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Correlation of virtual touch tissue imaging quantification with the Nottingham prognostic index and immunohistochemical features in breast invasive carcinoma

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Summary

Objectives: To investigate the correlation of virtual touch tissue imaging quantification (VTIQ) with the Nottingham prognostic index (NPI), and immunohistochemical features in breast invasive ductal carcinoma. *Materials and Methods:* In all, 134 patients with breast invasive ductal carcinoma confirmed by histopathology were included. All patients underwent conventional ultrasound and VTIQ to record the average shear wave velocity (SWV) as elasticity mean value (Emean) before the operation; then, postoperative clinical pathology, immunohistochemical features, and NPI score were recorded in order to investigate the correlation of Emean with NPI and immunohistochemical features. *Results:* Univariate analysis showed that the Emean was in positive correlation with NPI, tumor size, axillary lymph node metastasis, lymph node staging, histology grade, HER-2, and ki67 expressions in breast invasive ductal carcinoma, and the correlation coefficients were positive (r = 0.714, 0.308, 0.635, 0.668, 0.590, 0.221, and 0.559) ($p < 0.05$). It was in negative correlation with estrogen receptor (ER) (r = -0.373) ($p > 0.05$) and with progesterone receptor (PR) (r = -0.145) ($p > 0.05$). Multivariate analysis indicated that only NPI and ki67 were independently positively correlated with the Emean (r = 0.928, r = 0.276) ($p < 0.05$). *Conclusions:* The biological characteristics of breast carcinoma can affect its elastic tissue structure, which showed significant association with NPI. Moreover, VTIQ can be used to supplement conventional US to judge the prognosis of breast carcinoma.

Key words: Breast invasive ductal carcinoma; Immunohistochemistry; Nottingham prognostic index; VTIQ.

Introduction

Breast cancer is one of the most common malignant tumors among women, and its incidence has been steadily rising in China. Known as the "thirdeye" of clinicians, ultrasonography (US) possesses major advantages, such as simple operation, no radiation and low cost, and has become the most common method in clinical practice [1]. Numerous novel technical approaches, such as ultrasonic elastography have been widely used in the diagnosis of lesions in the breast, liver, thyroid, and other organs [2, 3]. Conventional ultrasound and mammography provide much information about breast tumors, such as their shape, margin, boundary, echo intensity, microcalcification, and blood flow. However, they do not allow measurement of tissue stiffness, and in the breast, cancers tend to be stiffer than benign lesions [4-6]. It is known that the stiffness of an inherent feature, has a close relationship with the different pathological types of breast lesion [7]. Based on this principle, elastography, which indirectly reflects the varying stiffness levels in lesions, is considered to be helpful in the diagnosis and predict the prognosis of breast cancer. Breast invasive ductal carcinoma is the most common type of carcinoma of the breast. Elasticity imaging, a newly developed ultrasonic technique, can quantitatively measure the tissue by evaluating the change in flexibility. Molecular factors determine the biological behavior and histopathological changes associated with metastatic breast cancer, which determines the morphology. Virtual touch tissue imaging quantification (VTIQ) is one of the latest methods of quantitatively measuring the acoustic radiation force impulse (ARFI) elasticity imaging [8]. VTIQ is a novel two-dimensional (2D) ultrasonic elastography system and its diagnostic accuracy for breast lesions has so far been confirmed in preliminary studies [9, 10].

The Nottingham prognostic index (NPI), which combines tumor size, nodal status, and histological grade, is a useful tool to predict the prognosis of breast carcinoma [11]. The NPI is an excellent tool for stratifying the prognosis of patients. The benefits of adjuvant systemic treatments, such as chemotherapy, are proportional to the risk of death from breast cancer [12].

In this study, the author used VTIQ to explore the correlation of VTIQ with the NPI and immunohistochemical fea-

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tures, such as estrogen receptor (ER), progesterone receptor (PR), and proto-oncogenes, HER-2, and ki67, in order to provide reference for comprehensive treatment and prognostic assessment of breast carcinoma.

Materials and Methods

In all, 134 patients with breast invasive ductal cancer who underwent surgery in the present hospital, and the diagnosis was were included from December 2014 to December 2015. The diagnosis was confirmed by pathological and relevant immunohistochemical examinations. Those patients who underwent preoperative radiation and chemotherapy or endocrine treatment were excluded from the analysis (Figure 1). The mean age of the cohort was 57.68 ± 12.20 (age range, 26–92) years. The authors adopted the standard histologic grade set forth by WHO (2003) to classify patients into grades I, II, and III. According to the tumor node metastasis (TNM) staging of WHO, clinical stage can be divided into I~IV periods. Applying the hospital medical record inquiry system, the authors recorded the maximum size, lymph node metastasis, histological grade, and immunohistochemical feature. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Tongji University. Written informed consent was obtained from all participants.

All examinations were performed by a sonographer with at least five-years' experience in breast US and elasticity ultrasound examination, by using a probe with a 9L4 linear array, 4-9 MHz frequency ultrasound system [8]. The patients were instructed to lay in the supine position with their chest completely exposed. The ultrasonic probe was then gently placed on the breast, with the fan probe applying gentle pressure, leading routine US examination, found after lumps to observe and record the size of the lesions, boundary, parts, and internal blood supply of the echo, and for BI-RADS classification. Then the selection was switched to the ARFI mode. Next, the sonographer switched to the VTIQ pattern, which displays the VTIQ model of the quality and velocity, and captured the respective images. VTIQ-obtained image quality model can monitor the quality of distribution of elastic quality from high to low, respectively, for the green-yellow-red spectrum, and select an effective shear wave velocity (SWV) measurement area (i.e., the area presented for the green and evenly distributed as per the VTIQ quality control chart). The region of interest (ROI) was marked and placed within the lesion, measuring a minimum of 1×1 mm. Patients were told to hold their breath while the VTIQ velocity model images were captured. Under VTIQ velocity mode, the two-dimensional space distribution of the lesion shear wave elasticity imaging from the high to low SWV were red, yellow, green, to blue. After adjusting the SWV range (maximum 10 m/s), such model is considered for VTIQ standard velocity imaging: the surrounding background of the lesion shows even light blue or light green, red or yellow appearance on the center of lesions. Similarly, multiple SWV data measurement (5–7 group) in the effective area were recorded, and the average value was considered in m/s. All conventional US and VTIQ images were stored for further analysis.

Pathology inspection for resection of lesions included the following aspects: (1) tumor diameter measurement, (2) statistically significant number of ipsilateral axillary lymph node metastasis, (3) microscopic observation for hematoxylin-eosin (HE) staining,

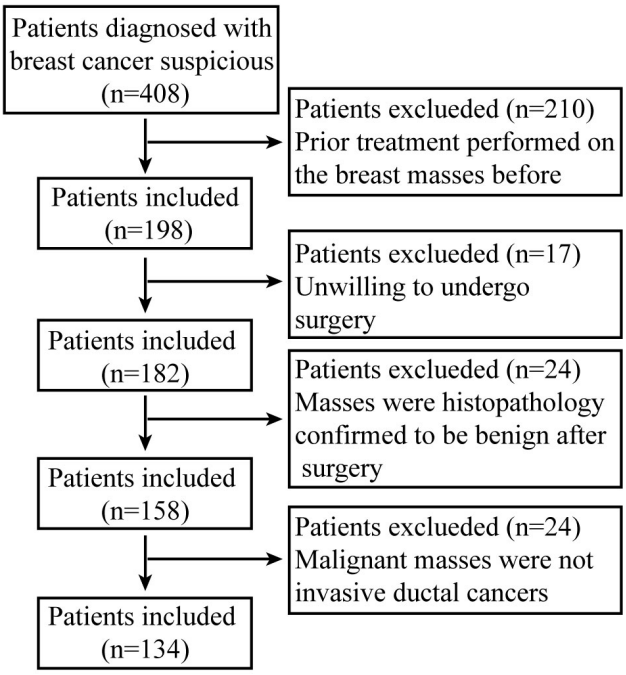

Figure 1. — Flowchart of the selection of the patients with breast invasive ductal cancer.

and histological classification. According to the tumor diameter (cm), lymph node staging (1: no lymph node metastasis, 2: 1–3 lymph node metastasis, 3: ≥ 4 lymph node metastasis), and histological grade (1-3), the authors calculated the NPI as follows: size (cm) × 0.2 + lymph node staging (1–3) + histological grade. The NPI score was divided into three groups, namely < 3.4, 3.4–5.4, and > 5.4.

Information on ER, PR, and HER2 status was assessed by IHC and extracted from pathology reports submitted from the Pathology Department. Detection and expression of ER, PR, HER-2, and ki67 was done using immunohistochemical (IHC) staining methods such as with oxide enzyme anti peroxidase (PAP). Among these, ER, PR, and ki67 were regarded positive with brown yellow particles in the tumor cell nucleus. According to the percentage (positive cells/all tumor cells), ER cells < 1% were negative, < 10% (+), 10–30% (++), and > 30% (+++). PR-negative tumors were defined as those with reactivity of < 10%, and PR-positive tumors as those with reactivity ≥ 10%, 10-30% (+), 30–50% (++), and > 50% (+++). HER2 expression status was determined with IHC and/or in situ hybridization. HER2 cells 0% were negative, < 10% (+), 10–30% (++), and > 30% (+++). In situ hybridization (fluorescence (FISH)) was usually used to confirm HER2 status if IHC yielded 2+ results. If IHC was 2+ and FISH were missing, or if IHC was missing, but FISH tumor was classified as HER2-positive. If IHC was 2+ and FISH were negative, the tumor was regarded as HER2-negative. Ki67 expression was measured by an experienced pathologist and presented as a percentage score (range 0–100%) of positive tumor cells. The number of Ki 67 positive cells 0% were negative, < 15% (+), 15~30% (++), and > 30% (+++). < 15% was regarded as low expression group, ≥ 15% as more than high expression group.

Statistical analysis was performed using the Statistical Package for the Social Sciences, version 18.0 (SPSS). The clinical charac-

Table 1. — *Expression of ER, PR, C-erB-2, and ki67 in breast invasive ductal carcinoma (n).*

Immunohistochemical	ER	PR	C-erB-2	Ki67
(-)	42	70	56	10
(+)	70	58	35	30
(++)	17	3	19	56
(+++)	5	3	24	38
Positive rate (%)	68.7	47.8	58.2	92.5

ER: estrogen receptor, PR: progesterone receptor, HerB-2: progesterone receptor human epidermal growth factor receptor 2, ki-67: cell proliferation associated antigen.

Table 2. — *Correlation of the Emean value with other factors.*

Factors	Correlation coefficient (r)	*p*
NPI	0.714	0.000
Maximum diameter	0.308	0.000
Lymph node metastasis	0.635	0.000
Lymph node staging	0.668	0.000
Histology grade	0.590	0.000
ER	-0.373	0.000
PR	-0.145	0.096
HER-2	0.221	0.01
Ki67	0.559	0.000

Note: For this model of analysis of variance, $p < 0.05$ was statistically significant.

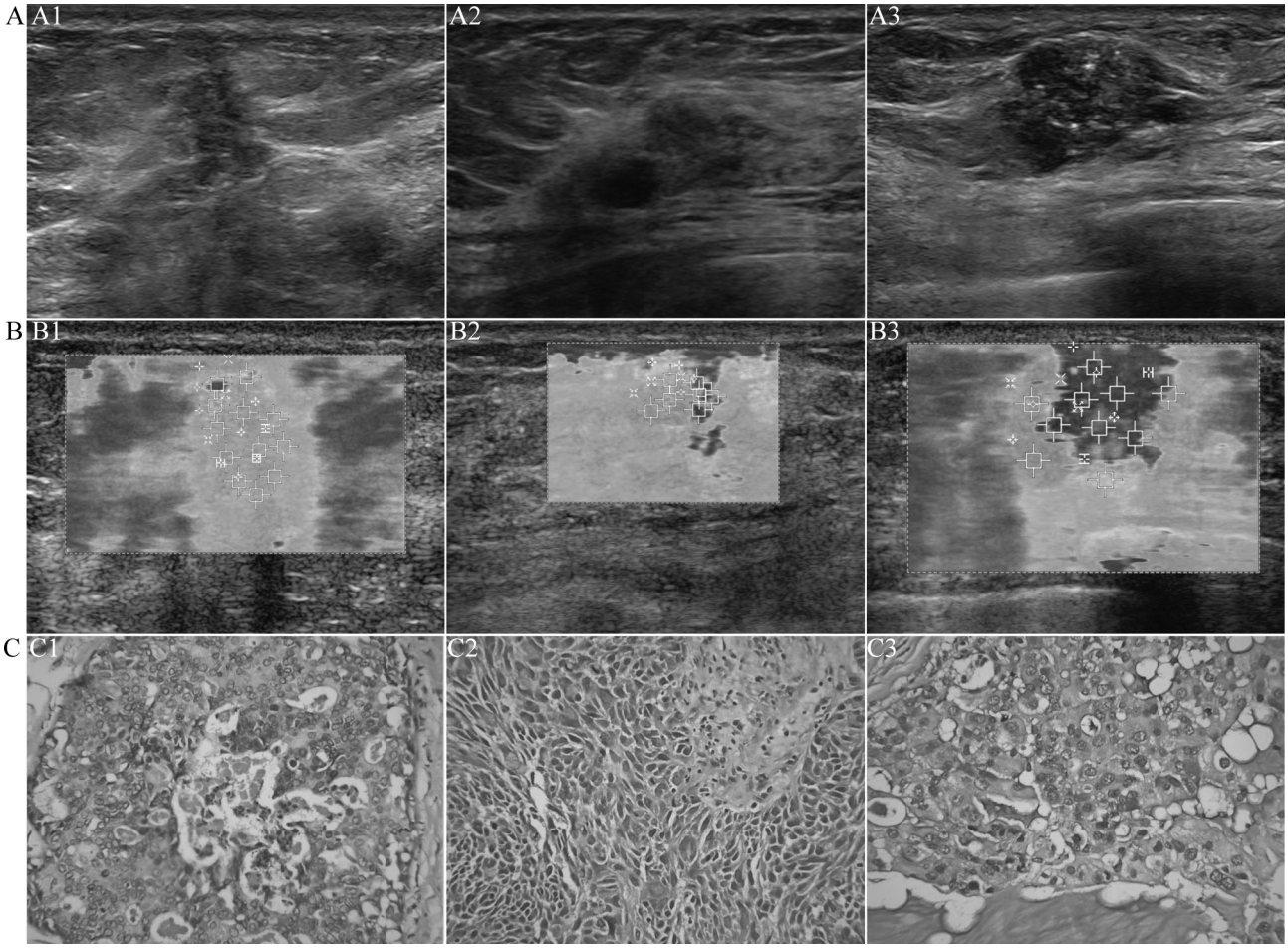

Figure 2. — Gray-scale images. Gray-scale images (A1), virtual touch tissue imaging quantification (VTIQ) imaging (B1), and histopathology (C1) in a 62-year-old woman with Grade 2 invasive ductal carcinoma measuring 15 mm, and negative for lymph node metastasis and human epidermal growth factor receptor 2 (HER-2) expression and ki-67 protein expression, but positive for estrogen receptor (ER) and progesterone receptor (PR) expression. VTIQ of the mass (B1) shows the mean SWV is 2.69 m/s. NPI score is 3.3. Gray-scale images (A2), VTIQ imaging (B2), and histopathology (C2) in a 64-year-old woman with grade 3 invasive ductal carcinoma measuring 20 mm, and negative for ER, PR expression, HER-2 expression, but positive for lymph node metastasis and ki-67 protein expression. VTIQ of the mass (B2) shows the mean SWV is 4.26 m/s. NPI score is 5.4. Gray-scale images (A), VTIQ imaging (B) and histopathology (C) in 64-year-old woman with a 20 mm, grade 3, negative ER and PR expression, negative-human epidermal growth factor receptor 2 (C-erbB-2) expression , positive-lymph nodal metastasis, and ki-67 protein expression. VTIQ of the mass (B) shows the mean SWV is4.26 m/s. NPI score is 5.4. Gray-scale images (A3), VTIQ imaging (B3), and histopathology (C3) in a 67-year-old woman with Grade 3 invasive ductal carcinoma measuring 55 mm, and negative for ER and PR expression, but positive for lymph node metastasis and expressions of HER-2 ki-67 protein. VTIQ of the mass (B3) shows the mean SWV is 8.96 m/s. NPI score is 6.1.

Table 3. — *Regression analysis of Emean value with other factors (multivariate analysis).*

Factor	Correlation coefficient (r)	*P*
NPI	0.928	0.030
Ki67	0.276	0.011

Note: For this model of analysis of variance, p <0.05 was statistically significant; the coefficient of determination r^2=0.662.

teristics and SWV measurements of patients with breast invasive ductal cancer were expressed as means ± standard deviations (SD). Relationships between SWV, SWV ratio, and clinicopathologic variables were investigated by using univariate analysis. Multiple linear regressions were applied to perform multivariate analysis to evaluate which clinicopathologic variables were most influential on SWV and its ratio. $P < 0.05$ was considered statistically significant.

Results

NPI-dependent distribution among the 134 patients with breast invasive ductal cancer was as follows: NPI < 3.4 in 45 cases, 3.4–5.4 in 60 cases, and > 5.4 in 29 cases. The mean NPI was 4.26 ± 1.28, and tumor diameter ranged from 0.4–6.0 (mean, 2.29 ± 1.17) cm. Fifty-six (41.8%) patients showed lymph node metastasis; the lymph node staging was Stage I in 78 cases (58.2%), Stage II in 31 cases (23.1%), and Stage III in 25 cases (18.7%). Histological grading was Grade I in 18 cases (13.4%), Grade II in 71 cases (23.1%), and Grade III in 45 cases (33.6%). Immunohistochemical features are shown in Table 1 and Figure 2.

The mean Emean value was 4.79±1.34 m/s (range, 2.11–8.12 m/s), which was associated with the NPI, maximum diameter, lymph node metastasis status, histological grade, lymph node staging, ER, HER-2, and expression of ki67. univariate analysis showed that the Emean was positive correlation with the NPI ($r = 0.714$), tumor size ($r = 0.308$), axillary lymph node metastasis ($r = 0.635$), lymph node staging ($r = 0.668$), histology grade ($r = 0.590$), HER-2 ($r = 0.221$), and ki67 ($r = 0.559$) ($p < 0.05$) expressions in breast invasive ductal carcinoma. It was negatively correlated with ER ($r = -0.373$) ($p < 0.05$) and PR ($r = -0.145$) ($p > 0.05$) (Table 2). The NPI and expression of ki67 independently correlated with Emean in the regression analysis (Table 3). Multivariate analysis indicated that only NPI and ki67 were independently positively correlated with the Emean ($r = 0.928$, $r = 0.276$) ($p < 0.05$).

Discussion

The stiffness of a tissue is related to pathological changes and the histological components of any lesions present [13]. Therefore, its measurement allows detection of various diseases that are associated with changes in the mechanical properties of tissue. ARFI is a relatively novel technology for non-invasive assessment of a tissue's elastic properties, including stiffness. Its quantitative implementation is called VTQ and this provides an objective numerical value for the shear wave velocity (SWV), which mainly depends on the stiffness of the target tissue [14-16]. Another method of shear wave elastography is VTIQ: [17] this was developed from second-generation VTQ technology and provides many advantages, such as a smaller ROI, a wide range, high reliability, and better reproducibility [2].

Breast cancer causes great harm to female health, and invasive ductal cancer is the most common pathological type of the disease. Judging the prognosis of breast cancer includes the tumor size, clinical stage, histological classification, and grade of and lymph node metastasis. Tumor molecular biology is the decisive factor of biological behavior and histopathological appearance, and then has a great influence on elastic features. Correlations between radiologic findings and pathologic prognostic factors of breast invasive ductal cancer have shown that there is a poor correlation between mammographic and US size of invasive lobular carcinoma with the histologic size [18]. Breast cancer has higher cure rates when detected early and treated appropriately. The present research shows that SWV and its ratio are related with clinicopathologic factors, which would help to initiate early intervention to this malignant disease.

Breast cancer is a kind of hormone-dependent tumor; with the development of molecular biology, ER, PR, HER-2, and ki-67 are increasingly commonly used biological markers to evaluate the behavior and prognosis of these tumors, and their expression can provide a theoretical basis for postoperative endocrine therapy as well. In the present study, the positive rate of ER in breast cancer tissue was 68.7%, PR 47.8%, HER-2 58.2%, and ki67, 92.5%, which is consistent with literature [19].

Correlation between NPI with the elasticity in breast invasive ductal carcinoma: of all breast cancer evaluation systems used for prognosis, NPI, established by Haytittle *et al.* [20] in 1982, has had the greatest influence. In his retrospectively study, 387 cases with complete data were enrolled and factors with independent prognostic significance were selected in the nine prognostic factors to build a prognosis model based on the Cox's regression model [20] that was suitable for the group confirmed by the data of evidence-based research. The three selected independent prognostic factors were tumor size, lymph node status, and histological grade. NPI as a recognized prognostic indicator was divided into < 3.4, 3.4–5.4, and > 5.4 levels which has significant influence on the prognosis of assessment, treatment, and treatment effect of monitoring [21, 22]. The higher score indicates the worse prognosis. This study showed NPI < 3.4 in 45 cases, 3.4–5.4 in 60 cases, and > 5.4 in 29 cases. NPI with Emean correlation coefficient was $r = 0.714$ ($p < 0.05$), indicating there was a positive correlation between them, such that the Emean increases proportionally with NPI increase. With respect to the three

independent prognostic factors, NPI was closely associated only with clinical pathology. A lesion's internal pathological structure to a certain extent can be reflected by the lesion's degree of hardness, then converted into quantitative Emean to indirectly predict the NPI degree, assisting in predicting the prognosis of breast invasive ductal carcinoma.

Correlation between the clinical pathological characteristics and stiffness in breast invasive ductal carcinoma: univariate analysis of this study showed there was significant positive association among Emean with the maximum diameter, lymph node metastasis status, histological grade, and lymph node staging in breast invasive ductal carcinoma. Whether benign or malignant, the mass size of a tumor can affect the elasticity value, and different grade have different elasticity [23]. Quantitative VTIQ elastic average reflects the internal pathological structure of the lesions, which can help predict the prognosis of breast invasive ductal carcinoma.

Correlation between immunohistochemical features and stiffness in breast invasive ductal carcinoma: univariate analysis showed ER, HER-2, and ki67 expression were correlated with stiffness in breast cancer. Among them, the expression of ER was negatively correlated with Emean, while HER-2, and ki67 showed positive correlation, which indicates breast cancer tissue with ER (-), HER-2 (+), and ki67 (+) has higher tissue hardness. Zellars *et al.* [24] found that for local recurrence, ER (-) was higher than ER (+), and the state of the ER expression was associated with invasive breast cancer. ER (-) status with tumors and perhaps indicate a high degree of malignant cells, higher probability of relapse, and lymph node metastasis, whereas ER (+) status indicates good prognosis. HER-2 is a type of proto-oncogene, also named CerbB-2 or neu, has a lower or even nil expression in normal tissue, and was reported for the first time by Slamon *et al.*, as the influence of over-expression being predictive of an unfavorable prognosis in breast cancer in 1987 [25]. The ki67 protein is a nuclear antigen associated with cell proliferation, whose expression is relevant with the development, metastasis, and prognosis of malignant tumors. It is also reportedly closely related with lymph node metastasis and tumor growth activity in breast cancer [26]. The results of multiple factors analysis showed that ER and HER-2 expression in breast cancer were not independent correlation with the hardness of the tumor, while Ki67 expression has independent correlation. The reason may be that the expression of ER, PR, HER-2 in breast cancer is closely related to the pathological type, tumor size, number of lymph node metastasis and clinical stage. From the results of single factor and multifactor analysis, with the increase of ki67 expression, the elastic mean value of breast invasive ductal carcinoma also increased. The higher expression of ki67 in tissues indicated that the higher the degree of malignancy of breast cancer, the more prone to invasion, adhesion to the surrounding tissue, decreased flexibility, and increased stiffness. Hanprasertpong *et al.* [27] showed that the expression of ki67 was associated with lymph node metastasis and tumor stage. When ki67 expression is positive, the cell is likely to possess unique biological characteristics, including active cell proliferation, the higher degree of malignancy, a larger probability to be invasive, a rapid pace of development, more transfer opportunities, and poor prognosis.

In conclusion, there was a significant correlation of NPI and ki67 with stiffness in breast invasive ductal carcinoma. The biological characteristics of breast cancer is associated with the hardness, and lesions' hardness reflects the biological characteristics to an extent. VTIQ, a newly non-invasive technique used for quantitative analysis of hardness will be helpful to evaluate the prognosis of breast cancer. Nevertheless, the occurrence and development of breast cancer is a result of the combined multiple factors, and there are many uncertain factors and missing links between the morphology and molecular biology of the disease [28]. There were some limitations to this study. The number of samples was relatively small and all cases originated from a single hospital. This study offers a preliminary research outlook, which requires further strengthening and validation with future research.

Acknowledgements

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