

Color-Doppler Using Contrast Medium in Evaluating the Response to Neoadjuvant Treatment in Patients with Locally Advanced Breast Carcinoma

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Abstract. *Background:* The aim of our study was to assess the color-Doppler ultrasound (CDU) pattern in the analysis of neoadjuvant preoperative treatment of patients with locally advanced breast carcinoma, improvement after injection of contrast medium (Levovist) and possible correlations between morphological and vascular aspects of the neoplasm and postoperative histopathological findings. *Materials and Methods:* We studied 50 patients affected by locally advanced breast carcinoma (T3a e b-T4), using CDU before and after injection of Levovist, prior to and after neoadjuvant chemotherapeutic treatment. *Results:* The use of Levovist for ultrasound examinations prior to treatment revealed a higher number of vascular signals in 94% of the lesions compared to the basic color-Doppler examination; in only 3 cases (6%) were no modifications observed after injection of the contrast medium. This finding was also evident after neoadjuvant treatment, as a greater number of vessels in 28 lesions were observed, in addition to residual vascularization in 9 patients in whom the basic color-Doppler examination demonstrated substantial avascularity. Histopathology revealed that this method was more sensitive in disclosing the presence of active neoplastic tissue. *Conclusion:* Color-Doppler ultrasound is the first step in assessing the efficacy of neochemotherapeutic treatment in patients affected by locally advanced breast carcinoma. Levovist increases sensitivity and improves the diagnostic precision, thus allowing for a better image of the vessels, which is an important index of the biological activity of the neoplasm, compared to the basic color-Doppler examination.

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Locally advanced breast carcinoma (T3-T4) is a pathology that has a poor long-term prognosis. The most common therapeutic protocol includes, first, chemotherapy and/or hormone therapy, sometimes followed by surgery if necessary. Total remission or a significant partial remission makes it possible to perform surgery on otherwise inoperable patients and reduces the number of radical mastectomies, causing only minor debilitating effects on the patients. Furthermore, the average overall survival improves after neoadjuvant treatment; it is greater than 50% after three years (3, 4).

Vascular changes induced by treatment are of particular importance. Some authors have made a correlation between neoplastic vascularization and tumor growth (12), as well as the rate of metastatic dissemination during surgery (6).

Many malignant tumors are characterized by the development of autonomous blood vessels, and this type of vascularization, which is recognized as a distinct sign of malignity (neoangiogenesis), represents a significant indicator of the biological activity of the tumor itself. Direct assessment of not only tumor volume but also vascularization provides precise evidence of the patient's response to the neoadjuvant therapy.

Materials and Methods

From January to December 2002, at the National Tumor Institute of Naples, Italy, 50 patients with locally advanced carcinoma of the breast were studied. Tumor volume ranged from 8 to 57 ml, and the patient age ranged from 44 to 80 years (mean 55 years). All patients underwent the following: complete routine staging including, first, color-Doppler ultrasound, followed by intravenous injection of contrast medium (Table I); in all cases we performed a core biopsy for micro-histological examination of the tumor, using a UNICUT needle (14 G).

The patients were included in one of two protocols, either hormonal or neoadjuvant chemotherapy. Next, we performed a restaging, using color-Doppler ultrasound before and after intravenous injection of the contrast medium (Table II) (9). Finally, all patients underwent surgery; 60% underwent a radical mastectomy, while the remaining 40% underwent a conservative procedure.

Table I. *B-mode and color-Doppler ultrasound before and after pre-neoadjuvant treatment, in patients with locally advanced breast carcinoma.*

B-Mode		PDU (vascularization)					Levovist		
Patients	Vol. cc	Site	Grade	Poles	PI	RI	SP cm/s	Dose	Increase
1	22	2	2	+	1	0.6	46	300	2
2	18	1	2	+	1.1	0.7	34	300	1
3	33	1	2	+	1.1	0.7	45	300	2
4	67	2	2	+	1.9	0.8	23	300	2
5	12	2	2	+	1.4	0.8	28	300	2
6	15	-	0	0	-	-	-	2x300	1
7	20	1	1	+	1.2	0.7	28	300	2
8	32	2	2	+	1.4	0.8	31	300	2
9	18	1	1	+	1.3	0.7	22	2x300	1
10	16	1	1	+	1.9	0.9	18	2x300	1
11	33	2	2	+	1.7	0.8	33	300	1
12	28	2	2	+	1.6	0.8	27	300	1
13	27	2	2	+	1.4	0.7	35	300	1
14	8	-	0	-	-	-	-	3x300	0
15	32	2	2	+	1.2	0.7	21	300	2
16	14	2	1	+	1.0	0.7	24	2x300	2
17	12	1	1	+	1.5	0.7	25	2x300	1
18	52	2	2	+	1.4	0.8	32	300	2
19	14	1	1	+	1.1	0.6	28	2x300	1
20	44	2	2	+	1.2	0.7	20	300	1
21	17	2	1	+	1.3	0.8	27	300	2
22	8	-	0	0	-	-	-	3x300	0
23	33	2	1	+	2.2	0.9	20	300	2
24	32	2	1	+	1.3	0.6	25	300	1
25	37	2	1	+	1.3	0.8	30	300	3
26	15	1	1	1	1.9	0.9	23	300	1
27	34	1	1	+	0.9	0.6	45	300	1
28	39	2	0-1	+	1.4	0.8	41	300	3
29	24	1	1	+	1.7	0.8	33	300	1
30	8	2	1	+	1.2	0.7	28	300	1
31	42	2	2	+	1.6	0.8	35	300	2
32	22	1	1	+	1.8	0.7	16	2x300	1
33	18	1	1	1	1.6	0.8	24	2x300	1
34	32	2	1	+	1.5	0.9	36	2x300	3
35	10	-	0	-	-	-	-	3x300	0
36	45	2	2	+	1.5	0.8	46	300	1
37	42	2	2	+	1.4	0.8	41	300	1
38	35	2	2	+	2.1	0.7	37	300	2
39	58	2	2	+	1.8	0.6	28	300	2
40	16	1	1	1	2.0	0.6	16	2x300	1
41	45	2	1	+	1.8	0.8	24	300	3
42	32	2	1	+	1.8	0.9	22	2x300	1
43	35	2	2	+	1.8	0.9	36	300	2
44	28	1	1	1	1.2	2.0	27	2x300	1
45	26	1	2	+	1.3	1.8	24	300	2
46	24	1	1	1	1.7	1.4	33	2x300	1
47	18	1	2	+	1.6	1.5	36	2x300	2
48	42	2	2	+	1.8	1.6	29	300	1
49	18	1	2	1	1.7	1.8	32	300	1
50	26	2	2	+	1.2	1.8	27	300	1

Table II. *B-mode and color-Doppler ultrasound before and after Levovist post-neoadjuvant treatment, in patients with locally advanced breast carcinoma.*

B-Mode		CDU					Levovist		
Patients	Vol. cc	Grade	Poles	IP	IR	SP cm/s	Dose	Increase	
1	1.4	0	-	-	-	-	3x300	0	
2	0.34	0	-	-	-	-	300	1	
3	2.8	1	+	1.2	0.7	27	300	2	
4	5.1	0	-	-	-	-	300	1	
5	-	0	-	-	-	-	3x300	0	
6	1.1	0	-	-	-	-	3x300	0	
7	0.46	0	-	-	-	-	300	1	
8	3.6	1	+	1.1	0.6	22	300	1	
9	1.3	0	-	-	-	-	3x300	0	
10	0.8	0	-	-	-	-	3x300	0	
11	1.6	0	-	-	-	-	3x300	0	
12	2.7	1		1.3	0.7	21	300	2	
13	3.4	1	+	1.4	0.6	26	300	2	
14	3.0	0	-	-	-	-	3x300	0	
15	10.4	1	1	1.6	0.7	16	300	1	
16	0.6	0	-	-	-	-	3x300	0	
17	1.2	0	-	-	-	-	300	0	
18	7.9	1	+	1.2	0.7	10	300	2	
19	3.8	1	+	1.6	0.7	10	300	1	
20	13.3	2	+	1.0	0.8	22	300	1	
21	1.7	1	+	1.5	0.8	21	300	1	
22	2.7	0	-	-	-	-	3x300	0	
23	10.1	1	+	1.5	0.7	25	300	2	
24	12.0	1	1	1.2	0.5	18	300	1	
25	2.4	0	-	-	-	-	300	2	
26	4.8	0	-	-	-	-	3x300	0	
27	3.8	1	1	1.4	0.6	45	300	1	
28	7.1	1	+	1.3	0.6	7.6	300	1	
29	16.1	0	-	-	-	-	300	2	
30	10.0	1	+	1.2	0.6	22	300	1	
31	1.5	0	-	-	-	-	300	1	
32	6.4	0	-	-	-	-	3x300	0	
33	0.8	0	-	-	-	-	3x300	0	
34	7.0	0	-	-	-	-	3x300	0	
35	2.0	0	-	-	-	-	3x300	0	
36	5.0	0	-	-	-	-	3x300	0	
37	7.4	1	+	1.5	0.7	18	2x300	1	
38	3.2	0	-	-	-	-	3x300	0	
39	7.3	1	+	1.6	0.8	16	300	1	
40	2.1	0	-	-	-	-	3x300	0	
41	1.0	0	-	-	-	-	3x300	0	
42	-	0	-	-	-	-	3x300	0	
43	4.5	1	+	1.4	0.6	24	300	1	
44	0.7	0	-	-	-	-	3x300	0	
45	2.5	0	-	-	-	-	300	1	
46	3.4	1	+	1.6	0.8	18	300	2	
47	1.6	0	-	-	-	-	300	2	
48	1.9	0	-	-	-	-	300	1	
49	2.2	0	-	-	-	-	300	1	
50	3.4	1	+	1.6	0.8	23	300	2	

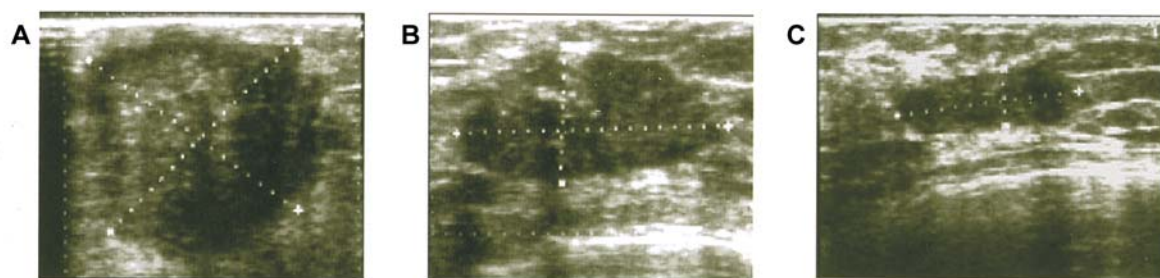


Figure 1. Carcinoma of the breast: B-mode ultrasound. A) Base morphology and volume. B) After two cycles of neoadjuvant chemotherapy: decrease in tumor volume of approximately 50%. C) After four cycles of chemotherapy: decrease in tumor volume > 50%.

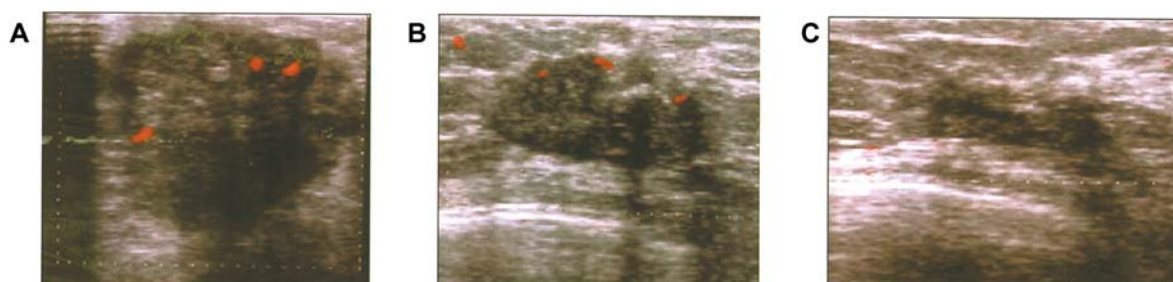


Figure 2. Carcinoma of the breast: color-Doppler ultrasound. A) Baseline: grade 1 vascularization. B) After two cycles of chemotherapy: moderate peripheral vascular signals. C) After four cycles of chemotherapy: no evidence of vascular spots.

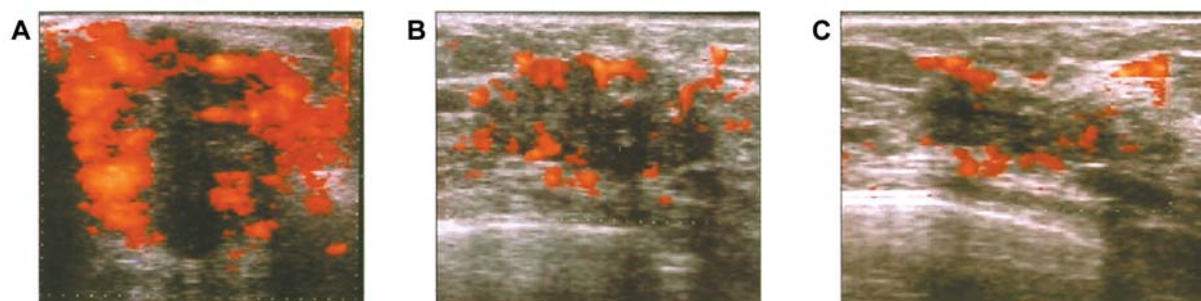


Figure 3. Carcinoma of the breast: color-Doppler ultrasound after Levovist injection. A) Grade 3 increase in contrast with rich intralesional and perilesional vascularization. B) After two cycles of chemotherapy: grade 2 increase compared to baseline. C) After four cycles of chemotherapy: moderate residual vascularization evident, but not evident with basic color-Doppler.

Ultrasound was performed using an Aloka 2000 with 7.5 MHz convex probes, before and after slow continuous *i.v.* infusion of Levovist (1 ml/s); one to three doses of Levovist were used per patient, at a concentration of 300 mg/ml (3, 10). The ultrasound machine was set with PRF and filters to detect slow flows. When Levovist is injected into the vein, it causes a reproducible and dose-dependent increase in back-scatter; the result is an amplified Doppler signal (approximately 10-20 dB).

Morpho-structural aspects and volume of the tumors were assessed using B-mode ultrasound; color-Doppler was used to assess the site and grade of vascularization, as well as the number

of vascular poles, pulsatility index (PI), resistance index (RI) and velocity of the systolic peak (SP).

Finally, after injection of Levovist, we assessed the increase in contrast. The grade of vascularization (0=absent, 1=moderate, 2=considerable, 3=marked) and the increase in contrast after injection of Levovist (0=absent, 1=moderate, 2=considerable, 3=marked) were quantified using an optical-visual scale in relation to the amount of color pixels visualized.

The instrumental examinations were always carried out by the same operator. Morpho-structural and vascular aspects were correlated with postoperative histopathological findings.

Results

Of the 50 patients, 41 were affected by infiltrating ductal carcinoma and 9 by lobular carcinoma; 70% presented axillary lymph nodes. B-mode ultrasound (Figure 1-A) revealed that all neoplasms had a hypo-echogenous and dishomogenous aspect with irregular margins. In 4 cases (8%), the basic color-Doppler examination did not reveal any vascularization (Figure 2 A); in 18 cases (36%), only peripheral vascularization and in 28 cases (56%) vascularization that was both intralesional and perilesional, with the presence of multiple vascular poles in 40 cases (80%) were identified. In 4 cases (8%), the grade of vascularization was 0, in 23 it was 1 (46%) and in 23 it was 2 (46%). The PI was between 0.9 and 2.2 (mean 1.46), the RI between 0.4 and 0.9 (mean 0.75), and SP between 16 and 46 cm/s (mean 30.15).

After injections of Levovist, only in 3 cases (6%) were no vascular changes observed, while in the remaining 47 cases (94%) an increase in contrast was observed (Figure 3-A), which was classified as grade 1 (52%), grade 2 (34%) or grade 3 (8%). Some patients were examined using CDU-Levovist in the middle of treatment (Figure 1-2-3 B).

Following neoadjuvant chemotherapy, the patients presented a partial response, with a reduction in the tumor that was greater than 50% (Figure 1-C) in 44 cases (88%), and less than 50% in 3 cases (6%); a complete response was observed in 2 cases (4%). Only one patient (2%) demonstrated a progression of the disease during the course of treatment.

The basic color-Doppler ultrasound examination (Figure 2-B) that was performed after neochemotherapy revealed the absence of vascularization in 31 out of 50 cases (62%), while in the other 38% of cases the grade of vascularization was 1 in 16 cases (32%), 2 in one case (2%) and 3 in 2 cases (4%). The pulsality index in these cases was between 1.0 and 1.7 (mean 1.39), the resistance index between 0.6 and 0.8 (mean 0.67), and the systolic peak between 10 and 45 cm/s (mean 20.5).

After injection of Levovist in 22 cases (44%), no vascular changes were observed, while in the other 28 cases (56%) an increase in contrast was observed (Figure 3-C), classified as grade 1 (36%) or grade 2 (20%). We did not observe any complications due to intolerance to the Levovist.

The visualization of vascular signals as a parameter of residual tumor biological activity was confirmed by post-surgical histopathological observations. Indeed, the presence of active tumor tissue was revealed in all cases in which vascular signals had been detected echographically (19 cases with basic color-Doppler alone and 28 cases after Levovist). On the other hand, in 6 out of 22 cases, which were negative according to the ultrasound, we observed the absence of active cells with a fibrotic-type residual mass (echo-histological agreement $34/50=68\%$).

Discussion

A preoperative assessment of the response to neoadjuvant chemotherapy in patients with locally advanced carcinoma of the breast using traditional methods (clinical examination, B-mode ultrasound and mammography) is mainly based upon the morphology and volume of the tumor. A detailed vascular map, that is both qualitative and quantitative, is an important indicator of the biological activity of the residual neoplasm and appears to be necessary for a more accurate assessment of the efficacy of the neoadjuvant treatment.

Ultrasound using color-Doppler is the examination of choice. Ultrasound is a non-invasive procedure, relatively inexpensive and easy to perform. In addition, despite technological advances, it is still difficult to evaluate slow blood flows in small vessels present in the residual tumor tissue after treatment (5, 8-11); as such, the use of contrast medium has increased the possibilities of basic color-Doppler.

Our results confirmed the efficacy of CDU in assessing patient response to neoadjuvant treatment. The use of Levovist permitted us to identify, prior to neoadjuvant treatment, a greater number of vascular signals in almost all of the lesions (94%) with grade 1, 2 and 3 increases in contrast (52%, 34% and 8%, respectively). Only in 6 cases (12%) were we unable to observe changes after injection of Levovist. This observation was equally evident after neoadjuvant treatment, as we were able to visualize a greater number of vessels in 28 lesions and observe the presence of vascular signals in 9 neoplasms that appeared to be primarily avascular during the basic color-Doppler examination.

Conclusion

In carcinoma of the breast, traditional analytic methods are primarily based upon monitoring the volume of the tumor during neoadjuvant therapy. Nevertheless, the significance of morphological and volumetric changes cannot be considered without considering the vascular aspect of a lesion as well. Consequently, the value of a quantitative analysis is limited without also incorporating a qualitative assessment. The present study demonstrated that color-Doppler ultrasound (CDU) with Levovist is sufficiently accurate in assessing vascular changes in carcinomas of the breast following preoperative therapy. CDU makes it possible to obtain a precise qualitative and quantitative vascular map of the tumor, which is a parameter of fundamental importance in establishing the efficacy of the patient's response to neoadjuvant therapy.

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