Table 3) and survival analysis was performed. The results showed that only X1 (classification of Ki–67), X4 (strain ratio) and X5 (Young's modulus) entered into the final equation. All of them were statistically different in different subgroups (Table 4) (Figure 3). The results of survival analysis indicated that the Cox model was RR (t) = Exp (1.104* X1+0.901*X4+0.972*X5).

Prognosis nomogram and c-index

A nomogram was developed to predict 5-years survival of clinic stage Ⅰ breast cancer using the above three covariates identified in the multivariate Cox model[14]. Each point of independent covariates could be determined according to the intersection of a vertical line drawn from the covariate to the point axis and then the total risk scores were calculated by adding each covariate point (Figure 4). 5-years survival probability of clinic stage Ⅰ breast cancer could be read on the total point axis. The predictive accuracy of the nomogram relative to our data, using Harrell's c-index, was 0.764 before calibration and 0.762 after calibration. A calibration curve illustrated the comparison between the prediction from the nomogram and the actual outcomes of the 112 patients (Figure 5).

Discussions

Our study showed that the all the UE parameters in subgroup of ⅠC were significantly different compare to the other two subgroups. Only three parameters (classification of Ki–67, strain ratio and Young's modulus) entered into the final equation which represented tumor markers, semi-quantitative parameter and quantitative parameter of UE respectively. The nomogram was successfully established for predicting the prognosis of clinic stage Ⅰ breast cancer and showed that classification of Ki–67 has the greatest influence among the three parameters. The combination of Ki–67 and UE multi-parameter enabled accurate assessment and prediction of clinic stage Ⅰ breast cancer and the nomogram has proved that the combination of the above two made the model most effective. Therefore, it was a novel predictive modality for the prognosis of clinic stage Ⅰ breast cancer.

Although pathological diagnosis was the gold standard through analyzing expression of tumor markers in evaluating the efficacy of neoadjuvant chemotherapy (NAC) in advanced breast cancer, many studies still attempted to use imaging methods (mammography, MRI and ultrasound) to achieve this goal[15–17]. Nevertheless, these conventional techniques provided unsatisfactory findings in predicting the prognosis after NAC by only monitoring dynamic changes of echo, shape and size of lesions because of that the stiffness of the lesions might have changed slightly while these morphological characteristics did not change significantly on screen. These subtle changes could be captured by UE and the results of

our study proved that the numerical value of multi-parameter of UE changed progressively among the subgroup of clinic stage □ breast cancer, which was consistent with the previous studies. Meanwhile, we also obtained the corresponding tumor markers' information of the lesions by immunohistochemical analysis and the results showed that Ki−67 has the most significant difference among these tumor markers, which was verified by Yan Ma et al. Therefore, our study had greater clinical significance than previous studies on the correlation between tumor stiffness and pathological results[18].

Current evidence supported the hypothesis that development and progression of advanced breast cancer involve cancer cells autonomous proliferation and biological cross talk between cancer cells and extracellular microenvironment, such as surrounding stromal cells and extracellular matrix[19, 20]. That was the complex interaction between breast cancer cells and their surrounding microenvironment determined the prognosis after NAC. Tumor makers represented tumor cell features while UE predominantly represented extracellular matrix features. The Ki−67 index provides information about cellular proliferation and multi-parameters of UE could extracellular matrix features deeply. Consequently, it provided new evidence for clinical research.

Reasons for the three parameters entered into the Cox model might be as follows: (1) Ki−67 was selected as a tumor marker of breast cancer cell proliferation. (2) Strain ratio and Young's modulus as semi-quantitative and quantitative parameter could objectively reflect the hardness of the lesion itself and the difference between the lesion itself and the surrounding tissues. It was almost consistent with other research findings except the cutoff point of our study was higher than that of previous studies because of the particularity of the sample[16]. In addition, elastic score, blue area percentage and shear wave speed were not included in the final model in our study. The possible reasons were listed as follows: (1) Elastic score and blue area percentage, as qualitative parameters, limited by operators' experience and manipulation, were only able to evaluate stiffness of the lesions preliminarily and the reliability of results were often questioned. (2) As a quantitative parameter, both shear wave velocity and Young's modulus were measured by ultrasonic conduction velocity to measure the biomechanical properties and hardness of lesions. Because of some homogeneity, it was difficult for them to enter the equation at the same time, which means that once Young's modulus entered the final Cox model, the shear wave velocity would be eliminated[21, 22].

In present, the nomogram, as a weighted model, is widely used in oncology to improve decision making and accurately predict the potential interaction because of its visual advantages among predictors and enable an evaluation of the extent of the impact of each predictor on the probability of survival[23]. Ki−67 was highly significant in our analysis which has been correlated with survival reproducibly among patients in several large studies, followed by Young's modulus and strain ratio. Thus, for example, a patient's Ki−67 index was 68% which belonged to Ki−67 □ (almost 70 points), value of strain ratio was 3.56 (almost 41 points), value of Young's modulus was 100kPa (almost 49 points) and a total points was 160 that converts to a 5-year survival probability of 15%. We believe that our nomogram could be a simple and easy tool for both the physician and patients for estimating the disease outcome and could contribute to decision making regarding adjuvant chemotherapy[24].

There were still some shortcomings in this study. The potential of selection bias can't be excluded because it was a retrospective design. It was necessary to have a further validation by using other institutional data although our nomogram showed well-validated results. The biological characteristics and biomechanical characteristics of clinic stage breast cancer were significantly related to the prognosis of patients. They were not only an important reference for the clinical monitoring it, but also a reliable prediction for the life and death risk of the patients. The authors will collect more samples to improve the Cox model and strive for more accurate prediction.

Conclusions

Our study explored a novel modality in predicting the prognosis of clinic stage breast cancer combining a tumor marker with breast UE imaging. A multivariable Cox model was established and confirmed that the combination of the UE parameters and Ki–67 index was an efficient protocol for predicting the prognosis of clinic stage breast cancer. A nomogram was developed to prove that the model was helpful to estimate the 5-year survival of the patients. Our ultimate goal was to assist physician to tailor chemotherapeutic modalities and regimens for each individual patient with advanced breast cancer based on risk assessment.

Abbreviations

UE: ultrasonic elastography; UGB: ultrasound guided biopsy; ANOVA: Variance of analysis; c-index: concordance index; NAC: neoadjuvant chemotherapy.

Declarations

Acknowledgements

Not applicable

Authors' contributions

Rui Du and Yuefeng Li designed this study. Baoding Chen, Xin Zhang, Xincai Wu, Hao Zhang, and Zhongqun Wang were responsible for data collection. Mengyuan Shang and Zheng Zhang were responsible for image processing. Rui Du and Yuefeng Li participated in the analysis of data and drafted the manuscript. All authors read and approved the final manuscript.

Funding

This study was funded by National Natural Science Foundation of China (CN) (Grant No. 81871343) and Maternal and Child Health Research Project of Jiangsu Province（F201822）.

Ethics approval and consent to participate

Clinical investigations were performed according to the declaration of Helsinki. The study protocol was approved by the ethics committee of Affiliated Hospital of Jiangsu University. The inform consents were signed by all subjects.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Clinical and pathological characteristics of three subgroups at baseline

Characteristic	A (n=20)	B (n=41)	C (n=51)	*P* value
Age (years)	42.31±9.38	48.47±6.59	47.78±8.70	0.05
Largest diameter (mm)	52 (32-60) [a]	38 (29-46) [a]	44 (31-56) [a]	0.05
Menopausal status				
Premenopausal, n (%)	9 (45.0)	19 (46,3)	17 (33.3)	
Postmenopausal, n (%)	11 (55.0)	22 (53.7)	34 (66.7)	
Position of lesions				
outer upper quadrant, n (%)	13(65.0)	29 (70.7)	33 (64.7)	
inner upper quadrant , n (%)	3 (15.0)	6 (14.6)	10 (19.6)	
lower inner quadrant , n (%)	2 (10.0)	2 (4.9)	3 (5.9)	
lower outer quadrant , n (%)	1 (5.0)	2 (4.9)	4 (7.8)	
area behind the areola , n (%)	1 (5.0)	2 (4.9)	1 (2.0)	
Family history of cancer				
Yes, n (%)	17 (85.0)	35 (85.3)	45 (88.2)	
No, n (%)	3 (15.0)	6 (14.7)	6 (11.8)	
Immunohistochemical marker				
Ki-67 percentage (%)	19 (15-33)[a]	43 (34-49) [a]	66 (28-74) [a]	0.01[b]
ER positive, n (%)	11 (84.6)	15 (78.9)	32 (88.9)	0.05[b]
PR positive, n (%)	10 (76.9)	15 (78.9)	31 (86.1)	0.05[b]
HER2 positive, n (%)	6 (46.1)	10 (52.6)	19 (52.8)	0.05[b]
Molecular subtype, n (%)				
Triple negative	8 (40.0)	14 (34.1)	10(19.6)	0.05[b]
HER2	8 (40.0)	7 (17.1)	1 (2.0)	NA[c]
Luminal A	0 (0)	6 (14.7)	9(17.6)	NA[c]
Luminal B	4 (20.0)	14 (34.1)	31 (60.8)	0.05[b]

ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2, NA, not applicable;

a. Values are given as median (25^{th}-75^{th} percentile).

b. There is a significant difference for pairwise comparison among three subgroups, respectively.

c. More than one cells have expected count less than 5.

Table 2 Comparison of multiple parameters of UE in different clinic stage of breast cancer

Clinic stage of breast cancer	elastic score	blue area percentage	strain ratio	Young's modulus	shear wave speed
A	3.40±0.68	0.70±0.14	3.54±0.34	95.28±5.13	4.78±0.58
B	3.90±0.80●	0.76±0.13	3.73±0.63	98.67±3.56●	5.04±0.70
C	4.41±0.73●▲	0.84±0.11●▲	4.10±0.74●▲	103.40±6.28●▲	5.43±0.71●▲
F value	14.364	11.435	6.765	20.207	7.669
p value	0.05	0.05	0.05	0.05	0.05

Data are mean±SD or median (interquartile ranges) .●p<0.05 vs Ki-67 , ▲p<0.05 vs Ki-67 .

Table 3 Parameter classification processing assignment

Parameter	Condition	Category (assignment)
X1classification of Ki-67	Ki-67	0
	Ki-67	1
	Ki-67	2
X2elastic score	3	0
	4	1
	5	2
X3classification of blue area percentage	0.81	0
	≥0.81	1
X4classification of strain ratio	3.55	0
	≥3.55	1
X5classification of Young's modulus	99.9	0
	≥99.9	1
X6shear wave speed	5.16	0
	≥5.16	1

Table 4 Test results of survival analysis

.eserved variables	B	S.E.	Wald	df	Sig.	EXP□B□	95.0% C.I.for EXP (β)	
							Lower	Upper
X1	1.014	0.190	28.617	1	0.000	2.757	1.902	3.998
X4	0.901	0.256	12.357	1	0.000	2.462	1.490	4.070
X5	0.972	0.260	14.016	1	0.000	2.645	1.589	4.400

Figures

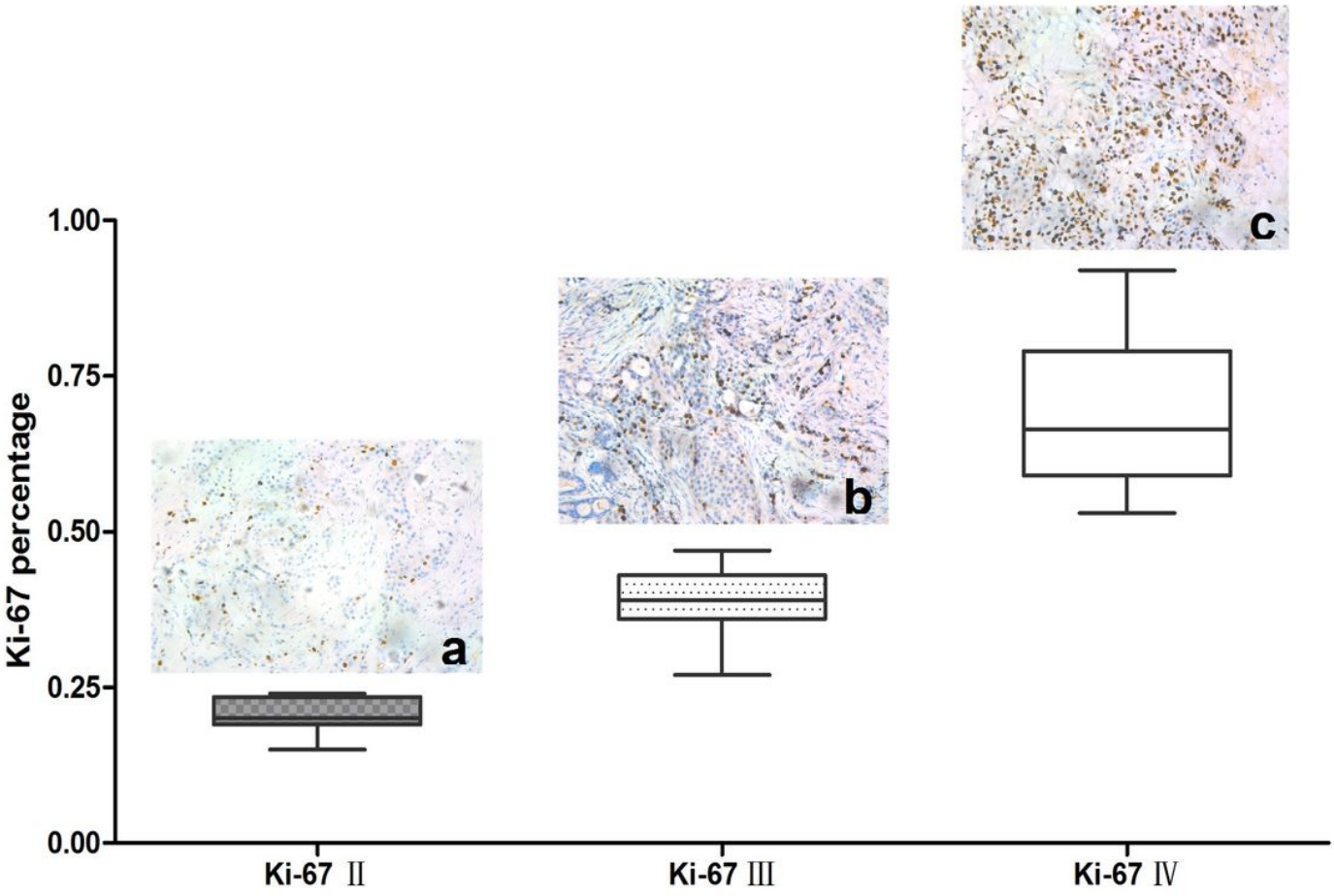

Figure 1

Box diagram of Ki-67 distribution in different classification. Fig a, b and c were Immunohistochemical staining diagram of Ki-67 □, Ki-67 □ and Ki-67 □ respectively. The immunohistochemical images were observed under a magnification of 20×.

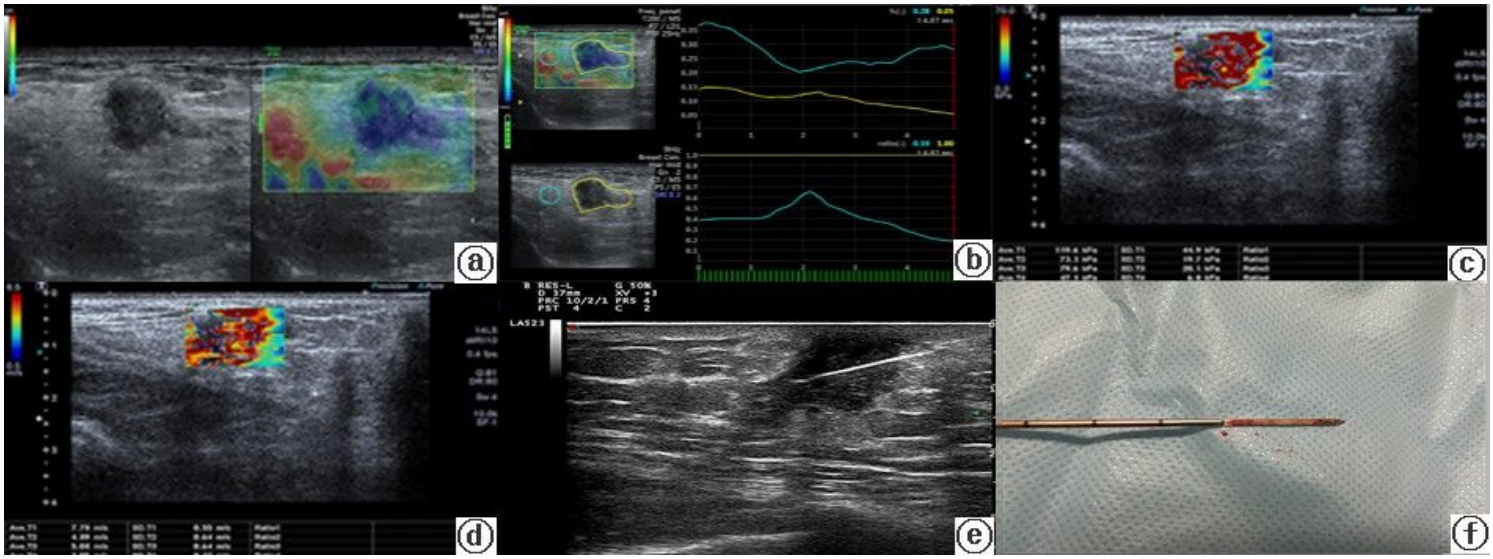

Figure 2

Elastography and biopsy. (a) Elastic score and blue area percentage were gained from CE. The region of the tumor was mainly blue. (b) Strain ratio were gained from CE. The blue sampling frame was normal breast area, and the yellow sampling frame was the tumor area. The amplitude of blue line fluctuation was significantly larger than that of yellow line, indicating that the elasticity of the blue region was greater than that of the yellow area. The tumor area was more rigid than the normal breast region.(c) Young's modulus were gained from SWE.(d) Shear wave speed were gained from SWE.(e) UGB of the tumor region.(f) A yellow white tumor tissue strip obtained from UGB.

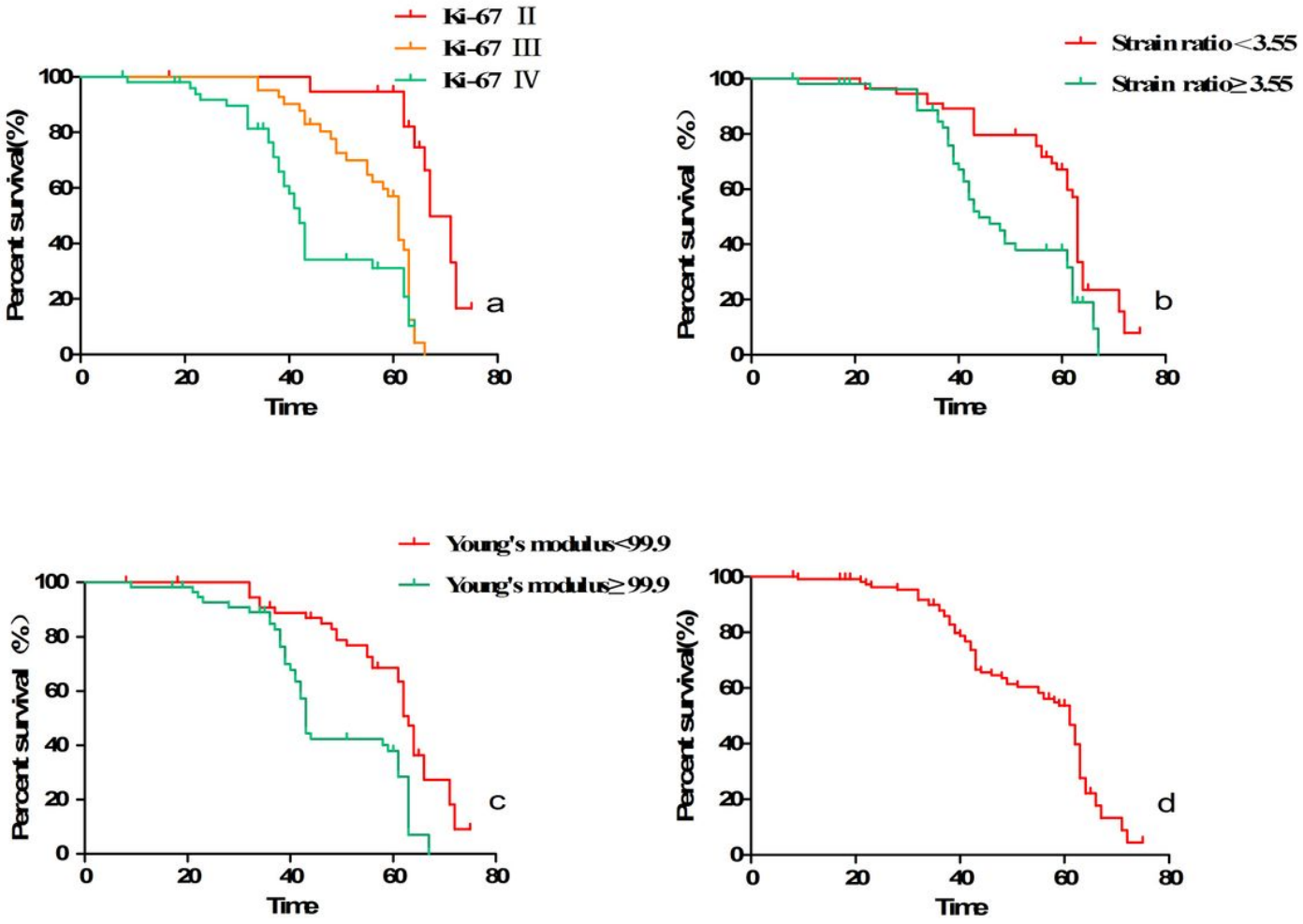

Figure 3

Survival curves of all parameters entering Cox model and the final survival curve of our study. (a) Survival curves between different classification of Ki-67 and Ki-67 Ⅳ had the shortest survival time. (b) Survival curves between different subgroups of strain ratio and the survival time of group with higher strain ratio was shorter than the lower one. (c) Survival curves between different subgroups of Young's modulus and the survival time of group with higher Young's modulus was shorter than the lower one. (d) The final survival curve was obtained according to the equation.

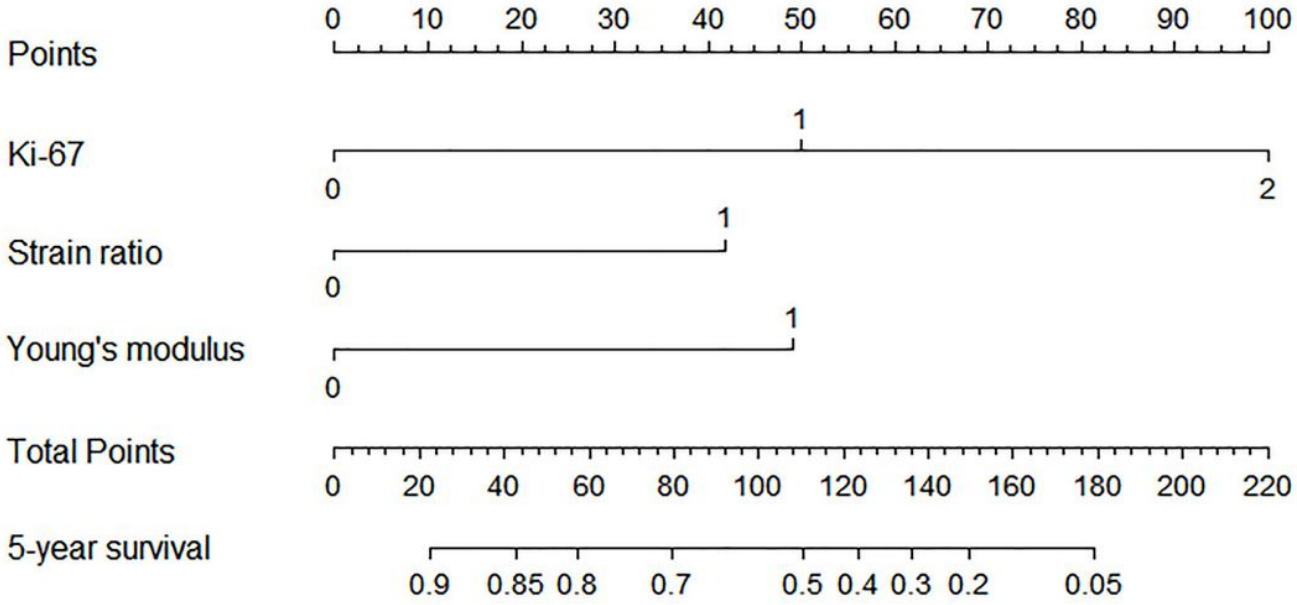

Figure 4

Nomogram predicting the probability of 5-years survival of clinic stage breast cancer

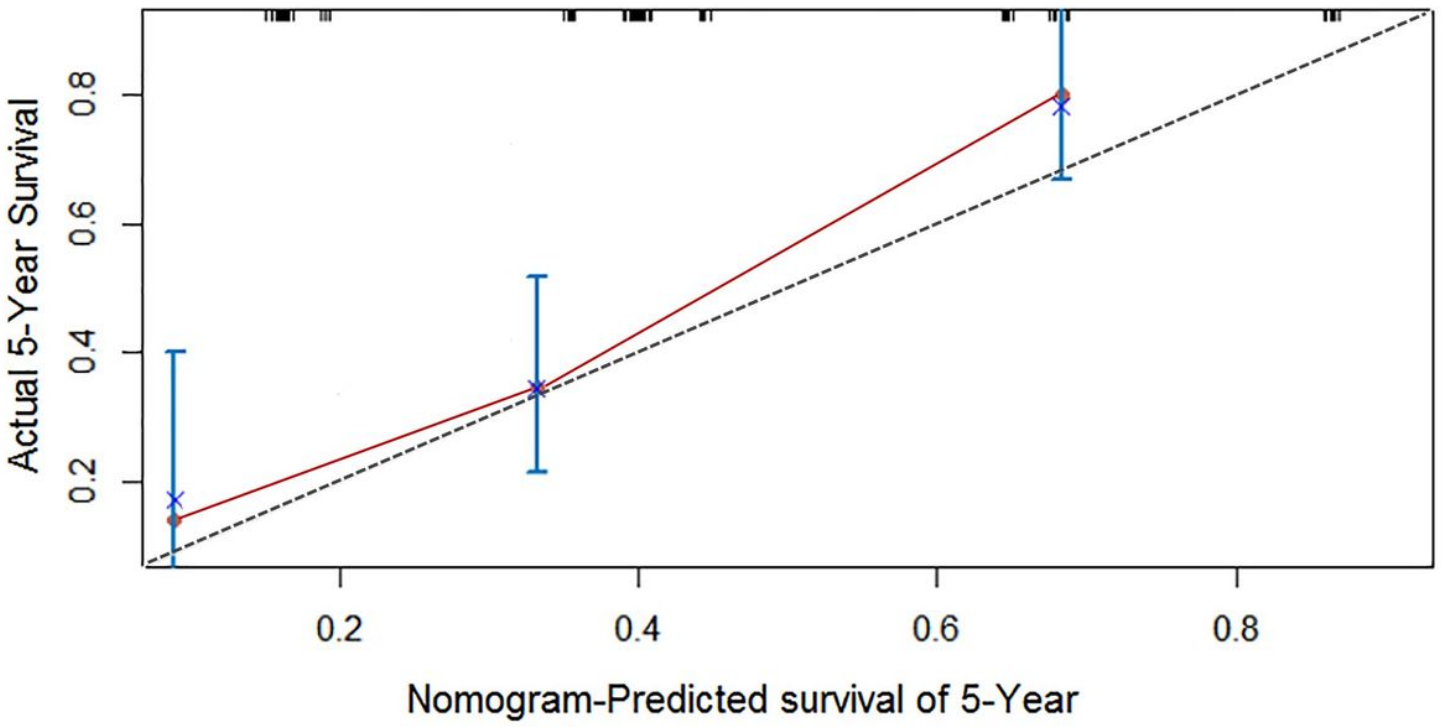

Figure 5

Calibration curve for 5-year breast cancer specific survival. The concordance index was 0.762. The solid line represents the performance of the present nomogram, and the dashed line represents the performance of an ideal nomogram.