MRI evaluation of neoadjuvant low-dose fractionated radiotherapy with concurrent chemotherapy in patients with locally advanced breast cancer

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Objectives: We address the diagnostic performance of breast MRI and the efficacy of neoadjuvant radiochemotherapy (NRC) treatment (NRC protocol) vs conventional neoadjuvant chemotherapy (NAC) in patients with locally advanced breast cancer.

Methods: The NRC protocol consists of six anthracycline/taxane cycles and concomitant low-dose radiotherapy on breast tumour volume. Breast MRI was performed at baseline and after the last therapy cycle in 18 and 36 patients undergoing the NRC protocol or conventional NAC (propensity matching).

Results: In both groups, we observed reduced tumour dimensions after the last cycle ($p < 0.001$), and the response evaluation criteria in solid tumours (RECIST) class directly correlated with the tumour regression grade class after the last cycle ($p < 0.001$). Patients in the NRC group displayed a higher frequency of complete/partial response than those in the NAC group ($p = 0.034$). 17 out of 18 patients in the NRC group met the criteria for avoiding mastectomy based on final MRI evaluation. The RECIST classification displayed a superior diagnostic performance in the prediction of the response to treatment [area under the receiver operating characteristic curve (AUC) = 0.72] than time-to-intensity curves and apparent diffusion coefficient (AUC 0.63 and 0.61). The association of the three above criteria yielded a better diagnostic performance, both in the general population (AUC = 0.79) and in the NRC and the NAC group separately (AUC = 0.82 and AUC = 0.76).

Conclusions: The pathological response is predicted by MRI performed after the last cycle, if both conventional MRI and diffusion imaging are integrated. The NRC treatment yields oncological results superior to NAC.

Advances in knowledge: MRI could be used to establish the neoadjuvant protocol in breast cancer patients.

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Neoadjuvant chemotherapy is currently widely employed in patients with locally advanced breast cancer (LABC) in order to improve the rate of breast-conserving surgery (up to 98% of patients) and systemic control of the disease [1, 2]. The coupling of pre-operative radiotherapy (RT) cycles with neoadjuvant chemotherapy has been proposed for other cancer types. In particular, taxanes could have a synergistic effect with RT when administered concurrently [3–5]. Nonetheless, few data are currently available on the efficacy of concurrent neoadjuvant RT in patients with LABC, although evidence exists that such a strategy is safe and feasible [6], and is supported by preliminary investigations [7, 8]. Radiation doses below 0.5 Gy have been demonstrated to enhance the effectiveness of continuous-infusion taxanes.

This phenomenon has been termed low-dose hyper-radiosensitivity [9, 10].

MRI is a reliable tool to evaluate the breast cancer response to chemotherapy by measuring tumour diameter changes and by assessing the viability of residual tumour areas [11–13]. Nevertheless, MRI may under- or overestimate the burden of residual tumour by confounding a fibrotic scar with viable tumour tissue, or *vice versa*. Diffusion-weighted imaging (DWI) has been shown in such contexts to improve the diagnostic performance of MRI [14]. It has not been clarified whether MRI retains its diagnostic performance even in the context of breast RT. The latter is known to trigger tissue oedema, which may potentially impair the diagnostic accuracy [15, 16]. The purpose of the present work is to ascertain (1) the diagnostic performance of MRI and DWI-MRI in the context of concurrent low-dose fractionated RT (LD-FRT) and chemotherapy in the prediction of response to neoadjuvant treatment; (2) whether the adoption of concurrent neoadjuvant LD-FRT

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and chemotherapy yields better oncological results in LABC than neoadjuvant chemotherapy alone.

Methods and materials

Study population

The neoadjuvant protocol consisting of chemotherapy and concomitant low-dose RT on breast tumour volume (NRC) has been used in our institution since August 2008, and is indicated for patients with histopathologically proven LABC (core needle biopsy) and scheduled for neoadjuvant treatment. To be eligible for the NRC protocol, patients had to fulfil the following criteria: women aged between 18 and 75 with previously untreated LABC; performance status (PS) = Eastern Cooperative Oncology Group PS score 0/1 or Karnofsky PS ≥ 60 ; no evidence of distant metastases; normal haematopoietic, liver, renal and cardiac functions. Exclusion criteria were *c-erbB2* positivity; the presence of an extensive intraductal component; pregnancy or lactation at the time of diagnosis; previous or current malignancies other than breast cancer. The patients enrolled herein received the NRC protocol in the period August 2008 to February 2011. The control group consisted of patients who received the same neoadjuvant chemotherapy (NAC) alone for LABC during the same time period. For all patients, study design comprised pre-treatment MRI (within 4 weeks before the start of neoadjuvant treatment), post-treatment MRI and definitive surgery within 4 weeks after the completion of neoadjuvant therapy. Intermediate-term MRI was routinely performed, but it was not included in the present analyses.

The final diagnosis of tumour response to neoadjuvant treatment was classified according to the histopathological Mandard's tumour regression grade (TRG) criteria after surgical excision [17]. The diagnostic performance of either conventional MRI or DWI was weighted against the results of TRG classification.

The local review board approved the study protocol, and the patients provided informed consent to both NRC treatment and enrolment in the study.

Treatment schedule

Patients receiving the NRC treatment underwent six cycles of non-pegilate liposomal doxorubicin (Myocet) [50 mg m^{-2} of body surface area (BSA)] plus docetaxel (75 mg m^{-2} of BSA) every 21 days, with concurrent LD-FRT (2 fractions of 0.4 Gy day^{-1} for 2 days, every 21 days, for 6 cycles; total dose 960 cGy) (linear accelerator). We adopted liposomal doxorubicin with the purpose of minimising the risk of cardiotoxicity due to concurrent RT and chemotherapy. The gross tumour volume (GTV) was based on pre-treatment imaging and clinical examination during the simulation. The clinical target volume was obtained by the visible tumour as defined by instrumental tests (GTV) plus a 1-cm margin in order to compensate for set-up errors and movement of the internal margin (planning target volume). Axillary lymph nodes were not included in the treatment even if clinically or radiologically positive. Tangential fields were commonly included to cover the area of the

tumour. Patients receiving NAC treatment underwent the same chemotherapy protocol (six cycles of doxorubicin 50 mg m^{-2} of BSA plus docetaxel 75 mg m^{-2} of BSA every 21 days), without concurrent LD-FRT.

After clinical and radiological re-evaluation, all patients underwent surgery, either wide excision or mastectomy plus axillary node dissection. If the tumour was still too large for breast conservation, modified radical mastectomy was recommended. 4–6 weeks after surgery, all women started conventional adjuvant therapy according to the prognostic markers and risk factors. The study design is summarised in Figure 1.

MRI protocol

MRI was performed with a 1.5 T unit with 23 mT m^{-1} gradient intensity (Signa Excite; GE Medical System, Milwaukee, WI) using a dedicated breast coil with women in the prone position. The following sequences were acquired:

- short-time inversion-recovery (STIR) axial sequence: repetition time (TR), 5900 ms; echo time (TE), 68 ms; echo train length (ETL), 17; bandwidth 41–67 kHz; matrix, 512×512 ; thickness, 4 mm; interval, 0 mm; field-of-view (FOV), 32–34 cm; number of excitations (NEX), 1–2.
- DWI axial sequence: TR, 5150 ms; TE = minimum, matrix, 256×256 ; thickness, 4 mm; interval 0 mm; FOV, 32–34 cm; NEX, 2. Sensitising diffusion gradients were applied sequentially in the *x*-, *y*- and *z*-directions with *b* values of 0 and 1000 s mm^{-2} according to the pertinent literature [18, 19].
- three-dimensional (3D) fast spoiled gradient-echo (FSPGR) saturated fat coronal sequence: flip angle (FA), 15° ; TR < 30 ms; TE < 5 ms; NEX, 0.5; thickness, 2–3 mm; interval, 0 mm; matrix 512×512 ; FOV, 34–38 cm before and five times after intravenous administration of 0.1 mmol kg^{-1} of gadopentetate dimeglumine (Gd-DTPA; Bracco Diagnostics Inc., Milan, Italy). The temporal resolution was 46 s. Contrast medium was

Figure 1. Study design. LABC, locally advanced breast cancer; NRC, neoadjuvant radiochemotherapy; NAC, neoadjuvant chemotherapy; LD-FRT, low-dose fractionated radiotherapy.

injected with a 10-s timing delay into the antecubital vein with a 18 to 20-G needle at a flow rate of 2 ml s⁻¹ followed by flushing with 20 ml of saline solution.

- 3D FSPGR sagittal post-contrast fat-suppressed sequence: TR<30 ms; TE<5 ms; FA, 15°; matrix, 512×512; thickness, 2–3 mm; interval, 0 mm; FOV, 22–26 cm; NEX, 2.
- 3D FSPGR axial post-contrast fat-suppressed sequence: TR<30 ms; TE<5 ms; FA, 30°; matrix 512×512; thickness, 2–3 mm; interval 0 mm; FOV, 34–38 cm; NEX, 2.

Acquisition time of this complete MRI protocol was 18–20 min.

Dynamic and DWI sequences were evaluated using a dedicated workstation (GE Healthcare®, Advantage Windows 4.1, Waukesha, WI) and consensus of three radiologists (PB, MC and EB) experienced in breast imaging.

The tumour response to treatment was assessed using RECIST 1.1 (response evaluation criteria in solid tumours) classification [20], based on the largest diameter of the target lesion (post-contrast fat-suppressed 3D FSPGR *T*₁ weighted images). In the presence of multifocal or multicentric disease, the largest lesion was considered as the target.

Time-to-intensity curves were built after contrast agent injection and placement of the region of interest within the tumour bed, both before and after treatment. For each target lesion, we evaluated the DWI sequence and measured the ADC values before and after chemotherapy. One single region of interest was applied according to previous evidence [14].

Pathological examination

Breast surgical specimens were cut into 5-mm slices, fixed in 10% neutral-buffered formalin and stained with haematoxylin and eosin for evaluation. Macroscopic inspection was used to identify gross tumour areas for subsequent microscopic assessment. If gross tumour was not macroscopically evident, each paraffin block was sliced and the tumour bed was identified by correlation with imaging findings and radiography of specimens.

Residual disease post-NAC was assessed according to Mandard's classification [17]. For the purposes of data analysis, we defined "responders" as patients having TRG Class 1, 2 or 3, and "non-responders" as patients having TRG Class 4 or 5. Similarly, under the profile of morphological MRI, we defined responders as patients having complete response or partial response, and non-responders as patients having stable or progressive disease (RECIST 1.1). We categorised time-to-intensity curves as either persisting type (expressing response to treatment) or plateau type and wash-out type (expressing no response to treatment). According to previous evidence, we adopted a cut-off value of >20% increase in ADC value after neoadjuvant treatment as indicative of response to treatment itself. The >20% increase in ADC value had been identified as the cut-off with the best diagnostic performance [14].

Statistical analysis

A total of 19 patients received NRC treatment, whereas 55 patients underwent NAC treatment using anthracyclines

and taxanes during the study period. For each patient in the NRC group, a logistic regression was built including baseline variables (Table 1) in order to model the likelihood of each patient to enter the NRC or the NAC group, and the propensity score was calculated for each of them. For each patient receiving the NRC treatment, we selected two from the largest pool of patients who received the NAC treatment having the closest propensity score (propensity matching with a 1:2 ratio) (maximum allowable difference: 0.1). One patient from the NRC group and 19 patients from the NAC group were excluded because it was impossible to find an adequate match in the other group. Thus, the final study groups comprised 18 (NRC group) and 36 patients (NAC group).

Statistical analysis was performed using SPSS® v. 11.0 for Windows (SPSS, Chicago, IL) and SAS software for propensity matching (SAS/STAT v. 8, SAS Inc., San Antonio, TX). Continuous data are presented as mean ± standard deviation and categorical variables as percentages. Intergroup comparison was performed using the two-tailed Student's *t*-test or the χ^2 test for continuous and categorical variables, respectively. Kendall's rank correlation coefficient was used to analyse the correlation between the Mandard class and the RECIST class, the ADC value and the type of time-to-intensity curves. Diagnostic performance of MRI parameters for the evaluation of the response or non-response status of a tumour lesion was performed by building ROC curves. The ROC curves obtained with either method were statistically compared according to Hanley and McNeil methodology (MedCalc software, Gent, Belgium). The α -level was 0.05.

Table 1. Baseline characteristics of the entire study population

Characteristic	NRC group	NAC group	<i>p</i> -value
Age (years)	50.83±9.7	49.53±8.2	0.62
Ki67	43.8±30.2	39.2±29.1	0.35
<i>p53</i> ^a	27.3±32.1	24.1±19.5	0.7
<i>bcl-2</i> ^a	15.4±16.6	13.5±15.5	0.5
Histology			0.9
Ductal invasive	12 (66.7%)	22 (61.2%)	
Lobular invasive	2 (11.1%)	7 (19.4%)	
Mixed ^b	4 (22.3%)	7 (19.4%)	
Phenotype ^c			0.98
Luminal	13 (72.2%)	25 (69.4%)	
Triple negative	5 (27.8%)	11 (30.6%)	
Grading			0.31
G1	1 (5.6%)	3 (8.3%)	
G2	6 (33.3%)	14 (38.8%)	
G3	11 (61.1%)	19 (52.7%)	
Staging			0.15
Stage 2	14 (77.8%)	24 (66.7%)	
Stage 3	4 (22.2%)	12 (33.3%)	

NAC, neoadjuvant chemotherapy; NRC, neoadjuvant radiochemotherapy.

^aAverage percentage of cells expressing either the mutated form of *p53* or the *bcl-2*.

^bIncludes invasive and *in situ* carcinoma.

^cTriple negative: demonstration at core needle biopsy of oestrogen receptor, progesterone receptor and HER2 negativity. Luminal: demonstration at core needle biopsy of oestrogen receptor positivity.

Results

Table 1 summarises the baseline characteristics of the study population. The two groups were confirmed to be similar with respect to the baseline features. The most frequent tumour histotype was ductal invasive carcinoma; the tumours were most frequently of grade 3 and of luminal type (*i.e.* showing oestrogen receptor positivity at core needle biopsy specimen). In both groups, we observed a statistically significant reduction in the average breast lesion diameter from the baseline MRI to the last imaging performed at the end of the neoadjuvant treatment (from 42.33 ± 19.3 mm to 8.06 ± 12 mm in the NRC group, and from 55.03 ± 23.7 mm to 28.03 ± 24.3 mm in the NAC group; $p < 0.001$ both). Hence, the average lesion diameter was lower in the NRC group at MRI performed before surgery: such differences were statistically significant ($p < 0.001$). Table 2 includes the MRI features obtained at this time point as well as the final histopathological report. The final pathological response indicated that the patients in the NRC group displayed a response to neoadjuvant treatment more frequently ($p = 0.022$). Figures 2 and 3 show a case of pre-treatment and post-treatment MRI evaluation in the NRC group, and Figures 4 and 5 show a case in the NAC group. A better response on MRI evaluation is evident in the NRC group. No cases of disease progression were observed. 15 out of 18 patients in the NRC group and 30 out of 36 in the NAC group actually avoided mastectomy. Nonetheless, two additional patients in the NRC group met the criteria for avoiding mastectomy (RECIST 1 class at final MRI and complete response at final histopathological assessment). Taking into analysis the major MRI-derived parameters obtained at the end of neoadjuvant treatment, we disclosed a statistically significant correlation between the histopathological response (TRG class) and the RECIST class (direct correlation, $\tau = 0.77$, $p < 0.001$) and the time-to-intensity curves after contrast

Table 2. MRI characteristics of the study groups at the time of the last imaging at the end of neoadjuvant treatment and final histopathological results

Characteristic	NRC group	NAC group	p-value
RECIST class			0.034
Complete response	10 (55.6%)	10 (27.8%)	
Partial response	6 (33.3%)	15 (41.7%)	
Stable disease	2 (11.1%)	11 (30.6%)	
ADC increase >20%	10 (55.6%)	20 (55.6%)	–
Time-to-intensity curve			0.014
Wash-out type	1 (5.6%)	4 (11.1%)	
Plateau type	1 (5.6%)	8 (22.2%)	
Persisting type	6 (33.3%)	16 (44.4%)	
Complete response type	10 (55.6%)	8 (22.2%)	
TRG class			0.022
TRG1	2 (11.1%)	3 (8.3%)	
TRG2	8 (44.4%)	7 (19.4%)	
TRG3	4 (22.2%)	6 (16.7%)	
TRG4	4 (22.2%)	17 (47.2%)	
TRG5	–	3 (8.3%)	

ADC, apparent diffusion coefficient obtained from diffusion-weighted imaging maps; NAC, neoadjuvant chemotherapy; NRC, neoadjuvant radiochemotherapy; RECIST, response evaluation criteria in solid tumours; TRG, tumour regression grade.

Figure 2. Maximum intensity projection (a); diffusion-weighted imaging sequence (b); enhancement kinetics (c); and apparent diffusion coefficient map (d) for a pre-treatment patient in the neoadjuvant radiochemotherapy group.

agent administration ($\tau = -0.52$, $p < 0.001$) (Figure 6a,b). The correlation between the ADC value increase and the TRG class did not reach significance ($\tau = -0.17$, $p = 0.095$) (Figure 2c). In the overall population, we observed an increased percentage of persisting-type time-to-intensity curves and increased average ADC value after completion of the neoadjuvant treatment. Accordingly, the RECIST classification obtained at the same time point displayed a better tumour response in patients belonging to the NRC group than the NAC group ($p = 0.034$). This finding was confirmed by the distribution of types of enhancement kinetics in the two groups ($p = 0.014$, underscoring a higher prevalence of tumour response in the NRC group). Conversely, the percentage of cases who displayed response to treatment on the basis of $\geq 20\%$ ADC value increase was similar among the groups.

By conducting separate analyses for the NRC and the NAC groups, it emerged that the direct correlation between the TRG class and the RECIST class obtained at the last MRI is maintained in both ($p < 0.001$). The same

Figure 3. Maximum intensity projection (a); diffusion-weighted imaging sequence (b); enhancement kinetics (c); and apparent diffusion coefficient map (d) for a post-treatment patient in the neoadjuvant radiochemotherapy group.

Figure 4. Maximum intensity projection (a); diffusion-weighted imaging sequence (b); enhancement kinetics (c); and apparent diffusion coefficient map (d) for a pre-treatment patient in the neoadjuvant chemotherapy group.

Figure 5. Maximum intensity projection (a); diffusion-weighted imaging sequence (b); enhancement kinetics (c); and apparent diffusion coefficient map (d) for a post-treatment patient in the neoadjuvant chemotherapy group.

could be said for the time-to-intensity curves after administration of contrast agent ($p < 0.001$ for both). An inverse correlation exists between the TRG and the ADC variation only in the NRC group ($\tau = -0.31$, $p = 0.045$), while no statistically significant correlation exists in the NAC group ($\tau = 0.15$, $p = 0.32$).

We subsequently addressed the diagnostic performance in the overall population for each of the MRI-derived parameters in predicting the final histopathological result. Sensitivity, specificity, positive and negative predictive value (PPV, NPV) and accuracy are reported in Table 3 ("Total" lines). The RECIST class displayed the best performance [area under the receiver operating characteristic (ROC) curve (AUC)=0.72] compared with the time-to-intensity curves (AUC=0.63) and the ADC variation (AUC=0.61) (Figure 7a). We then tested the association of two parameters (the established RECIST class and the time-to-intensity curves) to predict the response to treatment; this strategy yielded an inadequate result (AUC=0.65, lower curve in Figure 7b). With the aim of improving the diagnostic performance we decided to integrate a third parameter, *i.e.* the ADC value. The integration of these three imaging parameters as concurrent criteria to predict the TRG class yielded a superior performance (AUC=0.79) in the overall study population, mainly due to improved specificity (Table 3). Subsequently, we addressed the diagnostic performance separately in each group. Table 3 compares sensitivity, specificity, PPV, NPV and accuracy across the NRC and the NAC group. The association of RECIST class and time-to-intensity curves displayed a lower diagnostic performance (AUC=0.59) in the NRC group than the NAC group (AUC=0.72) ($p = 0.01$, Figure 8). In such settings, the integration of the ADC value variation as a concurrent diagnostic parameter allowed to re-establish adequate performance in both groups, mainly due to increased specificity (AUC=0.82 for the NRC group and AUC=0.76 for the NAC group, $p = 0.76$), thus confirming the observation achieved in the overall population (Figure 8, Table 3). After splitting the analysis of diagnostic performance into subgroups (responders *vs* non-responders), it was evident that the RECIST classification retains the highest sensitivity among the criteria for detecting responders. On the other hand, we confirm that the ADC value allows a remarkable improvement in

Figure 6. Kendall's rank-coefficient correlation. Correlation between the tumour regression grade (TRG) class and the RECIST class (a); the time-to-intensity curves after contrast agent administration (b); and the ADC variation (c). ADC, apparent diffusion coefficient; RECIST, response evaluation criteria in solid tumours.

Figure 7. Entire study population. Receiver operating characteristic curves and area under the curve (AUC) values for each of the three MRI-derived evaluation parameters (a) and for the association of either two or three of the above-mentioned parameters (b). ADC, apparent diffusion coefficient; RECIST, response evaluation criteria in solid tumours.

specificity by enhancing detection of non-responders (Table 4).

Discussion

Conventional MRI is a mainstay for the reassessment of breast cancer during neoadjuvant treatment protocols. It has been suggested that morphological and dynamic contrast-enhanced MRI alone has limited capability to differentiate among scar tissue and viable tumour, and it may over- or underestimate tumour size [2, 11, 12, 21, 22]. Recent investigations have indicated DWI as a

significant diagnostic improvement, mainly due to better specificity [14]. The rationale of DWI here is based on the cytotoxic response induced by chemotherapy, followed by an increase in the ADC value. Concurrent LD-FRT and chemotherapy is an emerging option for neoadjuvant treatment of breast cancer [6–8]. Nonetheless, there are few clinical data available for the efficacy of such a strategy in LABC. Additionally, concerns have been raised over the diagnostic reliability of conventional MRI associated with DWI in the setting of concurrent LD-FRT. Full-dose adjuvant RT may impair the diagnostic performance of MRI due to tissue oedema, glandular swelling and diffuse parenchymal enhancement [15, 16].

Table 3. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of either one of the three MRI-derived parameters or their association (see the text for details) as predictors of the status of responder to treatment according to the tumour regression grade classification

Characteristic	RECIST	CURVE	ADC	Association 2/3 criteria	Association 3/3 criteria
Sensitivity					
Total	(29/30) 97%	(24/30) 80%	(23/30) 77%	(27/30) 90%	(19/30) 63%
NRC	(14/14) 100%	(13/14) 93%	(10/14) 71%	(13/14) 93%	(10/14) 71%
NAC	(15/16) 94%	(11/16) 69%	(13/16) 81%	(14/16) 88%	(9/16) 56%
Specificity					
Total	(11/24) 46%	(9/24) 38%	(15/24) 63%	(13/24) 54%	(21/24) 88%
NRC	(2/4) 50%	(0/4) –	(2/4) 50%	(1/4) 25%	(3/4) 75%
NAC	(9/20) 45%	(9/20) 45%	(13/20) 65%	(12/20) 60%	(18/20) 90%
PPV					
Total	(29/42) 69%	(24/39) 62%	(23/32) 72%	(27/38) 71%	(19/22) 86%
NRC	(14/16) 88%	(13/17) 76%	(10/12) 83%	(13/16) 81%	(10/11) 91%
NAC	(15/26) 58%	(11/22) 50%	(13/20) 65%	(14/22) 64%	(9/11) 82%
NPV					
Total	(11/12) 92%	(9/15) 60%	(15/22) 68%	(13/16) 81%	(21/32) 66%
NRC	(2/2) 100%	(0/13) –	(2/6) 33%	(1/2) 50%	(3/7) 43%
NAC	(9/10) 90%	(9/14) 64%	(13/16) 81%	(12/14) 86%	(18/25) 72%
Accuracy					
Total	(40/54) 74%	(33/54) 61%	(38/54) 70%	(40/54) 74%	(40/54) 74%
NRC	(16/18) 89%	(13/18) 72%	(12/18) 67%	(14/18) 78%	(13/18) 72%
NAC	(24/36) 67%	(20/36) 56%	(26/36) 72%	(26/36) 72%	(27/36) 75%

ADC, apparent diffusion coefficient; CURVE, type of time-to-intensity curves; NAC, neoadjuvant chemotherapy; NRC, neoadjuvant radiochemotherapy.

Association 2/3 criteria: response evaluation criteria in solid tumours (RECIST) class and time-to-intensity curves; association 3/3 criteria: RECIST class, time-to-intensity curves and rise in ADC value.

Figure 8. Study subgroups. Receiver operating characteristic curves and area under the curve (AUC) values for the association of either three (upper) or two (lower) of the above-mentioned parameters. Neoadjuvant radiochemotherapy (NRC) treatment group on the left and neoadjuvant chemotherapy (NAC) group on the right. See the text for details.

Given the anticipated diffusion of concurrent LD-FRT-chemotherapy neoadjuvant protocols, we aimed at ascertaining the clinical efficacy of concurrent LD-FRT and chemotherapy neoadjuvant protocols in LABC, and the reliability of MRI in evaluating the tumour response to treatment.

The present data suggest that the neoadjuvant protocol based on concurrent LD-FRT and chemotherapy yields superior oncological results in LABC than established chemotherapy alone. Patients in the NRC group had

statistically lower tumour dimensions at MRI performed before surgery, as confirmed by the final histopathological results. Mastectomy was performed in three patients in the NRC group but breast-conserving surgery would have been feasible in all cases, since the final histopathological assessment displayed complete response (TRG1). Conversely, none of the six patients in the NAC group who underwent mastectomy was envisaged to potentially avoid this treatment on the basis of final histopathology. Although the present study has insufficient power to

Table 4. Diagnostic performance of either one of the three MRI-derived parameters or their association (see the text for details) as predictors of the status of responder to treatment

	RECIST	CURVE	ADC	Association 2/3 criteria	Association 3/3 criteria
Sensitivity					
Responder	(29/30) 97%	(24/30) 80%	(23/30) 77%	(27/30) 90%	(19/30) 63%
Specificity					
Non-responder	(11/24) 46%	(9/24) 38%	(15/24) 63%	(13/24) 54%	(21/24) 88%
Accuracy					
Responder	(29/54) 54%	(24/54) 44%	(23/54) 43%	(27/54) 50%	(19/54) 35%
Non-responder	(11/54) 20%	(9/54) 17%	(15/54) 28%	(13/54) 24%	(21/54) 39%

ADC, apparent diffusion coefficient; CURVE, type of time-to-intensity curves; RECIST, response evaluation criteria in solid tumours.

The population is split according to the responder or non-responder status.

Association 2/3 criteria: RECIST class and time-to-intensity curves. Association 3/3 criteria: RECIST class, time-to-intensity curves and ADC value raise.

draw conclusions about such an issue, it is clinically evident that the NRC protocol could be useful to avoid mastectomy in a significant number of patients. Larger investigations are needed to clarify this point. The biological basis of better tumour response in the LD-FRT group is not well ascertained, but could be justified by the hyper-radiosensitivity phenomenon [23, 24].

We confirm that the RECIST classification is a reliable tool in predicting the tumour response to neoadjuvant treatment, as supported by the close correlation with TRG class ($p < 0.001$) and by its diagnostic performance for the response to treatment in the overall population (Figure 7a). This is sustained predominantly by its excellent sensitivity (Table 3). We also confirm the known limitation of the RECIST class, *i.e.* its fairly low specificity (Table 3). This could be ascribed to its limited capability of discriminating between the scar and the residual viable tumour tissue, thus leading to a number of false-positive results and prompting a more aggressive surgical treatment in a proportion of cases who actually may not require it. Such a phenomenon is reasonably based on alteration of the tumour vascular bed and tumour fragmentation induced by chemotherapy drugs. The same mechanism could be at the root of the limited reliability we have observed for the time-to-intensity curves in the prediction of response to treatment. The enhancement kinetics showed the lowest specificity and global performance in the overall population (Table 3 and Figure 7a); this is particularly evident in the NRC subgroup (Table 3). The use of RT is known to induce not only tissue oedema and fibrosis, but also increased flow through the lymphatics and small blood vessels mainly in women subjected to concurrent RT and chemotherapy [15, 16, 22]. Nevertheless, this evidence has been obtained from adjuvant RT/chemotherapy studies; there are no available data about the neoadjuvant low-dose RT applications. In fact, the association of the time-to-intensity curve to the established RECIST criteria in the evaluation of response to treatment yielded a poorer performance not only in the overall population (AUC=0.65, Figure 7b) but particularly in the NRC subgroup (AUC=0.59, Figure 4).

In an attempt to improve the diagnostic capabilities of MRI, we tested the integration of the ADC value variation (after treatment *vs* before treatment) as an additional parameter. In the present population, we

observed no meaningful correlation between the ADC value and the TRG class in the NAC group, but not in the NRC group; such a finding was unexpected and probably due to the small sample size. If the ADC variation is implemented as a concurrent diagnostic criterion together with the contrast-enhanced MRI parameters, the diagnostic performance is remarkably improved in the general population (AUC=0.79, Figure 7b), mainly due to increased specificity (Table 3). The detrimental effects on the diagnostic performance associated with the concomitant LD-FRT are offset by the integration of the ADC value change, as outlined by the comparability of AUCs among the two subgroups (0.82 and 0.76 in the NRC and the NAC group, respectively) (Figure 8). Indeed, the ADC variations are the expression of cytotoxic response to treatment, with increased tissue water mobility due to cell shrinkage and breakdown of the plasma membrane [12, 25]. To the best of our knowledge, herein we present the first evidence that such a concept is confirmed in the setting of concurrent chemotherapy and LD-FRT.

We acknowledge the reduced sample size as a potential limitation of the study. This was because the radiochemotherapy neoadjuvant protocol is still an experimental strategy in breast cancer, and herein we provide initial evidence over its results. Additionally, we believe that the use of propensity-matching increases the methodological reliability of the study by selecting two highly similar subgroups (Table 1). Larger studies are expected to confirm these findings. Additionally, we did not present mid-term MRI evaluation, as we decided to employ the final post-operative histopathological results (TRG classification) as the gold-standard comparison for the MRI assessment. Mid-term histopathological data are not very reliable (core biopsy).

One point of strength of this investigation is the homogeneous study population concerning the chemotherapy regimen; the two subgroups were differentiated only by the addition of LD-FRT in one of them. In this second subgroup, the neoadjuvant strategy may yield superior oncological results in LABC and possibly increase the rate of breast-conserving surgery. Finally, this evidence suggests that MRI is generally capable of adequate diagnostic performance in the evaluation of response to treatment, despite the use of LD-FRT in association with the established neoadjuvant chemotherapy. Such an

outcome can only be achieved by the integration of both morphological and diffusion imaging parameters.

References

- Chen J, Feig B, Agrawal G, Yu H, Carpenter PM, Mehta RS, et al. MRI evaluation of a pathologically complete response and residual tumors in breast cancer after neoadjuvant chemotherapy. *Cancer* 2008;112:17–26.
- Johansen R, Jensen L, Rydland J, Goa PE, Kvistad KA, Bathen TF, et al. Predicting survival and early clinical response to primary chemotherapy for patients with locally advanced breast cancer using DCE-MRI. *J Magn Reson Imaging* 2009;29:1300–7.
- Zanelli GD, Quaia M, Robiux I, Bujor L, Santarosa M, Favaro D, et al. Paclitaxel as a radiosensitizer: a proposed schedule of administration based on in vitro data and pharmacokinetic calculations. *Eur J Cancer* 1997;33:486–92.
- Mason KA, Hunter NR, Milas M, Abbruzzese JL, Milas L. Docetaxel enhances tumor radioresponse in vivo. *Clin Cancer Res* 1997;3:2431–8.
- Dowell JE, Sinard R, Yardley DA, Aviles V, Machtay M, Weber RS, et al. Seven-week continuous-infusion paclitaxel concurrent with radiation therapy for locally advanced non-small cell lung and head and neck cancers. *Semin Radiat Oncol* 1999;9:97–101.
- Nardone L, Valentini V, Marino L, De Santis MC, Terribile D, Franceschini G, et al. A feasibility study of neo-adjuvant low-dose fractionated radiotherapy with two different concurrent anthracycline-docetaxel schedules in stage IIA/B-IIIa breast cancer. *Tumori* 2012;98:79–85.
- Formenti SC, Volm M, Skinner KA, Spicer D, Cohen D, Perez E, et al. Pre-operative twice-weekly paclitaxel with concurrent radiation therapy followed by surgery and postoperative doxorubicin-based chemotherapy in locally advanced breast cancer: a phase I/II trial. *J Clin Oncol* 2003;21:864–70.
- Chakravarthy AB, Kelley MC, McLaren B, Truica CI, Billheimer D, Mayer IA, et al. Neoadjuvant concurrent paclitaxel and radiation in stage II/III breast cancer. *Clin Cancer Res* 2006;12:1570–6.
- Chendil D, Oakes R, Alcock RA, Patel N, Mayhew C, Mohiuddin M, et al. Low dose fractionated radiation enhances the radiosensitization effect of paclitaxel in colorectal tumor cells with mutant p53. *Cancer* 2000;89:1893–900.
- Marples B, Lambin P, Skov KA, Joiner MC. Low dose hyper-radiosensitivity and increased radioresistance in mammalian cells. *Int J Radiat Biol* 1997;71:721–35.
- Loo CE, Teertstra H, Rodenhuis S, van de Vijver MJ, Hanneman J, Muller SH, et al. Dynamic contrast enhanced MRI for prediction of breast cancer response to neoadjuvant chemotherapy: initial results. *AJR Am J Roentgenol* 2008;191:1331–8.
- Yankeelov TE, Lepage M, Chakravarthy A, Boorme EE, Niermann KJ, Kelley MC, et al. Integration of quantitative DCE-MRI and ADC mapping to monitor treatment response in human breast cancer: initial results. *Magn Reson Imaging* 2007;25:1–13.
- Londero V, Bazzocchi M, Del Frate C, Puglisi F, Di Loreto C, Francescutti G, et al. Locally advanced breast cancer: comparison of mammography, sonography and MR imaging in evaluation of residual disease in women receiving neoadjuvant chemotherapy. *Eur Radiol* 2004;14:1371–9.
- Belli P, Costantini M, Ierardi C, Bufi E, Amato D, Mulé A, et al. Diffusion-weighted imaging in evaluating the response to neoadjuvant breast cancer treatment. *Breast J* 2011;17:610–19.
- Dershaw DD, Shank B, Reisinger S. Mammographic findings after breast cancer treatment with local excision and definitive irradiation. *Radiology* 1987;164:455–61.
- Schnitt SJ, Connolly JL, Harris JR, Cohen RB. Radiation-induced changes in the breast. *Hum Pathol* 1984;15:545–50.
- Mandard AM, Dalibard F, Mandard JC, Marnay J, Henry-Amar M, Petiot JF, et al. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. *Cancer* 1994;73:2680–6.
- Belli P, Costantini M, Bufi E, Magistrelli A, La Torre G, Bonomo L. Diffusion-weighted imaging in breast lesion evaluation. *Radiol Med* 2010;115:51–69.
- Woodhams R, Matsunaga K, Kan S, Hata H, Ozaki M, Iwabuchi K, et al. ADC mapping of benign and malignant breast tumors. *Magn Reson Med Sci* 2005;4:35–42.
- Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45:228–47.
- Kim HJ, Im YH, Han BK, Choi N, Lee J, Kim JH, et al. Accuracy of MRI for estimating residual tumor size after neoadjuvant chemotherapy in locally advanced breast cancer: relation to response patterns on MRI. *Acta Oncol* 2007;46:996–1003.
- Heywang-Koebrunner SH. Breasts. In: Bradley WG, Stark DD, eds. *Magnetic resonance imaging*. St. Louis, MO: Mosby; 1999. pp. 1401–28.
- Enns L, Bogen KT, Wizniak J, Murtha AD, Weinfeld M. Low-dose radiation hyper-sensitivity is associated with p53-dependent apoptosis. *Mol Cancer Res* 2004;2:557–66.
- Krueger SA, Joiner MC, Weinfeld M, Piasentin E, Marples B. Role of apoptosis in low-dose hyper-radiosensitivity. *Radiat Res* 2007;167:260–67.
- Morse DL, Galons JP, Payne CM, Jennings DL, Day S, Xia G, et al. MRI-measured water mobility increases in response to chemotherapy via multiple cell-death mechanisms. *NMR Biomed* 2007;20:602–61.