Int J Clin Exp Med 2015;8(10):18538-18542
www.ijcem.com /ISSN:1940-5901/IJCEM0012561

Original Article

Relation between qualitative and quantitative 3-dimensional ultrasound and ki-67 expression in breast cancer

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Received July 7, 2015; Accepted October 9, 2015; Epub October 15, 2015; Published October 30, 2015

Abstract: To investigate the relation between quantitative blood flow parameters on 3-dimensional (3D) color histogram, 3D ultrasound characteristics and Ki-67 expression in breast cancer. Three-dimensional ultrasound characteristics and histological classifications of 76 breast tumors in 75 confirmed cases were analyzed. Relations of tumor volume (V), vascularization index (VI), flow index (FI) and vascularization-flow index (VFI) on 3D color histogram to Ki-67 expression were studied by statistical methods. VI and VFI measurements of tumors in positive Ki-67 expression group were obviously increased compared with the negative expression group (P<0.05). V and FI measurements of positive expression group were higher than those of the negative expression group, but the difference was not significant (P>0.05). Cases showing positive expression of Ki-67 were more likely to have lymph node metastases (P<0.05), and Ki-67 expression positively correlated with histological classification (P<0.05). However, the two groups did not show significant differences in the findings of "sun-like symptom" (P>0.05). Qualitative and quantitative 3D ultrasound characteristics correlated with positive expression of Ki-67 in breast cancer. Quantitative analysis with 3D color histogram more accurately evaluates blood supply of breast tumors, providing references for predicting biological behaviors and prognosis of breast cancer.

Keywords: 3-dimensional ultrasound, color histogram, breast cancer, Ki-67

Introduction

Angiogenesis is crucial for the development of breast cancer. 3-dimensional (3D) color Doppler flow imaging technique overcomes the limitations of conventional 2-dimensional (2D) color Doppler imaging, which is now used to assess the spatial distribution of new tumor blood vessels and blood flow in tumors. Ki-67 index can serve as an important indicator of proliferative activity of tumor cells and also the basis for choosing treatment scheme and prognostic prediction [1]. We analyzed the relations between quantitative blood flow parameters, 3D ultrasound characteristics and Ki-67 expression for the above considerations.

Materials and methods

Subjects

A total of 76 breast tumors in 75 confirmed cases of breast cancer who did not receive chemotherapy or radiotherapy were examined by 3D color Doppler ultrasound from March to November 2013. Considering the limited scanning range of the probe, tumors with major diameter <5 cm were excluded. All subjects were females, aged 31-36 years with an average of 50.0±10.7 years.

Equipments and methods

GE Voluson E8 ultrasound equipment was used, with RSP6-16-D probe (frequency 6-12 MHz, scan angle 5-29°), medium-frequency Doppler energy, 0.9-0.6 kHz pulse repetition frequency, -0.4-0.6 color gain, and "low 1" wall filter. The subjects were told to breath slowly, and vascularization was submitted to 3D power Doppler imaging. Tumor delineation was done using VOCAL technique (each tangent plane rotated by 30°, 6 tangent planes). Then 3D color histogram generation program was started to calculate V, VI, FI and VFI. Measurements were performed for three times and averaged. V1 was the percentage of the colored voxels in the

Table 1. Relation between quantitative blood flow parameters on 3D color histogram and Ki-67 expression

Ki-67 expression (cases)	V (cm^3)	VI (%)	FI	VFI
Positive (46)	6.34±0.07	5.89±0.08	2.51±0.02	2.46±0.05
Negative (30)	2.86±0.04	1.45±0.02	2.24±0.06	0.32±0.06
P value	0.074	0.002	0.109	0.005

Table 2. Relation between 3D ultrasound characteristics, histological classification and Ki-67 expression

Item		Ki-67 expression (cases)		x^2 value	*P* value
		(+)	(-)		
Sun-like symptom	Yes	24	20	1.57	0.211
	No	22	10		
Axillary lymph node metastasis	Yes	29	8	11.46	0.001
	No	17	22		
Axillary lymph node metastasis	I	7	16	14.75	0.001
	II	24	12		
	III	15	2		

tumor to all colored voxels, which was approximately equal to microvessel density; FI was the average red blood cell density in unit voxel, or blood flow intensity; VFI=VI×FI, representing the overall blood perfusion.

Immunohistochemical test

En Vision two-step method was used for immunohistochemical test according to the kit instructions. Pathologists were blinded to analyze Ki-67 expression. Positive expression of Ki-67 was defined as the percentage of positive cells in the selected field of view to total cells. Percentage ≥20% was considered positive and <20% negative.

Statistical analysis

Statistical analysis was performed using SPSS16.0 software. Count data were analyzed by chi-square test, and measurement data by independent samples t-test. P<0.05 was considered as statistically significant.

Results

Pathological type and histological classification

Of the 76 tumors, 55 tumors belonged to invasive ductal carcinoma, 8 tumors invasive lobular carcinoma, 5 tumors intraductal carcinoma, 2 tumors invasive cribriform carcinoma, and 6 tumors nonspecific invasive carcinoma.

Histological classification according to WHO standard: Of all tumors, 23 tumors were classified as stage I (30%), 36 as stage II (47%), and 17 as stage III (23%).

Relation between quantitative indicators on 3D color histogram and Ki-67 expression

Positive expression of Ki-67 was found in 46 of 76 tumors (61%), and negative expression was found in 30 tumors (39%). Cases positive for Ki-67 showed a significant increase of VI and VFI values (P<0.05); V and FI values also increased compared with negative cases, but the difference was not significant (P<0.05, **Table 1**).

Relation between 3D ultrasound characteristics, histological classification and Ki-67 expression

It was shown that cases positive for Ki-67 were more susceptible to lymph node metastases (P<0.05). Ki-67 expression varied greatly between histological grades (P<0.05). No differences of statistical significance were found in the occurrence of "sun-like symptoms" between the two groups (P>0.05) (**Table 2**).

Discussion

Ki-67 antigen is a nuclear protein expressed in proliferating cells, which is also known as a nuclear proliferation marker. Positive expression of Ki-67 is related to the development, metastasis and prognosis of breast cancer [2]. Many studies have been carried out with respect to the relation between 2D ultrasound characteristics of breast cancer and Ki-67 expression. So far we have known only limited reports on the relation between 3D color histogram and Ki-67 expression in breast cancer. Compared with traditional 2D ultrasound, 3D ultrasound can more realistically reflect the relationship between tumors and the surrounding tissues and angiogenesis in tumors through multi-slice and multi-perspective scanning. Offering a new tool for quantifying new tumor blood vessels, 3D color histogram allows a more accurate evaluation of tumor angiogenesis. We investigated the relation between quantitative blood flow parameters on 3D color histo-

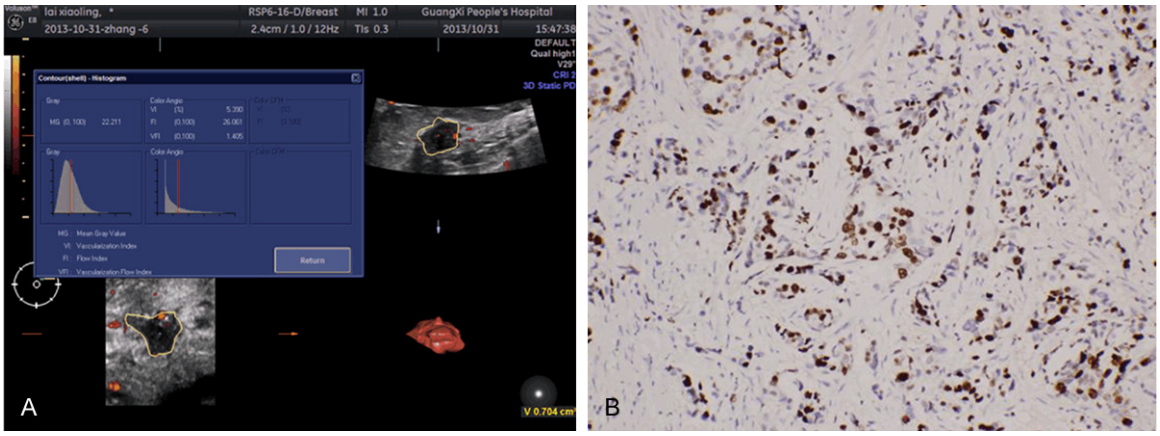

Figure 1. 3D color histogram and immunohistochemical staining for Ki-67-positive case. A: 3D color histogram; B: Immunohistochemical staining, Ki-67 positive (×20).

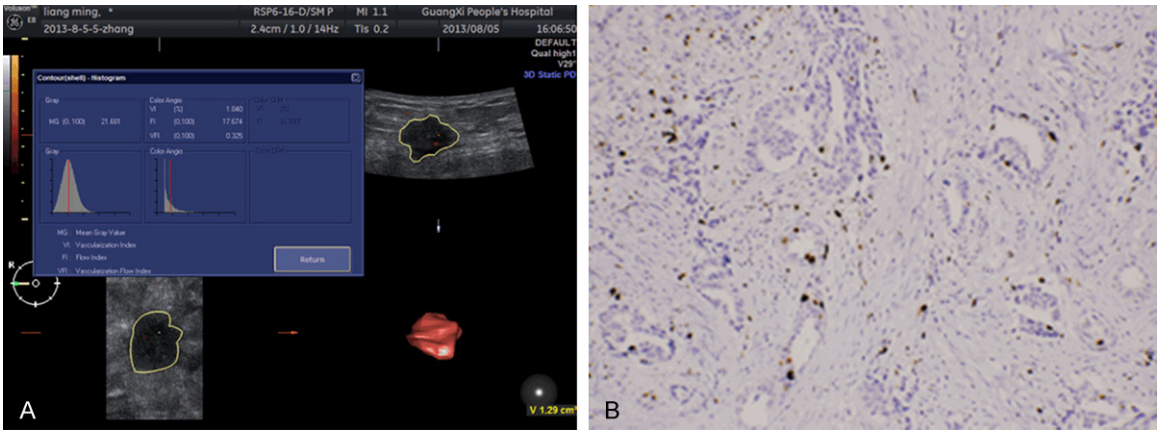

Figure 2. 3D color histogram and immunohistochemical staining for Ki-67-negative case. A: 3D color histogram; B: Immunohistochemical staining, Ki-67 negative (×20).

gram and Ki-67 expression so as to find reliable correspondence between biological behaviors and imaging characteristics of tumors.

Angiogenesis is the key step in the formation of solid tumors such as breast cancer, playing important roles in growth and infiltration of tumor cells. Tumor cells and the surrounding inflammatory cells produce angiogenic factors themselves or induce the formation of angiogenic factors. VEFG is the most effective factor inducing angiogenesis that has been known so far. It is found that Ki-67 and VEGF act in synergy during the occurrence and development of breast cancer and the expressions of the two show significant positive relation [3]. In our study, Ki-67-positive tumors contained cells with greater proliferative activity and had abundant blood supply, corresponding to higher indicators on 3D color histogram (**Figure 1**). In contrast, Ki-67-negative tumors had less blood supply and the indicators on 3D color histogram did no rise sharply (**Figure 2**). VI was the vascularization index of tumor, quantifying microvessel density not visible to naked eyes on ultrasound images. FI and VFI were average blood flow and total perfusion in the tumor, respectively. In the positive expression group, VI and VFI values were increased compared with those of negative expression group. The blood flow signals of tumors in the positive expression group were richer, which agreed with the findings by Huang et al. [4]. However, FI did not differ significantly between the two groups ($P>0.05$). Although microvessel density was increased in Ki-67-positive tumors, the blood flow, especially the blood flow in the microvessels was not necessarily increased by a large margin. Blood flow in tumors is not only affected by microvessel density, but also by blood flow rate in the microvessels. Since the blood flow in new tumor blood vessels encountered a

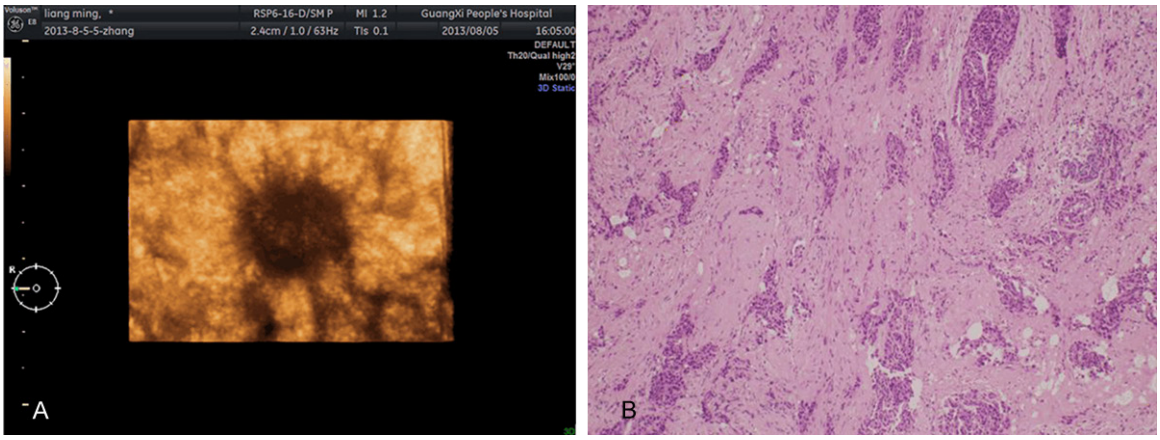

Figure 3. "Sun-like symptom" on coronal plane of 3D ultrasound image and histopathological pattern. A: "Sun-like symptom" in invasive ductal carcinoma; B: Infiltrative growth of tumor and stretching by surrounding fibrous tissues (HE, ×10).

high resistance, FI value was not increased obviously. Moreover, VI and VFI were increased but FI was not increased significantly, indicating that VI had a larger weight in the formula VFI=VI×FI than FI and the influence of VI was much greater than that of FI. That is to say, the increase of vascularization index led to the increase of total perfusion.

The mean size of the tumors positive for Ki-67 was greater than that of the tumors negative for Ki-67, but the difference was not significant (P>0.05), consistent with the results of Erden [5]. But there are oppositions to this view point [2, 6]. The disagreement may be attributed to differences in invasion fashion of tumors dependent on type and stage and the influence of stretching and restrictions by surrounding hyperplasic tissues. Therefore, high invasiveness of Ki-67-positive tumors is not directly correlated with tumor size. There is the possibility that 3D ultrasound is superior in detecting early-stage small-size breast tumors, or even the compact tumors that are otherwise misdiagnosed by mammary gland molybdenum target detection. "Sun-like symptom" on coronal plane is unique to 3D ultrasound imaging (**Figure 3A**), which has differentiating value between benign and malignant breast tumors [7]. However, we did not observe an obvious relation between "sun-like symptom" and Ki-67 expression (P> 0.05). One explanation is that positive expression of Ki-67 indicates strong invasiveness of tumors and the invasiveness of tumors varies with type and stage. "Sun-like symptom" is generally caused by proliferation and stretching of fibrous tissues and infiltration of cancer tissue into surrounding tissues (**Figure 3B**). Not all breast tumors show typical "sun-like symptom", and burr-like appearance may be observed in some cases.

Lymph node status and histological grade are two common prognostic indicators of tumors. After analysis, we found that positive expression of Ki-67 was correlated with lymph node metastasis and histological grade, consistent with some results by Azambuja [8]. The reason is probably that Ki-67-positive breast tumors contain more lowly differentiated cells, which may easily shed off to spread via lymphatic vessels and blood vessels. This explains the high histological grade and strong invasiveness.

Conclusion

To conclude, quantitative blood flow parameters on 3D color histogram and 3D ultrasound characteristics are correlated with Ki-67 expression. 3D ultrasound allows a preliminary prediction of biological behaviors and prognosis of tumors. However, many influence factors may interfere in the relations between the two. The feasibility of using 3D ultrasound to preclude the need for biopsies still needs confirmation from trials with large sample size.

Acknowledgements

This work was supported by the Guangxi scientific research and technique development Program (Grant number: GKG 0592007-2c & GKG 14124004-1-13); Guangxi medical and

health scientific research Program (Grant number: 200813).

Disclosure of conflict of interest

None.

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