

Original Article

Clinical Examination of Pathological Type and Intraductal Spread of Breast Cancer by Microbubble Contrast-Enhanced Color Doppler Ultrasonography—*Initial experience*—

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Abstract

(Objective) To assess the possibility of preoperatively diagnosing the pathological type of breast cancer by color Doppler ultrasonography using Levovist, an ultrasound contrast agent. (Subjects) Subjects were 19 female patients (aged 37 to 75 years) with primary breast cancer who underwent surgery between July and December 2001. Staging was 1 case of Tis, 5 cases of T1, 10 cases of T2, and 3 cases of T3 and above. As to pathological type, there were 7 cases of scirrhous carcinoma, 10 cases of papillo-tubular carcinoma, 1 case of lobular carcinoma, and 1 case of ductal carcinoma in situ. (Methods) Under optimal conditions for detecting intratumoral blood flow, 1,500mg/5ml of Levovist was administered intravenously, and color Doppler ultrasonography was performed without moving the probe. (Results) Intratumoral blood flow patterns, assessed by B-mode ultrasonography, were as follows: (1) "plunging" was the dominant pattern in 11 patients (58%); (2) "surrounding" was the dominant pattern in 4 patients (21%); and (3) no intratumoral blood flow was seen in 3 patients (16%). When Levovist was used in cases with results (1) and (2), intratumoral blood flow increased in 15 patients (79%) and increased markedly in 10 patients (53%). However, very little change was seen in the 4 individuals with no intratumoral blood flow. While a marked increase in intratumoral blood flow was seen in 7 of the 10 patients with papillo-tubular carcinoma (70%), it was only observed in 2 of the 7 patients with scirrhous carcinoma (29%). As to the relationship between scirrhous ratio and Levovist enhancement, the tendency was that the greater the scirrhous ratio, the lower the Levovist enhancement, and there was a significant difference between "high" and "very low" cases ($p < 0.01$). A high scirrhous ratio correlated with vascular resistance, namely increased PI, and decreased Vmax and Vmin. Furthermore, Levovist increased blood flow in an area with suspected intraductal spread only in one patient. (Conclusions) Even if US findings do not suggest attenuation

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(according to Kasumi's classification), when Levovist does not increase intratumoral blood flow, it is necessary to consider the presence of scirrhous carcinoma, and as a result, the use of Levovist could improve preoperative estimation of the pathological type of breast cancer. However, the use of Levovist had no effect on the preoperative diagnosis of intraductal spread of breast cancer.

(Key words : Ultrasonography, Microbubble, Pathological type, Intraductal spread, Scirrhous ratio)

Introduction

While mammography (MMG) is mostly used to screen and diagnose breast cancer in Europe and America, both ultrasonography (US) and (MMG) are performed at comparable frequencies in Japan. Unlike MMG, it is possible to noninvasively observe lesions in real time with US, and fine needle aspiration cytology (FNAC) can be performed under the guidance of US. US is also useful for estimating the pathological type of breast cancer and for assessing intraductal spread. However, in actual clinical settings, because breast cancer exhibits various morphologies, diagnosis of atypical tumors is sometimes difficult.

Contrast-enhanced echography was first used to improve the accuracy of noninvasive diagnostic techniques for hepatic tumors using CO₂ microbubbles¹⁾. In 1999, Levovist, an intravenous contrast agent, became commercially available, and contrast-enhanced color Doppler ultrasonography has been actively studied. Although there have been several reports on the usefulness of contrast-enhanced color Doppler ultrasonography for the diagnosis of breast cancer²⁾³⁾⁴⁾, no general consensus has been reached. This time, we investigated whether pathological type and intraductal spread of breast cancer can be preoperatively predicted by color Doppler ultrasonography using Levovist.

Subjects and Methods

Subjects were 19 female patients (mean age 53.4 years) who were diagnosed with primary breast cancer and underwent surgery at the Department of Surgery at Omiya Medical Center, Jichi Medical School, at some point between July and December 2001. According to General Rules for Clinical and Pathological Studies on Cancer of the breast⁵⁾, the patients were grouped into one of the three major pathological types (Scirrhous, Solid-tubular, and Papillo-tubular carcinoma), and when more than one type was observed, classification was based on the dominant pathological type. In addition, all tumors containing scirrhous carcinoma were divided with respect to the ratio of scirrhous as follows: "high" (>80%), "moderate" (50-80%), "low" (20-49%) and "very low" (<20%).

US was performed using a Toshiba SS-370A (Power Vision 6000) equipped with a 7.5 MHz electric linear probe. After performing B-mode (Power Doppler : PD) ultrasonography to identify optimal conditions for observing intratumoral blood flow, a 1,500mg/5ml bolus of Levovist was injected, and breast tissue was observed and videotaped for 5 minutes. Changes within 3 minutes postinjection were qualitatively compared to preinjection findings. According to Ueno's classification⁶⁾, patterns for intratumoral blood flow were divided into four groups :

plunging artery pattern (plunging), surrounding artery pattern (surrounding), spotty pattern (spotty), and absence of intratumoral blood flow (no flow). In this study, the plunging artery pattern included penetrating artery pattern. Using video images, the degree of Levovist enhancement was qualitatively assessed into three grades by four US specialists: marked (marked increase in intratumoral blood flow), moderate (moderate increase in intratumoral blood flow), and poor (relatively little increase in intratumoral blood flow). Among patients with marked and moderate enhancement in T2 or T3 cases, using the most well-defined blood flow waveform, PI (Pulsatility index = $V_{max} - V_{min} / V_{mean}$), RI (Resistive index = $V_{max} - V_{min} / V_{max}$), V_{max} (Peak velocity) and V_{min} (Minimum velocity) were measured. Kruskal-Wallis and Mann-Whitney U test were used for statistical analysis (Windows Stat Mate III).

As to intraductal spread, the probe was rotated in all directions around each tumor, and tubular structures extending from the tumor were identified. Intraductal spread was considered to be present when the tubular structures had irregular ductal structures or high echo spots, indicating internal calcification. With Levovist, a vessel extending from a tumor to a tubular structure indicated intraductal spread.

Results

Of the 19 patients studied, 6 were premenopausal (32%), and of these, 7 patients underwent breast conservative therapy (BCT: breast conservative therapy), 11 underwent Auchincloss' method, and 1 underwent Kodama's method. As to pathological types, 7 had scirrhous carcinoma, 10 had pap-tub carcinoma, and 2 had other types. Eight patients (42%) had breast cancer containing scirrhous carcinoma. As to breast cancer stage, there were 6 cases of T1 and less, 10 cases of T2, and 3 cases of T3 and above. As to clinical stage, there were 5 cases of stage I, 9 cases of stage II, 4 cases of stage III, and 1 case of stage IV. (Table 1)

The rate of preoperative diagnosis of pathological type by B mode was 71% (5/7) for scirrhous carcinoma and 70% (7/10) for pap-tub carcinoma. (Table 2) In cases in which the scirrhous ratio was "high", the rate of preoperative diagnosis of pathological type was 100% accurate, but two cases which were eventually diagnosed as having pap-tub carcinoma were initially misdiagnosed as having scirrhous carcinoma.

With regard to intratumoral blood flow patterns, "plunging" was the most common pattern (58%), followed by "surrounding" (21%) and "no flow" (16%). (Table 3) As to the relationship between flow pattern and pathological type, "plunging" accounted for about half of all cases of scirrhous and pap-tub carcinoma (46 and 56%, respectively), with no significant difference between the two types. (Table 4) As to the relationship between US flow patterns and Levovist enhancement, marked enhancement accounted for the majority of "plunging" and "surrounding" cases (70 and 75%, respectively), and as a result, Levovist enhancement was similar for these two patterns. (Table 5) In contrast, the degree of Levovist enhancement was poor in all 4 "no flow" cases, indicating that Levovist was not effective in those cases with no intratumoral blood flow, as assessed by PD ultrasonography. As to the relationship between pathological type and Levovist enhancement, marked enhancement accounted for 29% of the scirrhous cases

and 70% of the pap-tub cases. (Table 6) With regard to the relationship between scirrhous ratio and Levovist enhancement, the tendency was that the greater the scirrhous ratio, the lower the Levovist enhancement, and there was a significant difference between "high" and "very low" cases ($p < 0.01$). (Table 7) The two scirrhous carcinoma cases which were diagnosed preoperatively as pap-tub carcinoma by B mode showed marked or moderate enhancement by Levovist. Moreover, the greater the scirrhous ratio, the higher the PI, the lower the Vmin and the lower the Vmax. Between "high" and "very low", Vmin differed significantly ($p < 0.05$). (Figure 1) (Table 8) While US identified possible intraductal spread in 12 patients, intraductal spread was pathologically confirmed in 10. However, Levovist enhanced the blood flow in a suspected area in only one of the 10 patients (10%).

Table 1. Characteristics of the patients

- # Subjects: 19 female patients who were diagnosed with primary breast cancer and underwent surgery at the Department of Surgery at Omiya Medical Center, Jichi Medical School, at some point between July and December 2001.
- # Sex: all females
- # Average years: 53.4 years, Pre-menopausal: 6 (31.6%)
- # Operation: BCT *7, Auchincloss 11, Kodama 1
- # Pathological type: Scirrhous 7, Pap-tub 10, et al 2
- # T factor: $\leq T1$ 6, $T2$ 10, $T3 \leq 3$
- # Stage: I 5, II 9, III 4, IV 1

* Breast conservative therapy

Table 2. The rate of preoperative diagnosis of pathological type (scirrhous or pap-tub carcinoma) by B mode

Finally pathological type	Preoperative diagnosis rate
Scirrhous	5/7 (71%)
Pap-tub	7/10 (70%)
Total	12/17 (71%)

Table 3. Flow pattern of breast cancer by Ultrasound (Power Doppler Method)

Power Doppler Flow pattern	Cases
* Plunging artery	44 (58%)
Surrounding marginal	4 (21%)
Spotty artery	1 (5%)
No flow	3 (16%)
Total	19

* Including penetrating artery pattern

Table 4. The Correlation between US Flow pattern and Pathological type

Flow pattern	Cases	Pathological type		
		Scirrhous*	Pap-tub*	Others
Plunging	11	5 (46%)	6 (55%)	0
Surrounding	4	1 (25%)	2 (50%)	1 (25%)
Spotty	1	0	1 (100%)	0
No flow	3	1 (33%)	1 (33%)	1 (33%)
Total	19	7	10	2

* $p = 0.688$, Kruskal-Wallis test. "Plunging" accounted for about half of all cases of scirrhous and pap-tub carcinoma, with no significant difference between the two types.

Table 5. The correlation between US Flow pattern and Enhance grade using Levovist

Flow pattern	Cases	Levovist Enhancement		
		Marked	Moderate	Poor
Plunging*	10	7 (70%)	3 (30%)	0
Surrounding*	4	3 (75%)	1 (25%)	0
Spotty	1	0	1 (100%)	0
No flow	4	0	0	4 (100%)
Total	19	10 (53%)	5 (26%)	4 (21%)

*p=0.857, Kruskal-Wallis test. Between “Plunging” and “Surrounding”, Levovist Enhancement differed no significantly.

Table 6. The correlation between Pathological type and Enhance grade using Levovist

Pathological type	Cases	Levovist Enhancement		
		Marked	Moderate	Poor
Scirrhou*	7	2 (29%)	2 (29%)	3 (43%)
Pap-tub*	10	7 (70%)	3 (30%)	0
Lobular	1	0	0	1 (100%)
Non invesive	1	1 (100%)	0	0
Total	19	10	5	4

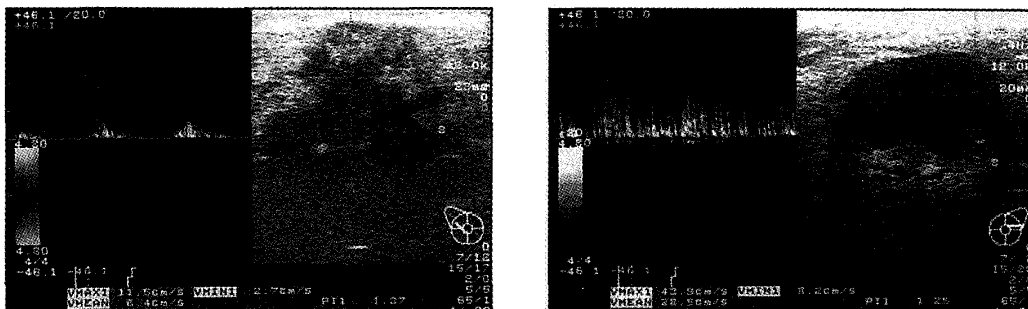
*p<0.05, Kruskal-Wallis test. Between “Scirrhou” and “Pap-tub”, Levovist Enhancement differed significantly.

Table 7. The correlation between scirrhou ratio and enhance grade using Levovist

Scirrhou ratio	Cases	Levovist Enhancement		
		Marked	Moderate	Poor
High*	4	0	1 (25%)	3 (75%)
Moderate	3	2 (67%)	1 (33%)	0
Low	1	0	0	1 (100%)
Very Low*	11	8 (73%)	3 (18%)	0
Total	19	10	5	4

*p<0.01, Kruskal-Wallis test. Between “high” and “very low”, Levevist Enhancement differed significantly.

a: scirrhou carcinoma (enhance grade:moderate) b: papillo-tubular carcinoma (enhance grade:marked)



Scirrhou ratio correlates with vascular resistance, namely increased PI, and decreased Vmax and Vmin.

Figure 1. Power doppler image of scirrhou carcinoma and papillo-tubular carcinoma

Table 8. The correlation of scirrhus ratio and PI, RI, Vmax, Vmin in marked and moderate enhancement grade cases using Levovist (T2, 3 cases)

Scirrhus ratio	Cases	Levovist Enhancement			
		PI	RI	Vmax (cm/s)	Vmin (cm/s)
High	1	1.38	0.77	11.5	2.7*
Moderate	2	1.23±0.18	0.76±0.08	18.6±10.4	3.7±3.52
Very Low	8	1.08±0.33	0.65±0.07	24.3±18.1	7.0±3.86*

* $p < 0.05$, Mann-Whitney U test. Between "high" and "very low", Vmin differed significantly.

Discussion

Levovist is a new agent that enhances detection of ultrasonic signals associated with blood flow by generating microbubbles. Since microbubbles generated by Levovist do not leak out of vessels, enhancement is believed to indicate rich intratumoral blood flow⁷. While studies on ultrasonography using Levovist have been mainly conducted on hepatic and cardiac lesions, the number of reports on body surface lesions has increased in recent years²⁾³⁾⁴. Enhancement of body surface lesions suggests angiogenesis and neovascularization, and it may aid the diagnosis of malignant cancer⁷, but no conclusive evidence has been obtained. This time, we performed color Doppler ultrasonography using Levovist on 19 females with primary breast cancer. When a feeding vessel was identified by PD ultrasonography, marked or moderate enhancement was seen in 70% of the cases, irrespective of US flow patterns. On the other hand, Levovist was not effective among the "no flow" cases. These findings suggest that Levovist enhancement requires intratumoral vessels that can be identified by PD ultrasonography, and it is difficult to identify minute angiogenesis.

The rate of preoperative diagnosis of pathological type of breast cancer by B-mode was about 70% for both scirrhus and pap-tub carcinoma, Diagnosis was correct in all cases in which the scirrhus ratio was "high", although 2 cases of pap-tub carcinoma were initially misdiagnosed as scirrhus carcinoma. As to the relationship between pathological type and Levovist enhancement, marked enhancement accounted for 70% of the pap-tub cases and 29% of the scirrhus cases, indicating that Levovist enhancement was poor in scirrhus cancer. As to the relationship between scirrhus ratio and Levovist enhancement, the tendency was that the greater the scirrhus ratio, the lower the Levovist enhancement ($p < 0.01$). The two pap-tub carcinoma cases were preoperatively misdiagnosed as scirrhus carcinoma by B-mode. With color Doppler ultrasonography using Levovist, one of these cases showed marked enhancement, while the other case showed moderate enhancement. Thus the preoperative use of Levovist strongly suggested that the scirrhus ratio was poor. This indicates the possibility that Levovist can improve the preoperative prediction of pathological type of breast cancer. As pap-tub carcinoma has a tendency towards ductal spread, it is necessary to closely follow cases in which Levovist shows enhancement.

The most notable difference between scirrhus carcinoma and other pathological types is that the ratio of fibrous compounds in tumor tissue is greater, and this morphological difference is reflected by the fact that US reveals attenuated posterior echo in many patients. One of the reasons for a poor response to Levovist by scirrhus carcinoma could be one of its histological characteristics, i.e., blood flow resistance due to intratumoral fibrosis. Vascular resistance can

be assessed by US in terms of PI, RI, Vmax and Vmin. While some studies have suggested a correlation between scirrhous compounds and PI¹⁰⁾ or Vmax¹¹⁾, and between intratumoral fibrosis and PI or Vmin¹¹⁾, no general consensus has been reached on the value of vascular resistance for diagnosing malignancy⁸⁾⁹⁾¹⁰⁾. Although only a small number of patients were enrolled in the present study, the results strongly suggest that a high scirrhous ratio correlates with vascular resistance, namely increased PI, and decreased Vmax and Vmin. Because there has been no definitive study on the relationship between pathological type and angiogenesis in breast cancer¹²⁾¹³⁾¹⁴⁾ and Levovist is an agent to enhance ultrasonic signals associated with blood flow and does not induce marked changes in vascular resistance, the following conclusions can be drawn : 1) poor Levovist response closely correlates with increased vascular resistance, and 2) US using Levovist is useful for the detection of scirrhous carcinoma. However, with quantitative analyses, the objectivity of machines needs to be established, and the problem of dealing with artifacts must be addressed. We are planning to quantitatively assess qualitative changes by studying a larger patient population.

As far as detecting intraductal spread was concerned, Levovist did not appear to be useful. US visualizes ductal spread as a low-echo tubular structure extending from a main tumor¹⁵⁾, but in the present study, Levovist enhanced the blood flow of a tubular structure in one patient only. Maeda and colleagues¹⁶⁾ reported angiogenesis in infiltrating carcinoma using microangiograms, while Gilles and colleagues¹⁷⁾ documented interstitial vascular proliferation in ductal carcinoma in situ (DCIS). One study found that the quantity and quality of intraductal cancer cells, vessels and interstitial components varied among different pathological subtypes¹⁸⁾. Ductal spread extends from the edge of a tumor, and consists mostly of angiogenesis, not the main feeder. Since Levovist is not very effective against vessels that cannot be identified by PD ultrasonography, it would be difficult to assess intraductal spread using Levovist.

Conclusions

1. B-mode (PD) ultrasonography showed no significant differences in intratumoral blood flow patterns between scirrhous and pap-tub carcinomas. However, with color Doppler ultrasonography using Levovist, enhancement was seen with pap-tub carcinoma, but not with scirrhous carcinoma.
2. Color Doppler ultrasonography using Levovist is a useful method for the detection of scirrhous carcinoma, thus it could improve preoperative prediction of the pathological type of breast cancer. As pap-tub carcinoma has a tendency towards ductal spread, it is necessary to closely follow cases in which Levovist shows enhancement.
3. Levovist was not very useful in the preoperative assessment of intraductal spread of breast cancer.

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超音波造影剤を用いた乳癌組織型，乳管内伸展の検討

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要 約

(目的) 乳癌組織型，乳管内進展に関して超音波造影剤（以下レボピスト）併用ドプラ法を行い検討したので報告する。(対象) 2001年7-12月に手術を施行した原発性乳癌19症例でTis 1例，T1 5例，T2 10例，T3以上3例。組織型は硬癌7例，乳頭腺管癌10例，その他2例。(方法) 腫瘍内血流の出現最適条件下でレボピストを1,500mg/5ml 静注し5分間の観察を行った。

(結果) 血流パターンは① plunging sign58%，② surrounding sign21%，③血流が観察されない症例16%。レボピスト使用で①②は79%に血流の増加を、また53%に強度の増加を認めたが、

③では殆ど変化は認められなかった。乳頭腺管癌では70%に強度の血流増加を認めたが、硬癌では29%であった。硬癌成分が増加すると造影効果は低下を示し、硬癌成分 High, Very low 間では有意差を認めた ($p < 0.01$)。乳管内伸展に血流増加を認めたのは1例のみであった。(結語) 通常 US で減衰型でない腫瘍像でも、レボピストで血流の増加を認めない症例では硬癌成分の合併は考慮すべきである。レボピスト併用で術前乳癌組織型推定率が上昇しうる可能性が示されたが、乳癌の乳管内伸展の診断には寄与しなかった。

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