**Diffusion-weighted magnetic resonance imaging of rectal cancer: tumour volume and perfusion fraction predicts chemoradiotherapy response and survival**

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Word count: xxxx

**Diffusion-weighted magnetic resonance imaging of rectal cancer: tumour volume and perfusion fraction predicts chemoradiotherapy response and survival**

**Background**

In locally advanced rectal cancer (LARC), responses to preoperative treatment are highly heterogeneous and more accurate diagnostics are likely to enable more individualised treatment approaches with improved responses. We investigated the potential of diffusion-weighted magnetic resonance imaging (DW MRI), with quantification of the apparent diffusion coefficient (ADC) and perfusion fraction (F), as well as volumetry from T2-weighted (T2W) MRI, for prediction of therapeutic outcome.

**Material and Methods**

In 27 LARC patients receiving neoadjuvant chemotherapy (NACT) before chemoradiotherapy (CRT), T2W- and DW MRI were obtained before and after NACT. Tumour volumes were delineated in T2W MRI and apparent diffusion coefficients (ADC) and perfusion fractions (F) were estimated from DW MRI using a simplified approach to the intravoxel incoherent motion (IVIM) model. Mean tumour values and histogram analysis of whole-tumour heterogeneity were correlated to histopathologic tumour regression grade (TRG) and 5-year progression-free survival (PFS).

**Results**

At baseline, high tumour F predicted good tumour response (TRG1-2) (AUC = 0.79, p = 0.012), with a sensitivity of 69% and a specificity of 100%. However, the combination of F and tumour volume (Fpre/Vpre) gave the highest prediction of poor tumour response (AUC = 0.93, p < 0.001) with a sensitivity of 88% and a specificity of 91%, and also predicted PFS (p = 0.002). Baseline tumour ADC was not significantly related to therapeutic outcome, whereas a positive change in ADC from baseline to after NACT, ΔADC, significantly predicted good tumour response (AUC = 0.83, p = 0.007, 83% sensitivity, 73% specificity), but not PFS.

**Conclusions**

The MRI parameter F/V at baseline was a remarkably strong predictor of both histopathologic tumour response and 5-year PFS in patients with LARC.

**Keywords**: apparent diffusion coefficient, diffusion-weighted magnetic resonance imaging, intravoxel incoherent motion, perfusion fraction, radiotherapy, rectal cancer

**Introduction**

Standard of care for locally advanced rectal cancer (LARC) is chemoradiotherapy (CRT) followed by surgical removal of residual tumour component [1]. Multimodal treatment gives excellent local control, but distant metastasis is a remaining challenge and disseminated disease is the major cause of death in LARC [2]

Tumour responses to CRT are highly heterogeneous and current LARC research is therefore focused on individualised treatment approaches. Watch-and-wait (deferred surgery) programs are running for patients who achieve complete or near-complete response to CRT [3]. Other individualised treatment options include induction (neoadjuvant) chemotherapy aimed at targeting distant metastasis in high-risk patients [4-5]. Further, radiotherapy may be omitted for selected patients who achieve good response to neoadjuvant chemotherapy [5].

Biomarkers predicting and monitoring treatment responses are indispensable for individualising LARC treatment. Predictive markers are essential when choosing between organ-preserving or intensified systemic treatment, and monitoring biomarkers are necessary to assure or adjust the planned treatment course. Magnetic resonance imaging (MRI) is integral in the diagnostic work-up and treatment response monitoring in LARC [2,6], where T2-weighted (T2W) and diffusion weighted (DW) MRI sequences are used in most examinations. Further use of these techniques, through extraction of quantitative measures, represents an easy and cost-efficient approach to facilitate individualised treatment.

There have been several attempts to use DW MRI with calculation of the quantitative marker apparent diffusion coefficient (ADC) for assessing tumour aggressiveness and predicting tumour response to CRT in LARC, but results have been inconsistent [7]. Results from baseline measurements have shown both positive [8] and negative [9] correlation to pathologic tumour response following CRT, as well as no statistically significant association [10].

DW MRI is sensitive to the motion of water molecules, where the signal decreases as the motion increases, and the residual signal reflects various tissue microstructures [11]. There are essentially two sources of motion of water molecules in tissue: diffusion and flow. Diffusion is the random motion in bulk water, in both extracellular as well as intracellular components. The mechanism of diffusion in tissue is not fully understood but is hindered by tissue microstructures, in particular the cell membranes. Therefore, in cancer diagnostics, DW MRI and ADC are often interpreted as an expression of the tumour cellularity. However, the motion of water molecules in blood (flow) is much faster than diffusion and do not reflect the cellularity. The standard mono-exponential method for calculating ADC does not differentiate the DW signal from tissue and blood [12]. The more complex intravoxel incoherent motion (IVIM) model seeks to estimate the perfusion fraction and the tissue diffusion separately [13]. Implementing the full model, however, requires time-consuming measurements with a high number of diffusion weightings, or b-values, which is difficult in clinical routine. In this study, we implemented a simplified approach to the IVIM-model in order to estimate both the perfusion fraction F and the ADC from a routine DW MRI acquisition in patients with LARC.

Our study aim was to evaluate the potential of tumour ADC and perfusion fraction, F, from DWMRI acquired both at baseline and during preoperative chemoradiotherapy, as predictors of therapeutic outcome in patients diagnosed with LARC. Because we previously found that tumour volume calculated from T2W MRI was a strong predictor of histopathological tumour response [14], the results from T2W- and DW MRI were compared to investigate the potential of combining volumetry and DW MRI.

**Material and Methods**

***Patients***

The prospective phase II trial LARC-Radiation Response Prediction (LARC-RPP) (ClinicalTrials.gov NCT00278694) was approved by the Institutional Review Board and the Regional Committee for Medical and Health Research Ethics South East. The study was performed in accordance with the Helsinki declaration, and written informed consent was required for participation. Inclusion of patients took place between July 26, 2007 and June 17, 2009 and the last follow-up was censored on August 8, 2013. The primary inclusion criterion was histologically confirmed rectal adenocarcinoma, either T4, T3 with mesorectal fascia margin of 3 mm or less or a tumour of any T stage with lymph node involvement. Diagnostic T and N stages (TNM version 5) were assessed by MRI. The current MRI analysis was performed on 27 of the 113 patients enrolled in the clinical study (Supplementary Figure S1).

***Treatment***

The experimental preoperative treatment consisted of neoadjuvant chemotherapy (NACT) followed by CRT. NACT was given as two cycles of Nordic FLOX (oxaliplatin 85 mg/m2 day 1 and bolus 5-fluorouracil 500 mg/m2 and folinic acid 100 mg days 1 and 2) in week 1 and week 3. In week 5 the patients commenced CRT. Radiotherapy was given in 2 Gy fractions 5 days per week; the initial 23 fractions to the macroscopic tumour volume and areas at risk followed by two final boost fractions adapted to the macroscopic tumour as planned by computed tomography. Concomitant chemotherapy was 50 mg oxaliplation every week, plus 825 mg capecitabine twice a day on days with radiotherapy. Patients were referred to curatively intended surgery 6-8 weeks after completed CRT. We previously reported that 90% of the patients enrolled in the LARC-RRP study obtained an R0 resection [15]. None of the patients received postoperative adjuvant chemotherapy.

***MRI***

MRI was obtained before and after NACT (including three 2 Gy fractions of RT). The study included 113 patients, with 79 patients eligible for analysis, (Supplementary Figure S1). The first patients were imaged using an older MRI scanner with insufficient image quality for quantitative DW MRI analysis. Hence, only DW MRI data from the last 27 patients, imaged using a 1.5T Siemens Espree scanner (Siemens, Erlangen, Germany) were of sufficient quality for quantitative calculations. Four of these lacked the follow-up MRI during treatment. All MR examinations were performed according to the standards of the MERCURY study [16]. The imaging parameters for the T2-weighted (T2W)- and DW sequences are summarized in Supplementary Table S1. For DW MRI, a spin-echo echo-planar-imaging sequence with b-values of 0, 300 and 900 s/mm2 in three perpendicular directions was used. All images were stored in the institutional picture archiving and communication system.

***Tumour delineation***

All T2W images were transferred to the Oncentra Masterplan treatment planning system (3.0 SP1; Nucletron, Veenendaal, Netherlands). Tumours (both solid and mucineous) were manually contoured in transversal T2W images by a radiologist with 12 years’ experience in pelvic MRI, who was unaware of therapeutic outcomes.

***Data analysis***

Tumour volumes were calculated by multiplying the contoured area with the slice thickness, adding the slice gap. The contours were transferred to calculated parameter maps for extraction of all pixel values into frequency histograms using MATLAB (R2015b). All parameters were calculated both at baseline (Vpre, ADCpre, Fpre), and after NACT (VNACT, ADCNACT, FNACT). In addition, the difference between the two time-points (ΔV, ΔADC, ΔF), were calculated as percentage change from baseline.

To ensure that the quality of the DW MR images was sufficient to perform quantitative calculations, the signal-to-noise-ratio (SNR) was calculated in the images with the highest b-values (b900):

where the mean signal was measured in the central slice of the tumour, and std denotes the standard deviation.

***Tumour apparent diffusion coefficient***

ADC values were calculated using MATLAB and a mono-exponential equation with the b-values 300 and 900 s/mm2,

where SI denotes the pixel signal intensity. This was done individually for each of the three DW MRI acquisition directions before averaging.

***Tumour perfusion fraction***

After calculating the ADC from the b300 and b900 images, a line back to the y-axis (b = 0) was extrapolated asymptotically, as a simplified IVIM-approach, previously described by Wirestam et al. [17]. The discrepancy between this extrapolated signal intensity and the original signal intensity at b0 was calculated and makes an approximation to the signal decay in the fast decaying phase of the signal. We denoted this as the tumour perfusion fraction, F (Supplementary Figure S2), calculated as

***Study endpoints***

Resected tumour specimens were prepared according to validated protocols [16], and tumour regression grade (TRG) was quantified using the five-point scale proposed by Bouzourene et al. [18]. Patients were defined as either good (TRG1-2) or poor (TRG3-5) responders. Long-term patient outcome was assessed by five-year progression free survival (PFS). PFS was calculated from study enrolment until date of diagnosis of local recurrence, distant metastasis, and death of any cause or end of follow-up.

***Statistical analysis***

The Mann-Whitney U test was used to compare MR parameters for the good and poor responders. Differences in PFS were assessed using the Kaplan–Meier method and log-rank test, after evaluating the optimal cut-off by receiver operating characteristic (ROC) curve analysis. In ROC analysis the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was calculated. The optimal cut-off was identified by giving equal weights to sensitivity and specificity (overall accuracy).

To investigate the predictive potential of tumour heterogeneity measures, promising parameters (significant at the 0.05 level), were further analysed with histogram analysis extracting all percentile values from 1-100, see Supplementary File 1. Data and statistical analysis were performed in MATLAB and SPSS (IBM SPSS Statistics 22). P-values < 0.05 were considered statistically significant.

**Results**

Of the 27 patients, 16 were defined as good responders (TRG1-2) and 11 as poor responders (TRG3-5). Long-term patient outcome data showed that 9 of the patients had PFS events (all metastatic disease) and 18 patients had no PFS event. In the subgroup of 23 patients with MRI after NACT, 12 were defined as good responders and 11 as poor responders. The 9 patients with PFS events were also in this cohort, accordingly 14 patients were without PFS event.

***Tumour apparent diffusion coefficient***

The mean SNR of the b900-images for the tumours at baseline was 21.4 ± 4.0. The absolute tumour ADCpre and ADCNACT were not significantly associated with TRG (Table 1), but the change, ΔADC, was. ROC-curve analysis showed that ΔADC differentiated good and poor responders with 83% sensitivity and 73% specificity (AUC = 0.83, p = 0.007, confidence interval (CI) = 0.67 - 1.00), PPV of 85% and NPV of 80%, at the optimal cut-off of 4%. Hence, an increase in ADC after NACT was associated with poor tumour response to CRT, complementary; a decrease in ADC was associated with good tumour response. Percentile analysis showed that this association was stronger for the lower percentiles of ΔADC (Supplementary Figure S3). Tumour ADC did not predict PFS at any time-point.

***Tumour perfusion fraction***

Baseline perfusion fraction, Fpre, was significantly associated with tumour response to CRT, with a high F associated with good response (Table 1). Figure 1 shows the ROC-curve for mean tumour Fpre predicting TRG1-2 (AUC = 0.79, p = 0.012, CI = 0.61 - 0.97), with a sensitivity of 69%, specificity of 100%, PPV = 100% and NPV = 69%. Percentile analysis did not further contribute to these results. Tumour Fpre did not predict PFS at any time-point.

***Tumour volume***

There was also an association between tumour volume and response to CRT (Table 1). Figure 1 shows the ROC-curve for tumour volume predicting TRG1-2 (AUC = 0.84, p = 0.004, CI = 0.67 – 1.00), with a sensitivity of 75%, specificity of 91%, PPV = 88%, NPV = 82%. Vpre was also associated to PFS (p = 0.034) with a cut-off at 13.7 cm3. This resulted in estimated 5-year survival of 83% versus 47% for patients with tumour volumes below and above 13.7 cm3, respectively.

***Perfusion fraction combined with volume***

The combination of Fpre with Vpre into Fpre/Vpre gave the most significant prediction of TRG1-2 (AUC = 0.93, p < 0.001, CI = 0.82 - 1.00) with a sensitivity of 88%, specificity of 91%, PPV = 93% and NPV = 83% (Figure 1). This combination also gave a significant prediction of PFS. When separating patients above and below the optimal cut-off of 0.5, the respective estimated 5-year PFS were 82% and 30% (p = 0.002) (Figure 2).

**Discussion**

In this study, we found that the tumour perfusion fraction, Fpre, estimated from DW MRI, significantly predicted histopathological tumour response to NACT and CRT in LARC. Combined with tumour volume the association was highly significant and also predicted 5-year PFS.

High Fpre was favourable for obtaining good tumour response. Although F is a parameter derived from DW MRI, it reflects water molecule movement due to flow, not diffusion (13). Hence, a high F indicates tissue with good vascularization, and a low F indicates poorly vascularized tissue. In rectal cancer, F has been shown to correlate with the vascular area fraction measured by CD31 staining [19]. A low F may also reflect tumour hypoxia, a known cause of therapy resistance [20]. Previously, high F from IVIM has been linked to pathologic complete response (pCR) in locally advanced breast cancer [21]. However, a very recent study in rectal cancer found no differences in F between pCr and non-pCr [22]. To assess perfusion-related parameters with MRI it is generally more common to use dynamic contrast-enhanced (DCE) MRI. Tong *et al*. has recently shown that the perfusion-related parameter Ktrans from DCE MRI, was associated with favourable treatment [23], supporting that well-vascularised tumours respond well to treatment.

We found that a decrease in ADC was associated with a favourable response. This differs from previous studies that have found increased tumour ADC during first [24], second [8, 10], and third [25] week of concomitant CRT in patients with favourable response. Low tumour ADC generally reflects high cellularity, therefore ADC is expected to increase during cytotoxic treatment. We measured ΔADC after a systemic induction treatment of two 2-week cycles of NACT, which caused extensive tumour shrinkage [26]. At the time of ADC measurement, most of the tumour cells were probably already replaced by ingrowth of fibrosis. Thus, the measured ADC no longer reflect tumour cell density, but the tissue composition following treatment-induced changes. Fibrotic tissue generally has low ADC. Hence, the early tumour shrinkage after NACT may explain why we found decreased ∆ADC in good responders.

The main limitations of our study are the low number of patients and the use of experimental NACT before CRT. Furthermore, the quality of the DW MR images was lower than current standards. Although three b-values for estimation of the tumour perfusion fraction may be an oversimplification, our results are promising. However, further investigations in other, independent cohorts are required. The main strengths of our study are experienced investigators in all disciplines, balanced response groups, and long-term follow-up with PFS as a highly relevant clinical endpoint.

In conclusion, the MRI parameter F/V at baseline was a remarkably strong predictor of both histopathologic tumour response and 5-year PFS for LARC patients. However, the study population was small and the study treatment was experimental. If our results can be confirmed in larger studies with standard treatment, F/V may prove to be a useful clinical tool for individualised treatment planning.

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**Acknowledgments**

Financial support was received from the South-Eastern Norway Regional Health Authority (Grant Nos. 2012002, 2014012, 2015048, 2016050) and the Norwegian Cancer Society (Grant No. 04085/003).

# **References**

1. **Weber GF, Rosenberg R, Murphy JE, et al.** Multimodal treatment strategies for locally advanced rectal cancer. 2012; 12(4): 481-494.

2. **van de Velde CJ, Boelens PG, Borras JM, et al.** EURECCA colorectal: multidisciplinary management: European consensus conference colon & rectum. *Eur J Cancer.* 2014; 50(1); 1.e1-1.e34.

3. **Habr-Gama A, Perez RO, Nadalin W, et al.** Operative Versus Nonoperative Treatment for Stage 0 Distal Rectal Cancer Following Chemoradiation Therapy: Long-term Results. *Annals of surgery.* 2004; 240(4): 711-717.

4. **Haustermans K, Debucquoy A, Lambrecht M.** The ESTRO Breur Lecture 2010: toward a tailored patient approach in rectal cancer. *Radiother Oncol.* 2011; 100(1): 15-21.

5. **D., Schrag.** Evolving role of neoadjuvant therapy in rectal cancer. *Curr Treat Options Oncol.* 2013; 14(3): 350-364.

6. **Schmoll HJ, van Cutsem E, Stein A, et al.** ESMO Consensus Guidelines for management of patients with colon and rectal cancer. A personalized approach to clinical decision making. *Ann Oncol.* 2012; 23(10): 2479-2516.

7. **Beets-Tan RGH, Beets, GL.** MRI for assesing and predicting response to neoadjuvant treatment in rectal cancer. 2014; 11(8): 480-488.

8. **Barbaro B, Vitale R, Valentini V, et al.** Diffusion-Weighted Magnetic Resonance Imaging in Monitoring Rectal Cancer Response to Neoadjuvant Chemoradiotherapy. *Int J Radiation Oncol Biol Phys.* 2012; 83(2): 594-599.

9. **Intven M, Reerink O, Philippens M.** Diffusion-weighted MRI in locally advanced rectal cancer. *Strahlenther Onkol.* 2013; 189(2): 117-122.

10. **Kim SH, Lee JY, Lee JM, et al.** Apparent diffusion coefficient for evaluating tumour response to neoadjuvant chemoradiation therapy for locally advanced rectal cancer. *Eur Radiol.* 2011; 21(5): 987-995.

11. **Padhani AR, Liu G, Mu-Koh D, et al.** Diffusion-Weighted Magnetic Resonance Imaging as a Cancer Biomarker: Consensus and Recommendations. *Neoplasia.* 2009; 11(2): 102-125.

12. **Koh DM, Collins DJ, Orton MR.** Intravoxel incoherent motion in body diffusion-weighted MRI: reality and challenges. *AJR Am J Roentgenol.* 2011; 196(6): 1351-1361.

13. **Le Bihan D, Breton E, Lallemand D, et al.** Separation of diffusion and perfusion in intravoxel incoherent motion MR imaging. *Radiology.* 1988; 168(2): 497-505.

14. **Seierstad T, Hole KH, Grøholt KK, et al.** MRI volumetry for prediction of tumour response to neoadjuvant chemotherapy followed by chemoradiotherapy in locally advanced rectal cancer. *Br J Radiol.* 2015; 88(1051): 20150097.

15. **Dueland S, Ree AH, Grøholt KK, et al.** Oxaliplatin-containing preoperative therapy in locally advanced rectal cancer: local response, toxicity and long-term outcome. Clin Oncol. 2016; 28(8): 532-539.

16. **MERCURY study group.** Diagnostic accuracy of preoperative magnetic resonance imaging in predicting curative resection of rectal cancer: prospective observational study. *BMJ.* 2006; 333(7572): 779.

17. **Wirestam R, Borg M, Brockstedt S, et al.** Perfusion-related parameters in intravoxel incoherent motion MR imaging compared with CBV and CBF measured by dynamic susceptibility-contrast MR technique. *Acta Radiol.* 2001; 42(2): 123-128.

18. **Bouzourene H, Bosman FT, Seelentag W, et al.** Importance of tumor regression assessment in predicting the outcome in patients with locally advanced rectal carcinoma who are treated with preoperative radiotherapy. *Cancer.* 2002; 94(4): 1121-1130.

19. **Bäuerle T, Seyler L, Münter M, et al.** Diffusion-weighted imaging in rectal carcinoma patients without and after chemoradiotherapy: a comparative study with histology. *Eur J Radiol.* 2013; 82(3): 444-452.

20. **Bristow RG, Hill RP.** Hypoxia, DNA repair and genetic instability. *Nat Rev Cancer.* 2008; 8(3): 180-192.

21. **Che S, Zhao X, Ou Y, et al.** Role of the intravoxel incoherent motion diffusion weighted imaging in the pre-treatment prediction and early response monitoring to neoadjuvant chemotherapy in locally advanced breast cancer. *Medicine.* 2016; 95(4): e2420.

22. **Zhu HB, Zhang XY, Zhou XH, et al.** Assessment of pathological complete response to preoperative chemoradiotherapy by means of multiple mathematical models of diffusion-weighted MRI in locally advanced rectal cancer: A prospective single-center study. *J Magn Reson Imaging.* 2016; 16 DEC 2016: 10.1002/jmri.25567

23. **Tong T, Sun Y, Gollub M, et al.** Dynamic contrast-enhanced MRI: Use in predicting pathological complete response to neoadjuvant chemoradiation in locally advanced rectal cancer. *J Magn Reson Imaging.* 2015; 42(3): 673-680.

24. **Sun YS, Zhang XP, Tang L, et al.** Locally advanced rectal carcinoma treated with preoperative chemotherapy and radiation therapy: preliminary analysis of diffusion-weighted MR imaging for early detection of tumor histopathologic downstaging. *Radiology.* 2010; 254(1): 170-178.

25. **Jacobs L, Intven M, van Lelyveld N, et al.** Diffusion-weighted MRI for Early Prediction of Treatment Response on Preoperative Chemoradiotherapy for Patients With Locally Advanced Rectal Cancer: A Feasibility Study. *Ann Surg.* 2016; 263(3): 522-528.

26. **Flatmark K, Saelen MG, Hole KH, et al.** Individual tumor volume responses to short-course oxaliplatin-containing induction chemotherapy in locally advanced rectal cancer – Targeting the tumor for radiation sensitivity? *Radiother Oncology.* 2016; 119(3): 505-511.

**Table and Figure Legends**

**Table 1** Mean tumour values for the different MRI parameters quantified for good responders (tumour regression grade (TRG) 1-2) and poor responders (TRG3-5) to neoadjuvant chemotherapy (NACT) followed by chemoradiotherapy (CRT), with associated p-values obtained from the Mann Whitney U test.

**Figure 1**. Receiver operating characteristic (ROC) curves for baseline tumour perfusion fraction (Fpre), tumour volume (Vpre), as well as the combined parameter Fpre/Vpre for prediction of tumour regression grade (TRG) 3-5, i.e. poor responders to neoadjuvant chemotherapy (NACT) followed by chemoradiotherapy (CRT). Area under curve (AUC) for the different parameters are indicated.

**Figure 2**. Differences in 5-year progression-free survival (PFS) for patients with baseline tumour perfusion fraction (Fpre) divided by baseline tumour volume (Vpre), i.e. Fpre/Vpre, above and below 0.5, respectively. The number of patients and p-values are indicated.

**Table 1.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Good responder**  **(TRG1-2)** | **Poor responder**  **(TRG3-5)** | **p-value1** |
| ADCpre (x 10-4 mm2/s) | 74.1 ± 22.7 | 61.0 ± 12.6 | >0.1 |
| ADCNACT (x 10-4 mm2/s) | 65.1 ± 35.6 | 69.3 ± 15.4 | >0.1 |
| ΔADC (%) | -15.7 ± 25.3 | 14.9 ± 23.4 | <0.01\* |
| Fpre (a.u.) | 25.4 ± 16.9 | 12.6 ± 4.1 | 0.01\* |
| FNACT (a.u.) | 23.0 ± 14.3 | 15.6 ± 3.9 | >0.1 |
| ΔF (%) | 41.7 ± 168.8 | 39.6 ± 67.1 | >0.1 |
| Vpre (cm3) | 17.1 ± 21.6 | 43.5 ± 31.1 | <0.01\* |
| VNACT (cm3) | 7.1 ± 11.3 | 24.9 ± 19.8 | 0.02\* |
| ΔV (%) | -70.3 ± 13.5 | -42.6 ± 35.3 | 0.06 |
| Fpre/Vpre | 3.5 ± 4.3 | 0.51 ± 0.6 | < 0.001\* |
| FNACT/VNACT | 11.4 ± 11.8 | 3.7 ± 9.4 | <0.01\* |
| Δ(F/V) | 26.4 ± 14.9 | 51.7 ± 36.5 | >0.1 |

1Parameters with significant difference between good and poor responders are marked with \*

**Figure 1.**

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**Figure 2.**

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**Supplementary File – Percentile analysis**

***Method***

To explore tumour heterogeneity in diffusion-weighted magnetic resonance imaging (DWMRI) parameters, we performed percentile analysis of the mean DWMRI parameters that showed a significant association with treatment response. The aim was to identify which percentile ranges contained the most predictive information and all percentile values were analysed using receiver operating characteristic (ROC) curve analysis in SPSS, and evaluated by their area under curve (AUC), p-value, sensitivity and specificity.

***Results***

For the change in the apparent diffusion coefficient (ΔADC) from baseline to after neoadjuvant chemotherapy, lower percentiles showed slightly higher AUC, sensitivity and specificity and lower p-value than the mean value, Figure S3. The 16th percentile showed the highest accuracy and predicted good histopathological tumour response to CRT with 83% sensitivity and 91% specificity (AUC = 0.92, p = 0.001, confidence interval = 0.81 - 1.00), positive predictive value of 91% and negative predictive value of 83%, at an optimal cut-off of 0.

There was also an association between ∆ADC and progression-free survival (PFS) at the lower percentile ranges, especially the 20-40th percentiles, Figure S3. The 23rd percentile was most significantly associated with PFS, when separating patients above and below the cut-off value of -6%, the respective estimated 5-year PFS were 33% and 91% (p = 0.008). In comparison, this association was not significant for mean ΔADC.

Percentile analysis did not contribute further to the analysis of the perfusion fraction F.

**Supplementary File – Tables and figures**

**Table and figure legends**

**Table S1**. Main image acquisition parameters for the MRI sequences.

**Figure S1**. Number of patients in the analysis amounted to 27 for baseline MRI (MRIpre) and 23 for the follow-up MRI after neoadjuvant chemotherapy (MRINACT).

**Figure S2**. The tumour perfusion fraction (F) was calculated as the discrepancy between the original signal intensity at b0 and the line extrapolated from the apparent diffusion coefficient (ADC) calculated from b300 and b900 s/mm2.

**Figure S3.** P-values (upper figure) and area under curve (AUC) values (lower figure) from receiver operating characteristics (ROC) curve analysis from each percentile for the change in tumour apparent diffusion coefficient (ADC) for the two study endpoints tumour regression grade (TRG) and progression free survival (PFS).

**Table S1.**

|  |  |  |
| --- | --- | --- |
| **Acquisition parameter** | **T2-weighted imaging** | **Diffusion-weighted imaging** |
| Repetition time (ms) | 3000 | 6500 |
| Echo time (ms) | 81 | 117 |
| Echo train length | 13 | -- |
| Number of averages | 2 | 10 |
| In-plane resolution (mm2) | 0.38 x 0.38 | 1.70 x 1.70 |
| Slice thickness (mm) | 4 | 4 |
| Slice gap (mm) | 1 | 5 |
| b-values (s/mm2) | - | 0, 300, 900 |

**Figure S1.**

**Figure S2.**

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**Figure S3**

