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Report

Monitoring response to primary chemotherapy in breast cancer using dynamic contrast-enhanced magnetic resonance imaging

Laura Martincich[1], Filippo Montemurro[2], Giovanni De Rosa[3], Vincenzo Marra[1], Riccardo Ponzone[4], Stefano Cirillo[1], Marco Gatti[5], Nicoletta Biglia[4], Ivana Sarotto[3], Piero Sismondi[4], Daniele Regge[1], and Massimo Aglietta[2]

[1]*Department of Diagnostic Imaging,* [2]*Department of Medical Oncology,* [3]*Department of Surgical Pathology,* [4]*Department of Gynecological Oncology,* [5]*Department of Radiation Therapy, Institute for Cancer Research and Treatment, Candiolo, Torino, Italy*

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Summary

Purpose. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) allows analysis of both tumor volume and contrast enhancement pattern using a single tool. We sought to investigate whether DCE-MRI could be used to predict histological response in patients undergoing primary chemotherapy (PCT) for breast cancer.

Patients and methods. Thirty patients with breast cancer, clinical diameter >3 cm or stage III A/B, received anthracycline and taxane based PCT. DCE-MRI was performed at the baseline, after two cycles and after four cycles of PCT, before surgery. Histological response was assessed using a five-point scheme. Grade 4 (small cluster of dispersed residual cancer cells) and grade 5 (no residual viable cancer cell) were defined as a major histopathological response (MHR).

Results. Univariate analysis showed that a >65% reduction in the tumor volume and a reduction in the early enhancement ratio (ECU) after two cycles of PCT were associated with a MHR. Multivariate analysis revealed that tumor volume reduction after two cycles of PCT was independently associated with a MHR (odds ratio [OR] 39.968, 95% confidence interval [CI] 3.438–464.962, $p < 0.01$). ECU reduction was still associated with a MHR (OR 2.50, 95% CI 0.263–23.775), but it did not retain statistical significance ($p = 0.42$). Combining tumor volume and ECU reduction after two cycles of PCT yielded a 93% diagnostic accuracy in identifying tumors achieving a pathological complete response (pCR) (histopathological grade 5).

Conclusions. DCE-MRI allows prediction of the effect of neoadjuvant chemotherapy in breast cancer. Although in our study tumor volume reduction after two cycles had the strongest predictive value, DCE-MRI has the potential to provide functional parameters that could be integrated to optimize neoadjuvant chemotherapy strategies.

Introduction

Primary chemotherapy (PCT) is administered to patients with breast cancer with the aim of inducing tumor regression before radical surgery [1–3]. The achievement of a pathological complete response (pCR) is associated with a survival advantage, and many current strategies employing PCT are aimed at maximizing the pCR rate [4, 5]. In fact, although clinical response rates range between 60 and 90% (including 20–40% complete remissions) with anthracycline and taxane-containing combinations, pCR occurs in a minority of the cases [5, 6]. Moreover, current methods of tumor response assessment during PCT do not predict pathological response accurately [7–9]. Serial monitoring of tumor response to PCT has the potential to increase the likelihood of achieving a pCR by switching to another regimen or treatment modality

those patients who are identified as poor responders to the initial regimen.

Magnetic resonance imaging (MRI) has a growing role in the management of breast cancer [10–13]. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) has recently emerged as a promising tool in cancer management [14–16]. Compared with morphological and volumetric parameters of conventional imaging modalities, the most important advantage of DCE-MRI is the ability to provide high-resolution images that depict functional parameters like perfusion and permeability of the tumoral capillary network [17]. The time–intensity curve represents temporal variations of flow and permeability of tumoral capillary network and reflect the absorption and clearance of the contrast. These data are correlated with tumoral neoangiogenesis and provide information on tumor-biological aggressiveness and response to medical therapy [18]. DCE-MRI has thus potential implications in the management of patients undergoing PCT, not only providing detailed morphological information (tumor shape, tumor size and changes during chemotherapy), but also functional parameters (contrast enhancement kinetics and perturbations in tumor blood supply) that can be related to the efficacy of the administered treatment. We were interested in evaluating whether morphological and functional parameters obtained by DCE-MRI could predict histological response in women with stage II and III ($T > 3$ cm) breast cancer receiving taxane-based PCT.

Patients and methods

Patients

At our Institution, PCT is offered to patients aged between 18 and 65 years, with fine needle aspiration biopsy proven stage II/III operable breast cancer ($T > 3$ cm), or with inoperable locally-advanced breast cancer. This study was conducted in compliance with the ethical regulatory issues of our Institution and patients were asked to provide written informed consent before study entry. A core biopsy of the tumor was performed after the baseline DCE-MRI of the breast in order to obtain adequate tumor tissue for histological diagnosis, determination of histological grade and hormone receptor status. Staging work-up was carried on according to Institutional guidelines. All of the patients were evaluated in our multidisciplinary clinic before PCT and upon completion of PCT.

Treatment schedule and disease assessment

The intended treatment for all the patients was a combination of doxorubicin 50 mg/m^2 bolus i.v. followed by paclitaxel 175 mg/m^2 as a 3 h i.v. infusion. In patients with baseline left ventricular ejection fraction (LVEF) <55%, doxorubicin was omitted and monochemotherapy with paclitaxel 225 mg/m^2 as a 3 h i.v. infusion was administered. Cycles were repeated every 21 days if ANC $\geq$ 1500/μl and platelets $\geq$ 100,000/μl, and otherwise delayed until resolution of hematologic toxicity. The planned number of cycles was four. Conventional evaluation of tumor response included palpation, mammography and/or ultrasonography. The clinical response was determined by calculating the change in the product of the to largest perpendicular tumor diameters [19]. Complete resolution of a palpable mass within the breast was termed a complete clinical response (cCR). A partial clinical response (cPR) was deemed to have occurred if the tumor size had been reduced by at least 50%. Progressive disease (cPD) was defined as an increase in tumor size of at least 25%. Patients in whom the breast cancers had undergone a clinical response other that defined by cCR, cPR or cPD were considered to have stable disease (cSD). The clinician assessing tumor response was blinded to the results of DCE-MRI. The results of the post-treatment DCE-MRI were available to the surgeon for surgical planning.

Definitive breast surgery had to be performed not less than 14 days and not more than 35 days after the completion of PCT.

The histopathological response to chemotherapy was evaluated using a five-point assessment scheme described by Smith et al. [5]: grade 1, some alteration to individual malignant cells but no reduction in overall numbers as compared with the pre-treatment core biopsy; grade 2, a mild loss of invasive tumor cells but overall cellularity still high; grade 3, a considerable reduction in tumor cells up to an estimated 90% loss; grade 4, a marked disappearance of invasive tumor cells such that only small clusters of widely dispersed cells could be detected; and grade 5, no invasive tumor cells identifiable in the sections from the site of the previous tumor, that is, only *in situ* disease or tumor stroma remained. Grade 5 response was deemed to represent a pCR of the primary cancer.

DCE-MRI

DCE-MRI was carried on before the first cycle of chemotherapy (baseline), within 2 week from the

second cycle (intermediate) and after the completion of the planned treatment, within 1 week before surgery. The radiologist carrying on the DCE-MRI examinations was blinded to the clinical outcome of the patients.

Magnetic resonance imaging of both breasts was acquired with 1.5T scanner by using a dedicated surface multicanal coil (GE Medical System, Milwaukee) with the patient in the prone position.

Morphological T1-weighted (slice thickness 5 mm) and FSEIR sequences in the sagittal plane (slice thickness 5 mm) were acquired before the dynamic evaluation.

The dynamic study was performed on the coronal plane using 3D SPGR fat suppressed sequence with TR 8.9 ms, TE 4.2 ms, flip angle 20°, slice thickness 2.6 mm without interval, matrix 256×256 and temporal resolution ranging from 60 to 90 s, according to the volume of the breasts and to the field of view (FOV). The 3D sequence was acquired before and five times continuously after i.v. injection of gadolinium chelate (Magnevist, Schering); a late acquisition was performed 2 min after the last sequence. Contrast-enhanced study was started simultaneously with the bolus injection of 0.1 mmol/kg of gadolinium chelate, infused in the antecubital vein by power injector, at a rate of 2 ml/s and followed by a saline flush.

The acquired images were transferred to a workstation (Advantage Window 3.0 and 4.1), for post-processing with dedicated software, such as Image Subtraction, Volume Analysis and Functool (GE Medical System, Milwaukee).

Image Subtraction of basal acquisition from all series of contrast-enhanced slices was performed to improve visualization of areas of contrast uptake and to evaluate its morphology.

The subtraction of basal acquisition from early contrast-enhanced slices was also used to calculate the volume of the lesion by a Volume Analysis software. In every subtracted slice, we defined the area of the tumor by a selection with electronic paintbrush. After this, the volume analysis software automatically calculated the volume of the selected areas, expressed in cm^3. If, in the context of the tumor lesion, necrosis was present, suggested by lack of contrast uptake during all the acquisitions of the dynamic exam, we calculated the volume of the necrotic portion and the value was subtracted from the entire volume of the lesion.

For the evaluation of the enhancement kinetic, we used the Functool Software (GE Medical System, Milwaukee). This is a color-coded software that visualizes the enhancement uptake in different shades of colors from blue to red: structures with early and fast enhancement show red color, whereas areas with late and slow uptake appear in different shades of blue-green. In addition this software allows the construction of the time–intensity (*T/I*) curve by positioning a 'region of interest' (ROI) within the area of enhancement. In every examination the region of interest (4 pixel – 7.9 mm^2) was positioned in standardized sites: in the center of the enhancing area in pattern 1 lesions and in the thickest point of the peripheral ring-like enhancement in pattern 2 lesions (see 'Results' for the definition of pattern 1 and 2 lesions).

The following parameters were evaluated: the volume of the lesions, the enhancement pattern, the early contrast uptake (ECU) in percent and the shape of the time–intensity curve. The ECU, was calculated by the following equation: $(SI_{max} - SI_{bas})/SI_{bas}$, where SI_{max} represents the maximum increase of signal intensity at the first sequence after contrast media administration, and SI_{bas} is the basal signal intensity. The shape of the curve was approximated to three reference types according to whether the signal intensity continued to increase after the initial upstroke (type I), whether it was cut off and reached a plateau (type II), or whether it washed out (type III).

Table 1. Patient demographics

	Number	Percentage
Median age in years (range)	49 (36–65)	
Median tumor diameter* (mm)	40 (30–70)	
Initial clinical stage		
IIA	6	20
IIB	14	47
IIIA	5	17
IIIB non inflammatory	2	7
IIIB inflammatory	3	10
Clinically involved nodes	22	73
IDC	27	90
ILC	3	10
Receptor status		
ER and/or PgR+	18	60
ER and PgR−	12	40

* Clinical assessment.
IDC, infiltrating ductal carcinoma.
ILC, infiltrating lobular carcinoma.
ER, estrogen receptor.
PgR, progesterone receptor.

Table 2. Clinical and histopathological response

Response	SD	Percentage[a]	PR	Percentage[a]	CR	Percentage[a]	Grade 4 and 5[b]	Percentage[a]
Clinical after two cycles	11	37	18	60	1	3	NE	–
Clinical after four cycles	4	13	20	67	6	20	NE	–
Histopathological	–		–		–		11	37

CR, complete response.
PR, partial response.
SD, stable disease.
[a] Because of rounding sum of percentages does not always equal 100.
[b] A major histopathological response was defined as a grade 4 or 5 histopathological response in the breast cancer. Six patients achieved a grade 5 and five a grade 4 response.

Signal intensity of normal glandular tissue before contrast medium administration was calculated both at the baseline and at the 2nd MRI to exclude potential biases introduced by signal intensity changes during chemotherapy.

Statistical methods

Our outcome of interest was a major histopathological response in the breast tumor, defined as either a grade 4 or 5 response according to the adopted system. The histopathological status of the axillary lymph nodes was not considered in the definition of the outcome of interest. For each of the primary breast cancers in the study, the following parameters obtained by DCE-MRI were evaluated: the baseline pattern of contrast uptake, the baseline shape of the *T*/*I* curve, the baseline tumor volume, the baseline ECU, the percent reduction in the tumor volume after two cycles of PCT, and the percent reduction in the ECU after two cycles of chemotherapy. ECU values were normally distributed. Comparison of the mean ECU values at the baseline and after two cycles of PCT was performed using a student's *t*-test for paired samples. Comparison of ECU values across different groups of lesions (pattern 1 and 2, *T*/*I* curve type 1 and 2) was performed using a student's *t*-test for independent samples. Because basal volume and basal ECU were not linearly associated with the outcome, we created four categorical variables using the quartiles as cutoff points. Percent volume and ECU reduction was dichotomized. To identify an optimal threshold for prediction of histopathological response, receiver operating characteristics (ROC) analysis was performed by incrementally increasing cutoff values and recalculating corresponding true-positive and false-negative rates. The chi-square test was used for univariate comparisons of categorical or dichotomous variables. Factors that were statistically associated with the outcome of interest ($p \leq 0.05$) were studied in a logistical regression analysis. Results of the latter are reported as odds ratio with their 95% confidence intervals (CI).

Results

Patient characteristics and clinical outcome

Thirty women entered the study between March 2000 and August 2002. Table 1 shows the patient characteristics. Median age was 49 years (range 36–65). Median number cycles of PCT was 4 (range 4–8). Twenty-nine women received doxorubicin–paclitaxel. One woman received paclitaxel alone because of borderline LVEF values. One patient with inoperable, locally advanced breast cancer, who had a partial response (PR) to four cycles of doxorubicin and paclitaxel, received two additional cycles of the same regimen before surgery. Definitive breast surgery was performed after a median of 30 days (range 19–36) from the completion of PCT, and consisted of modified radical mastectomy in 19 cases (63%), and conservative surgery in the remaining 11 cases (37%). All of the patients underwent level I–III axillary lymph node dissection. Overall clinical and pathological response is summarized in Table 2. At histopathological examination, 6 patients showed a grade 5, and 5 patients a grade 4 response, for a major histopathological response rate of 37% (95% CI 22–54%). Ten out of 19 patients achieving either a PR or a CR after two cycles of PCT, showed a grade 4 or 5 histopathological response. One patient failing to achieve a PR or CR after two cycles of PCT showed a grade 5 histopathological response. The achievement of either a PR or CR after two cycles of PCT was significantly associated with

the outcome of interest (univariate OR 11.111, 95% CI 1.778–104.809, $p = 0.04$).

DCE-MRI findings

Baseline findings

We studied a total of 30 focal enhancing lesions. The median basal volume of the lesions was 22 cm^3 (range 4–126 cm^3). The baseline DCE-MRI showed two distinct patterns of contrast enhancement: homogeneous (15 lesions, pattern 1), and peripheral ring-like (15 lesions, pattern 2) (Figure 1). Type 2 and type 3 *T/I* curves were seen in 6 and 24 lesions, respectively. The mean basal ECU was 231% (range 112–370%). Mean basal ECU values did not differ between pattern 1 and pattern 2 lesions (233% v.s. 230%, $p = 0.93$), or in lesions displaying a type 2 or 3 *T/I* curve (213% v.s. 236% $p = 0.51$).

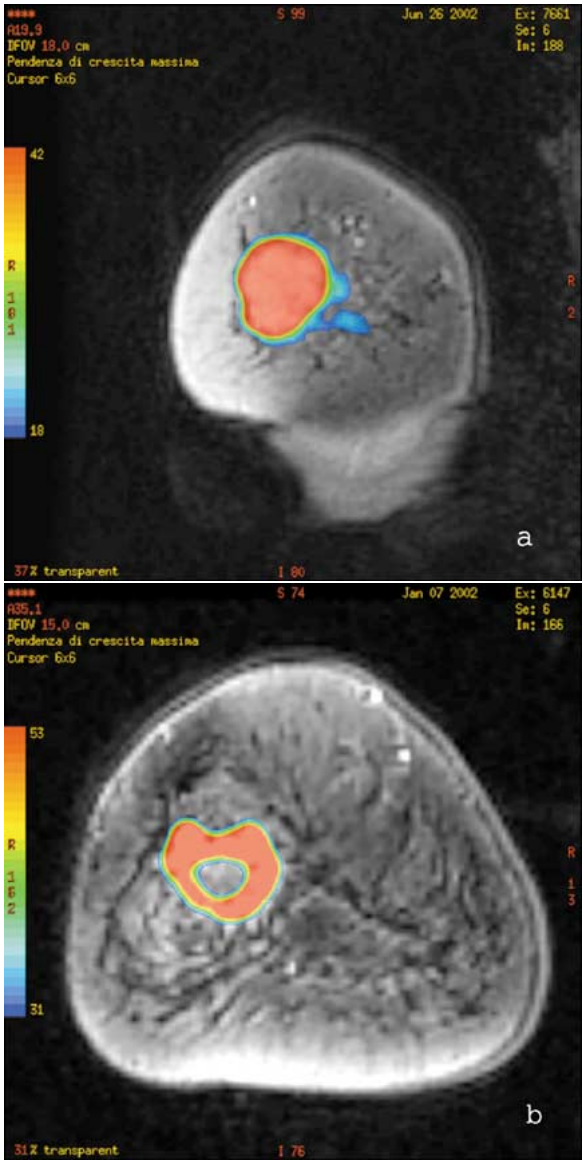

Figure 1. Contrast enhancement patterns. Panel a: example of a pattern 1 lesion, characterized by homogeneous enhancement. Panel b: example of a pattern 2 lesion, characterized by peripheral ring like enhancement.

After two cycles of PCT

The second DCE-MRI, which was performed a median of 11 days after the 2nd cycle of PCT (range 5–16), showed the following findings: the median tumor volume was 7 cm^3 (range 0–43 cm^3) and the mean tumor volume reduction was 62%. One lesion was no longer detectable (cCR). Mean tumor volume reduction was similar in pattern 1 and 2 lesions, and

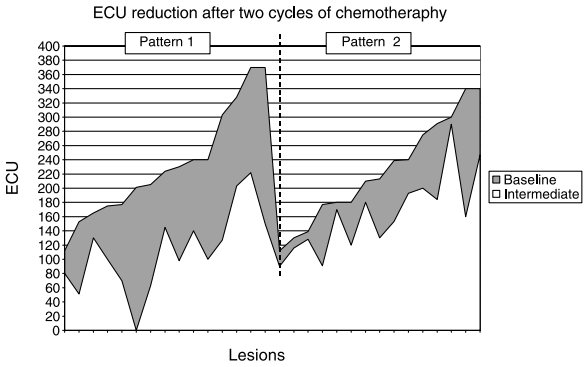

Figure 2. Changes in ECU between the baseline and after two cycles of PCT, grouped according their pattern of enhancement at the baseline (pattern 1 homogenous, pattern 2 peripheral ring-like). Each point on the abscissa represents a different lesion. The gray area corresponds to the percent reduction in ECU. Mean reduction values were significantly different between pattern 1 and 2 lesions (52% v.s. 27%, $p = 0.001$).

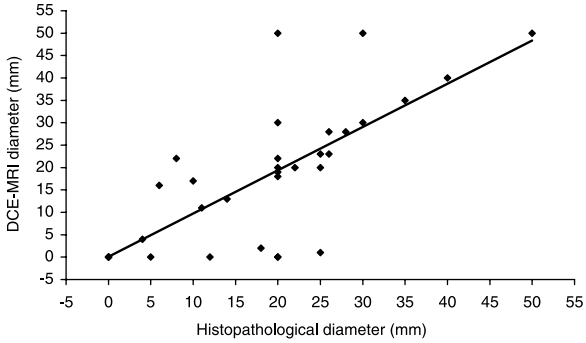

Figure 3. Histopathological tumor diameter (abscissa) and corresponding tumor diameter obtained by post-treatment DCE-MRI (ordinate). The correlation coefficient was 0.72, $p < 0.001$.

Table 3. Univariate analysis of factors associated with a major histological outcome

Variable		Success	Percentage[a]	Failure	Percentage[a]	p[b]
Basal enhancement pattern						
1	15	5	33	10	67	0.99
2	15	6	40	9	60	
Basal *T*/*I* curve type						
2	6	3	50	3	50	0.64
3	24	8	33	16	67	
Basal ECU values (%)						
≤176.5	7	1	14	6	86	0.10
176.6–218.5	8	5	62	3	37	
218.6–300,7	8	4	50	4	50	
>300.7	7	1	14	6	86	
Basal volume (cm^3)						
≤14.7	7	1	14	6	86	0.25
14.8–22.2	8	5	62	3	37	
22.3–38.2	8	3	37	5	62	
>38.2	7	2	29	5	71	
Volume reduction (%)						
≤65	17	1	6	16	94	<0.01
>65	13	10	77	3	23	
ECU reduction[c]						
0	19	4	21	15	79	0.05
1	11	7	64	4	36	

[a] Because of rounding sum of percentages does not always equal 100.
[b] Fisher's exact test.
[c] 0 = failure, defined as either a <58% or a <38% reduction in ECU after two cycles in group 1 and 2 lesions, respectively; 1 = success, defined as either a ≥58% or a ≥38% reduction in ECU after two cycles of PCT in group 1 and 2 lesions, respectively.

Table 4. Logistic regression analysis of factors associated with a major histopathological outcome

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	p	OR	95% CI	p
Volume reduction (%)						
≤65	1			1		
>65	53.301	4.815–585.592	<0.01	39.968	3.438–464.962	<0.01
ECU reduction						
0	1			1		
1	6.526	1.259–34.202	0.03	2.500	0.263–23.775	0.42

OR, odds ratio; CI, confidence interval.

in lesions displaying a basal type 2 or 3 curve (not shown). Overall, there was a significant reduction in ECU values after the second cycle of chemotherapy (Figure 2): mean ECU was 137% (range 0–290%, $p < 0.01$), corresponding to a mean 39% reduction with respect to the initial values. Notably, pattern 1 and pattern 2 lesions showed significantly different mean ECU values (112% v.s. 163%, $p = 0.02$) and

mean ECU reduction (52% v.s. 27%, $p = 0.001$). ECU reduction and volume reduction were not significantly correlated (Pearson Coefficient 0.28, $p = 0.13$). Separate analysis of correlation between ECU reduction and volume reduction in pattern 1 and 2 lesions yielded similar results (not shown).

Post-treatment DCE-MRI

After the completion of the planned therapy, DCE-MRI correctly detected absence of residual disease (histopathological grade 5) in three out of six lesions. In the remaining 3 cases DCE-MRI detected minimal residual enhancing areas showing a type 3 *T/I* curve shape. The presence of viable tumor was correctly identified in all of the 24 cases showing residual disease (histopathological grade 1–4). High-grade ductal carcinoma *in situ* (DCIS) was found in four cases with grade 1–3 response, in one case with grade 4 response, and in no cases with grade 5 response. In the case with grade 4 response and DCIS, DCE-MRI correctly evaluated the extension of residual disease, but failed distinguish between infiltrating and *in situ* carcinoma. The correlation coefficient between tumor diameter measured by histopathology and post-treatment DCE-MRI was 0.72 ($p < 0.001$) (Figure 3).

Univariate and multivariate analysis

We conducted a univariate analysis of factors associated with a major histopathological response. Percent volume reduction was dichotomized in $\leq$65% and $>$65% (see statistical methods). From now on a $>$65% reduction in tumor volume will be referred to as a 'volume reduction'. Because percent ECU reduction after two cycles of chemotherapy was significantly different in pattern 1 and 2 lesions, optimal thresholds were identified by running two separate ROC analyses. This variable was thus dichotomized in '1' ($\geq$58% reduction in pattern 1 lesions, or $\geq$38% reduction in pattern 2 lesions), or '0' ($<$58% reduction in pattern 1 lesions, or $<$38% reduction in pattern 2 lesions). From now on a $\geq$58% reduction in pattern 1 lesions or a $\geq$38% reduction in pattern 2 lesions will be referred to as an 'ECU reduction'. Table 3 shows the univariate analysis of factors associated with a major histopathological response. Volume reduction and ECU reduction were both significantly associated with a major histopathological response.

A multivariate logistic regression analysis was then conducted including both the above-mentioned factors. Results are summarized in Table 4. Volume reduction was strongly predictive of a major histopathological response (OR 39.968, $p < 0.01$). ECU reduction was also associated with histopathological outcome (OR 2.500), although it did not reach statistical significance ($p = 0.42$). No significant primary interaction was found between these two variables (not shown).

Combined volume and ECU reduction

A tumor volume reduction after two cycles of chemotherapy occurred in 10 out of 11 lesions achieving either a grade 4 or 5 histological responses (91%

Table 5. Summary of volume and ECU changes

Lesion ID	Pattern	ECU, reduction[a]	>65% reduction in volume	Final response
1	1	+	+	5
2	2	+	+	5
3	1	+	+	5
4	1	+	+	5
5	2	+	+	5
6	2	+	+	5
7	1	+	+	4
8	2	−	+	4
9	2	−	+	4
10	2	−	−	4
11	1	−	+	4
12	1	−	−	3
13	1	−	−	3
14	1	+	−	2
15	1	−	−	2
16	2	−	−	2
17	2	+	−	2
18	1	+	+	2
19	2	−	−	1
20	1	+	−	1
21	1	−	−	1
22	2	−	+	1
23	1	−	−	1
24	2	−	−	1
25	1	−	−	1
26	2	−	−	1
27	2	−	−	1
28	2	−	−	1
29	2	−	−	1
30	1	−	+	1

ECU, early contrast uptake.

[a] ECU reduction was defined as a $\geq$58% reduction in pattern 1 lesions or a $\geq$38% reduction in pattern 2 lesions.

sensitivity). Three patients showing volume reduction failed to achieve a major histopathological response (84% specificity). When combined with ECU reduction, all of the six cases showing a grade 5 histopathological response achieved both tumor volume and ECU reduction after two cycles of chemotherapy (Table 5). One lesion achieving both volume and ECU reduction had a grade 4 histopathological response. One lesion, which was an inflammatory breast cancer showing both volume reduction and ECU reduction after two cycles, failed to achieve a grade 5 histopathological response. The patient bearing this tumor received four cycles of PCT and achieved a cCR. DCE-MRI performed before surgery failed to show evidence of viable tumor. Histopathology of this case revealed regressive changes in the tumor bed, but persistence of multiple neoplastic emboli, which were scattered in the breast lymphatics over an area of 7 cm in major diameter. With respect to the achievement of a pCR (grade 5), the diagnostic accuracy of combined volume and ECU reduction was 93% (28 out of 30 correct predictions).

Discussion

This study shows that the integration of morphological and functional parameters provided by DCE-MRI has a potential role in predicting the histopathological outcome early in the course of PCT in patients with breast cancer. The strongest predictive factor for our outcome of interest (grade $4+5$ histopathological response) was a more than 65% tumor volume reduction after two cycles of PCT. To our knowledge this is the first report of such an association using MRI techniques. A 65% tumor volume reduction corresponds to a 50% reduction in the major diameter product and to a 30% reduction in the major diameter of tumors, which are criteria conventionally used to define a PR clinically [20]. One obvious question is whether a PR or CR after two cycles of PCT, as assessed conventionally (major diameters product measured by palpation, mammography and/or ultrasonography), could have predicted the histopathological outcome as effectively as DCE-MRI. In our study, the achievement of either a PR or CR after two cycles of PCT was significantly associated with the outcome of interest (OR 11.111), with a sensitivity, specificity, positive predictive value, and negative predictive value of 91%, 53%, 53%, and 91%, respectively. Despite similar sensitivity and negative predictive value, due to 1 false negative out of 11 negative results (91%), tumor volume reduction measured by DCE-MRI yielded less false positive results compared with conventional assessment (3 v.s. 9 cases) resulting in higher specificity (84% v.s. 53%), and positive predictive value (77% v.s. 53%). Superiority of DCE-MRI over conventional assessment of response is also suggested by its higher univariate OR (53.301 v.s. 11.111). However, as our study was not designed and powered to compare DCE-MRI with the other modalities of tumor response assessment, our results should be interpreted with caution and serve as a prompt for adequately designed prospective studies.

The correlation analysis between the histopathological and post-treatment DCE-MRI tumor diameter measurement yielded a coefficient of 0.72, similar to that reported recently by Wasser et al. [21] in 21 patients treated with PCT. Our findings, and that of other authors reporting even higher correlation coefficients [11, 22], confirm the worth of DCE-MRI in estimating the size of residual tumor after PCT.

DCE-MRI has the ability to provide additional information related to the dynamics of contrast uptake before and during chemotherapy, which could be exploited clinically in breast cancer patients undergoing PCT. We identified two baseline enhancement patterns: homogeneous and peripheral-ring like. The baseline enhancement pattern was not associated with histopathological outcome in our study. However, we found a statistically different ECU reduction in pattern 1 and pattern 2 lesions after two cycles of PCT. Studying appropriate cut-offs, which were different for pattern 1 and 2 lesions, we found that ECU reduction was predictive of histopathological response at the univariate analysis. This finding suggests that the initial pattern of contrast enhancement might have a complex relationship with tumor biology and response to treatments. Recent observations by Esserman et al. [23] using contrast-enhanced MRI seem to support this view. The authors reported five distinct MRI enhancement patterns, which they defined as 'MRI phenotypes' in 33 patients receiving PCT for four cycles. Seventy-seven percent of tumors appearing as a circumscribed mass had a change in the longest diameter fitting the criteria for a CR or a PR, whereas tumors showing one of the four other phenotypes had a 0–37.5% PR or CR rate.

Although our multivariate analysis showed lack of independent statistical value of ECU reduction, the upper CI of the hazard ratio suggests a potential relevance of such parameter, independently of tumor volume reduction. The ECU values are known to be

associated with tumor neovascularization, and their reduction is suggestive of regressive phenomena as a consequence of effective cytotoxic therapy [14].

The most interesting finding in our study is the fact that all of the lesions achieving a pathological complete remission (grade 5) achieved both volume and ECU reduction after two cycles of PCT, corresponding to a diagnostic accuracy of 93% (28 out of 30 correct predictions). One of the two misclassified cases was an inflammatory breast cancer. As described in the results section, this tumor showed a >65% volume reduction and an ECU reduction after two cycles of PCT, and a clinical and DCE-MRI complete remission after four cycles of PCT. However, histopathology revealed the persistence of extensive disease in the breast lymphatics. This false positive result is likely to be due to the fact that MRI techniques are not optimal to characterize inflammatory breast cancer [24]. Moreover, regressive phenomena occurring during chemotherapy might have further impaired the diagnostic accuracy of DCE-MRI in detecting residual disease [25].

One of the main limitations of our study was the semi-quantitative method that was used to study time/intensity curves. It is generally agreed that changes in the percentage of signal intensity at fixed time points after the contrast has entered the tumor region have a correlation with tumor vessels and the presence of viable cancer cells [14, 16]. Reduction in the percentage of signal intensity during chemotherapy is associated with tumor response. However, semiquantitative measures like signal intensity changes over time are reader dependant and suffer from lack of reproducibility [16]. Newer quantitative DCE-MRI parameters, like those correlated to the pharmacokinetics of the contrast agent, are now increasingly being studied [16, 26]. These methods are credited to be reproducible and to yield more detailed insights into the physiology and biology of individual tumors. Wasser et al. [21] used DCE-MRI with high temporal resolution in their study involving 21 patients with locally advanced breast cancer receiving PCT. The authors quantified the contrast enhancement by a pharmacokinetic two-compartment model previously described by Brix et al. [27]. One of their most intriguing findings was that one of the pharmacokinetic parameters, K_{ep} [distribution constant rate (min^{-1})], dropped significantly after the first cycle of chemotherapy in most of the patients showing post-treatment tumor regression. Notably, in responding patients, a tumor size reduction was not clearly evident until after the third cycle of chemotherapy. Some degree of reduction in pharmacokinetic parameters was also seen in patients without tumor regression, suggesting that this model of quantitative analysis could specifically identify early vascular changes due to chemotherapy, rather than direct effects on tumor cells [17, 28].

In conclusion, our study showed that tumor volume reduction measured by DCE-MRI after two cycles of PCT had a strong, independent association with histopathological response. Reductions in ECU after two cycles of PCT were significantly associated with tumor response at the univariate analysis, although we saw different magnitudes of reduction according to the initial MRI appearance of the lesion (homogeneous v.s. peripheral ring-like contrast enhancement). In a multivariate analysis model including both tumor volume and signal intensity reduction, the latter factor lost statistical significance. Combining tumor volume and ECU reduction after two cycles of PCT yielded a diagnostic accuracy of 93% in predicting a PCR. Further research is needed to establish whether newer DCE-MRI techniques and different timing of DCE-MRI assessment could provide information independent from tumor shrinkage, which can predict the final tumor response early in the course of PCT.

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Address for offprints and correspondence: Filippo Montemurro, MD, Institute for Cancer Research and Treatment, Strada Provinciale 142, 10060 Candiolo, Italy; *Tel.:* +39-11-9933263; *Fax:* +39-11-9933275; *E-mail:* fmontemurro@ircc.mauriziano.it