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Functional MRI for quantitative treatment response prediction in locally advanced rectal cancer

Trang Pham
University of New South Wales

Gary P. Liney
University of Wollongong, garyl@uow.edu.au

Karen Ms Karen Wong
University of New South Wales

Michael Barton
University of New South Wales, Michael.Barton@sswahs.nsw.gov.au

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Abstract

Despite advances in multimodality treatment strategies for locally advanced rectal cancer and improvements in locoregional control, there is still a considerable variation in response to neoadjuvant chemoradiotherapy (CRT). Accurate prediction of response to neoadjuvant CRT would enable early stratification of management according to good responders and poor responders, in order to adapt treatment to improve therapeutic outcomes in rectal cancer. Clinical studies in diffusion-weighted imaging (DWI) and dynamic contrast-enhanced (DCE) MRI have shown promising results for the prediction of therapeutic response in rectal cancer. DWI allows for assessment of tumour cellularity. DCE-MRI enables evaluation of factors of the tumour microvascular environment and changes in perfusion in response to treatment. Studies have demonstrated that predictors of good response to CRT include lower tumour pre-CRT apparent diffusion coefficient (ADC), greater percentage increase in ADC during and post CRT, and higher pre-CRT K^{trans}. However, the mean ADC and K^{trans} values do not adequately reflect tumour heterogeneity. Multiparametric MRI using quantitative DWI and DCE-MRI in combination, and a histogram analysis technique can assess tumour heterogeneity and its response to treatment. This strategy has the potential to improve the accuracy of therapeutic response prediction in rectal cancer and warrants further investigation.

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REVIEW ARTICLE

Functional MRI for quantitative treatment response prediction in locally advanced rectal cancer

[1,2,3,4]TRANG T PHAM, MB BS, FRANZCR, [1,3,4,5]GARY P LINEY, PhD, [1,3,4]KAREN WONG, PhD, FRANZCR and [1,3,4]MICHAEL B BARTON, MD, FRANZCR

[1]Department of Radiation Oncology, Liverpool Hospital, Sydney, NSW, Australia
[2]Sydney West Radiation Oncology Network, Westmead, Blacktown and Nepean Hospitals, Sydney, NSW, Australia
[3]Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia
[4]Ingham Institute for Applied Medical Research, Sydney, NSW, Australia
[5]Faculty of Radiation and Medical Physics, University of Wollongong, NSW, Australia

Address correspondence to: Dr Trang T Pham
E-mail: *trang.pham@sswahs.nsw.gov.au*

ABSTRACT

Despite advances in multimodality treatment strategies for locally advanced rectal cancer and improvements in locoregional control, there is still a considerable variation in response to neoadjuvant chemoradiotherapy (CRT). Accurate prediction of response to neoadjuvant CRT would enable early stratification of management according to good responders and poor responders, in order to adapt treatment to improve therapeutic outcomes in rectal cancer. Clinical studies in diffusion-weighted imaging (DWI) and dynamic contrast-enhanced (DCE) MRI have shown promising results for the prediction of therapeutic response in rectal cancer. DWI allows for assessment of tumour cellularity. DCE-MRI enables evaluation of factors of the tumour microvascular environment and changes in perfusion in response to treatment. Studies have demonstrated that predictors of good response to CRT include lower tumour pre-CRT apparent diffusion coefficient (ADC), greater percentage increase in ADC during and post CRT, and higher pre-CRT K^{trans}. However, the mean ADC and K^{trans} values do not adequately reflect tumour heterogeneity. Multiparametric MRI using quantitative DWI and DCE-MRI in combination, and a histogram analysis technique can assess tumour heterogeneity and its response to treatment. This strategy has the potential to improve the accuracy of therapeutic response prediction in rectal cancer and warrants further investigation.

INTRODUCTION

Advances in multimodality treatment strategies over the past decades have contributed to an improvement of outcomes for patients with rectal cancer. The combination of neoadjuvant chemoradiotherapy (CRT) and standardized surgical technique (total mesorectal excision) has led to an improvement in locoregional control, with local recurrence rates dropping from 20–30% to 7–10% for patients with locally advanced disease (T3–4 and/or N1–2).[1,2] Neoadjuvant CRT followed by a 6- to 8-week break prior to surgery has the advantage of tumour downstaging, with a pathological complete response (pCR) achieved in 15–27% of patients.[3] Despite these advances, tumour responses to CRT still vary considerably, with 54–75% of patients having tumour downstaging and the remainder having no treatment response.[4] The reason for this variation in treatment response is not well understood, and at present, there is no accurate method of predicting treatment response. Furthermore, distant metastases still predominate with a 5-year cumulative incidence of 30% in locally advanced (T3–4) resectable rectal cancer shown in a pooled analysis of five Phase III randomized trials.[2]

More accurate imaging biomarkers for the prediction and assessment of radiotherapy response would enable early stratification of patients into different prognostic groups and a personalized treatment approach. Identification of patients with a clinical complete response prior to surgery would enable optimization of the surgical approach with "organ-preserving" procedures, which would result in a reduction in surgical morbidity. It has been proposed that patients with a clinical complete response may be able to avoid surgery with a "wait and watch" policy;[5] however, accurate identification of patients with complete response is crucial for this approach to minimize the risk of recurrence. Early detection of poor responders to CRT would provide the opportunity for these patients to proceed directly to surgery thereby avoiding morbidity of CRT or for

intensified treatment regimens such as second-line chemotherapy or higher radiation dose to maximize therapeutic response.

Functional imaging predictive biomarkers may be able to guide individualization of patient treatment in order to maximize therapeutic outcomes and minimize treatment toxicity. MRI has the benefit of sampling the whole tumour and can be repeated on multiple occasions unlike biopsy. Conventional pre-operative staging investigations and histological examination of tissue biopsies are not capable of predicting individual treatment response. Although MRI using standard morphological sequences (T_2 weighted) is important for staging and treatment planning,[6] it is inadequate for prediction and assessment of individual response to CRT. Current predictive models require pathological staging, making them unsuitable for use as a pre-treatment decision support tool.[2,7] Recent research efforts have focused on using physiological information from functional MRI techniques to more accurately predict treatment response; however, the clinical role of functional MRI is yet to be defined.

This expert review assesses the current status on functional MRI, from the clinical utility in prediction of CRT response through to the *ex vivo* analysis and characterization of rectal cancer. This review summarizes the existing literature on clinical potential of diffusion-weighted imaging (DWI) and dynamic contrast-enhanced (DCE) MRI in prediction of CRT response, prognosis and biological characterization of tumour and the *ex vivo* high-field MR spectroscopy (MRS) in the metabolic characterization of rectal cancer.

Potential benefits of MRI/MR spectroscopy biomarkers

The development and discovery of MRI/MRS biomarkers can improve the clinical management of rectal cancer. The foreseeable benefits are:

(i) MRI/MRS provides a non-invasive technique that can assess the entire tumour region, thereby yielding information about tumour heterogeneity and avoiding sampling error associated with conventional biopsy techniques.
(ii) New MRI/MRS biomarkers will better reflect patient prognosis and response to different treatment options, thus facilitating individually tailored medical management and monitoring of outcomes. This can enable early stratification of patients into high-risk groups, for de-escalation of treatment (*e.g.* exclusion of surgery or sphincter-sparing techniques in patients with pCR) or low-risk groups for intensification of treatment.

FUNCTIONAL MRI FOR CLINICAL THERAPEUTIC RESPONSE PREDICTION AND ASSESSMENT IN RECTAL CANCER

The development of functional MRI sequences has enabled the assessment of different biological tumour characteristics and is an important advance in imaging evaluation in oncology. Standard morphological MRI (T_2 weighted) has been shown to have significant correlation with survival outcomes in the MRI and Rectal Cancer European Equivalence (MERCURY) study,[8] a large prospective trial assessing the prognostic significance of post-CRT MRI assessment of tumour regression grade (TRG). However, volumetric measurements based on standard morphological MRI lack sufficient accuracy for the differentiation of treatment responders from non-responders because of their inability to detect small residual tumour deposits within areas of radiation-induced fibrosis. Functional MRI biomarkers have been documented to have greater potential compared with standard T_2 weighted sequences in the assessment of therapeutic response in patients with locally advanced rectal cancer undergoing neoadjuvant CRT.[9] The clinical utility of these functional MRI biomarkers in rectal cancer is still yet to be defined.

DIFFUSION-WEIGHTED MRI

DWI is a functional MRI technique which is sensitive to the movement of water molecules through the body. The apparent diffusion coefficient (ADC), a quantitative parameter used for assessment of water diffusion through tissue, shows an inverse relationship with tissue cellularity.[10] Viable tumour cells restrict the mobility of water, whereas necrotic tumour cells allow increased diffusion of water molecules. Increasing tumour cellularity and architectural distortion of the extracellular space will contribute to decreased ADC values. The ADC values have been shown to correlate with tumour cellularity and grade.[11] The ADC has been shown to differentiate post-treatment persistent tumour from inflammation and necrosis, making it a useful tool to monitor effects of radiotherapy. Radiotherapy-related cellular damage leading to necrosis can occur within days of initiating therapy. The ability to detect changes in tumour microstructure allows DWI to be used for early treatment predictions.[12,13]

Prediction and assessment of primary tumour response

Prospective studies have evaluated the use of DWI in the prediction and assessment of primary response to CRT in rectal cancer. The majority of these studies demonstrated ADC to be useful for distinguishing good responders from poor responders, with the standard histological reference based on TRG. The key findings in prospective studies of DWI for assessment of CRT response are shown in Table 1. In general, studies demonstrated predictors for good responders have a lower pre-CRT ADC value and a greater percentage increase in ADC during and post CRT. Performance of pre-treatment ADC values for prediction of good responders is variable between small prospective studies, with sensitivities, specificities, positive-predictive values (PPVs) and negative-predictive values (NPVs) ranging from 62% to 100%, 86–91%, 67–79%, and 62–100%, respectively.[13–15] One study[16] demonstrated that pre-treatment ADC was not a significant predictor of treatment response. The performance of post-treatment percentage increase (41–59%) in ADC for detection of good responders produced PPV and NPV ranging from 82 to 91% and from 43 to 94%, respectively.[15,16] In another study,[17] a post-treatment mean ADC cut-off of $1.045 \times 10^{-3}\,mm^2\,s^{-1}$ was associated with a sensitivity and specificity of 75% and 100%, respectively. These DWI studies used echo planar imaging (EPI) and multiple *b*-values (range between 0 and 1000). However, there were a number of differences that may account for the variations in results observed between studies. The standardization of MRI protocols is important to compare quantitative studies; the differences in the number of *b*-values

Table 1. Diffusion-weighted imaging (DWI) MRI studies—assessment of neoadjuvant chemoradiotherapy response

Study	*n*	MRI time points	MRI variable	Histopathology response	Result—direction of correlation with histopathology	Significance
Primary tumour response						
Barbaro et al (2012)[13]	62	1.5 T Pre, during and post	ADC (median) %ΔADC	TRG (Mandard)	Pre-ADC $<1\times10^{-3}$mm^2 s^{-1} correlation (+) with TRG4	$p = 0.0011$
				Good responders TRG1–2 *vs* non-responders TRG3–5	During: higher %ΔADC correlation (+) with responders	$p < 0.0001$
Intven et al (2013)[15]	59	3 T Pre	ADC (median)	TRG (Mandard)	Pre-ADC (0.95 good response *vs* 1.12 moderate response)	$p < 0.001$
				Good response TRG1–2 *vs* moderate response TRG3–5	Post-ADC	NS
					ΔADC (%) (50 *vs* 23)	$p < 0.001$
					ΔADC (absolute) (0.43 *vs* 0.26)	$p < 0.001$
Monguzzi et al (2013)[16]	31	1.5 T Pre and post	ADC (median)	TRG (Mandard)	Pre-ADC	NS
			ΔADC	Good responders TRG1–2 *vs* non-responders TRG3–5	Post-ADC (1.3 *vs* 1.2)	$p = 0.004$
					ΔADC (%)	NS
					ΔADC (absolute) (0.5 *vs* 0.4)	$p < 0.05$
Kim et al (2011)[55]	34	3 T Pre, during and post	ADC	TRG (Mandard)	Pre-ADC	NS
				Good responders TRG1–2 *vs* non-responders TRG3–5	Early ΔADC	NS
Cai et al (2013)[56]	15	1.5 T Pre CRT, weekly during CRT	ADC (mean)	TRG (Dworak)	Pre-ADC	$p = 0.021$
				Good response TRG3–4	Weekly ADC—comparison with pre-ADC:	
				Poor response TRG0–2	Week 1 ADC	NS
					Week 2 ADC (increase-correlation with GR)	$p = 0.004$
					Week 3 ADC	NS
					Week 4 ADC	NS
					Week 5 ADC	NS
Musio et al (2013)[57]	22	3 T Pre, during, post	ADC (mean)	Histological responders: Downstaging/reduction in T or N staging Non-responders: stable or progressive disease	Pre-ADC—no significant difference	NS
					Responders: ΔADC from pre to during in responders	$p < 0.05$
					Non-responders: ΔADC	NS
Carbone et al (2012)[58]	14	1.5 T Pre, post	DWI volume	TRG (Mandard)	Pre-V_{DWI}	NS
			(V_{DWI}) *vs* T2W volume (V_c)	Good responders TRG1–2 *vs* non-responders TRG3–5	Post-V_{DWI} (4 responders *vs* 13 non-responders)	$p = 0.004$
Nodal response						
Lambregts et al (2011)[18]	30	1.5 T Post	ADC (mean)	Histological malignant nodes *vs* benign nodes	DWI *vs* T2W or DWI + T2W: no improvement in identification of malignant nodes	NS

ADC, apparent diffusion coefficient; NS, not significant; T2W, T_2 weighted; TRG, tumour regression grade.

and *b*-values used in the protocols could result in differences in the ADC maps produced, making it difficult to compare studies. The imaging analyses and quantification methods varied between studies; Lambrecht et al[14] defined the region of interest (ROI) on $b = 0$ and $b = 1000$ images; Barbaro et al[13] defined the ROI on ADC maps, matching with corresponding T_2 weighted images, and Intven et al[15] used in-house software for their analysis.

A meta-analysis by van de Paardt et al,[9] which included six DWI studies, has compared the performance of DWI with standard morphological MRI (T_2 weighted) in assessment of treatment response post CRT. DWI showed significantly better results for tumour re-staging compared with standard morphological sequences. They found that DWI had a mean sensitivity of 83.6% (95% CI 61.7–94.2%) and specificity of 84.8% (95% CI 74.2–91.5%). Studies with experienced observers showed significantly better results, with higher sensitivity for re-staging, than studies with less experienced observers.

There are limited prospective studies examining the value of DWI during CRT.[13,14] These studies showed that the percentage increase in ADC during Week 2 of CRT was predictive of response. This is potentially useful in identifying poor responders early during treatment, to provide an opportunity to change the treatment approach and to prevent them from proceeding with likely futile treatment. Further investigation of DWI performed during CRT is warranted.

ADC cut-off values for response in studies were chosen on an *ad hoc* basis and still require prospective validation in larger patient populations. Furthermore, there was a wide variation in performance of ADC values demonstrated between studies. Although promising, DWI currently lacks sufficient accuracy for clinical use to stratify patients into adaptive management pathways. The above rectal cancer diffusion studies used conventional single-shot EPI. More recent improvements in these sequences, such as readout segmented diffusion technique (RESOLVE), have improved detail and decreased image distortion compared with standard EPI diffusion. The benefit of RESOLVE for therapeutic response prediction warrants further investigation in rectal cancer.

Assessment of nodal response

The van der Paardt meta-analysis found that MRI, including standard morphological sequences and DWI studies, cannot discriminate nodal response to treatment.[9] However, it was not specified how many DWI studies formed part of this analysis. One DWI prospective study of 30 patients by Lambregts et al[18] specifically assessed the performance of ADC for nodal staging after completion of CRT. They found that DWI on its own was not reliable and did not improve accuracy of nodal staging when performed in addition to T_2 weighted sequences.

Identification of pathological complete response

Between 15% and 27% of patients will have a pCR following CRT. This represents an important subgroup of patients in whom surgery could be avoided with a "wait and watch" policy, or morbidity of surgery can be reduced with sphincter-sparing surgical techniques. Accurate identification of these patients is of great importance prior to considering more conservative management options. There are only 5 small prospective studies[14,15,19–21] assessing the role of DWI in detection of pCR; the remainder of studies are retrospective series.[22–24] The key findings from these studies are summarized in Table 2. The majority of these studies have shown that it is possible to identify patients with pCR through early prediction pre CRT or assessment post CRT.

Multiple studies[14,15,19] have found that a lower pre-treatment mean ADC had value in prediction of patients who went on to have a pCR, although this was not significant in Genovesi et al.[19] Lambrecht et al[14] studied DWI performed at multiple time points for the assessment of pCR in a prospective study of 20 patients. They found that pre-CRT ADC had a sensitivity of 100% and specificity of 86% in prediction of pCR.

In the post-CRT assessment of CRT response, Intven et al[15] and Genovesi et al[19] found that change in the ADC post CRT could identify pCR, with diagnostic accuracy of 98% and 91% in the studies, respectively. Lambrecht et al[14] found that ΔADC post CRT was also useful in the detection of pCR. These results have not always been replicated, with some investigators finding that DWI does not unequivocally determine pCR.[20] A large retrospective multicentre study[22] of 120 patients found that the addition of DWI to standard morphological sequences improved the selection of pCR after CRT. DWI analysis by observers in this study was qualitative and therefore did not use the ADC values. With T_2 weighted MRI alone, the sensitivity for identification of pCR was poor ranging from 0% to 40%. The addition of DWI improved the sensitivity to 52–65% and had a specificity of 89–98%.

It is difficult to accurately identify pCR on DWI alone, and this modality may need to be combined with another modality. It is expected that in patients with no residual tumour, diffusion will be freer and restriction will not remain on the post-CRT scan. However, a study[25] assessing the post-CRT DWI of patients with confirmed pCR has shown that this is not always the case. Jang et al[25] retrospectively reviewed post-CRT DWI of 43 patients who had undergone neoadjuvant CRT, subsequent surgery and achieved pCR. They reported diffusion restriction remained in 42% of patients with pCR. Radiation proctitis and fibrosis, demonstrated on histopathology, were significant independent predictors of diffusion restriction in patients achieving pCR after CRT. This study has shown that even in the absence of residual tumour, restricted diffusion can still occur as a result of radiation-induced fibrosis, making it difficult to accurately identify all patients with pCR on DWI alone. A study by Maas et al[21] on 50 patients found that post CRT, qualitative T_2 weighted imaging and DWI had low sensitivity of 35% and specificity of 94% for the prediction of pCR. However, combining MRI with clinical assessment, consisting of digital rectal examination and endoscopy, improved the prediction of pCR with a post-test probability for predicting pCR of 98%. This study demonstrated that DWI alone is poor at detecting pCR and needs to be combined with another modality.

Joye et al[26] performed a systematic review on the role of DWI in the prediction of pCR in response to CRT. Pooled data from

Table 2. Diffusion-weighted imaging (DWI) MRI studies—identification of pathological complete response (pCR)—ypT0 *vs* non-pCR after chemoradiotherapy

Study	*n*	MRI timing	MRI variable	Result	Significance
Lambrecht et al (2012)[14]	20	1.5 T Pre, during, post	ADC (mean)	Pre-ADC (0.94 pCR *vs* 1.19 non-pCR)	$p < 0.003$
			ΔADC (%)	During ΔADC (72 *vs* 16) and post-ΔADC (88 *vs* 26) higher in pCR	$p = 0.0006$
					$p = 0.0011$
Genovesi et al (2013)[19]	28	Pre, post	ADC (mean)	ΔADC (%) (77.2 pCR *vs* 36 non-pCR)	$p = 0.05$
			ΔADC (%)	ΔADC (%) better than T2W Δvolume (%) in detection of pCR	$p = 0.022$
Engin et al (2012)[20]	30	Pre, post	DWI qualitative—SI, ADC (mean)	Post-ΔSI and Post-ΔADC	NS
Maas et al (2015)[21]	50	1.5 T Post	T2W and DWI—qualitative	Detection of pCR: sensitivity 35%, specificity 94%	
				Combining T2W, DWI and clinical assessment improves detection of pCR post-test probability 98%	
Lambregts et al (2011)[22]	120 (multicentre)	1.5 T Post	Qualitative	Detection of pCR: DWI *vs* T2W alone (sensitivity 52–64% *vs* 0–40%, specificity the same 89–98%)	
Sassen et al (2013)[23]	70	1.5 T Pre, post	T2W *vs* addition DWI—qualitative	DWI improved interobserver agreement	$p = 0.005$
Curvo-Semedo et al (2011)[24]	50 (single centre)	1.5 T Pre, post	ADC ΔADC (%) ADC volume *vs* T2W volume	ADC and ΔADC (%)	NS
				Post-ADC volume (0.03 pCR *vs* 1.5 non-pCR) and Δvolume (−100 *vs* −90) better than T2W in detection pCR	$p < 0.001$ $p < 0.001$
Ha et al (2013)[59]	100	1.5 T Pre, post	V_{DWI} (pCR: Dworak grade 4)	Pre-V_{DWI}—no significant difference	NS
				Post-V_{DWI} (0.8 pCR *vs* 3.5 non-pCR)	$p < 0.001$
				Pre-ADC—no significant difference	NS
				Post-ADC (1.33 *vs* 1.13)	0.002

ADC, apparent diffusion coefficient; NS, not significant; SI, signal intensity; T2W, T_2 weighted; V_{DWI}, DWI volume.

9 studies with a combined total of 226 patients demonstrated that the ADC pre CRT was unable to predict for pCR with a sensitivity of 69%, specificity of 68%, PPV of 35% and NPV of 90%. Pooled data from 10 studies with a combined total of 315 patients found that the ADC post CRT had sensitivity of 78%, specificity of 72%, PPV of 47% and NPV of 91% for detection of pCR. However, the lack of standardization of MRI protocols and image quantification methods between different centres would limit the potential of pooled analysis.

DYNAMIC CONTRAST-ENHANCED MRI

DCE-MRI provides functional information on tumour microvessel perfusion, permeability and extracellular-extravascular space composition by assessing the changes in signal intensity over time following intravenous injection of a paramagnetic contrast agent. ROIs may be interrogated to produce enhancement–time curves with malignant tumours, due to their abnormal microvasculature, demonstrating a rapid washin and washout of contrast and a greater increase in signal intensity than in normal tissues.[10,27] DCE-MRI may be able to assess characteristics of the tumour vascular microenvironment, such as hypoxia and microvascular density that influence radiotherapy response, and also vascular changes induced by radiotherapy. DCE-MRI can potentially assess tumour downstaging, therefore distinguishing good responders from poor responders to treatment. The perfusion characteristics on DCE-MRI can be analysed either in a quantitative[28] or qualitative technique, hence the published studies are difficult to compare. Quantitative analysis involves modelling the

pharmacokinetics of an intravenously administered contrast agent and requires correction for T_1 pre-contrast. Investigation into DCE-MRI is sparse, and its clinical role in response prediction and assessment and optimal evaluation technique is yet to be established.

Prediction of therapeutic response

Several quantitative studies assessing tumour response with DCE-MRI have shown that higher contrast exchange rates pre CRT, which indicates higher tumour permeability, are associated with better therapeutic response to CRT. Key findings from studies evaluating the role of DCE-MRI in therapeutic response assessment are shown in Table 3. In a prospective study of 95 patients, a higher pre-treatment K_{21} (contrast medium exchange in the Brix pharmacokinetic model) was significantly associated with good treatment response, defined as post-treatment pT0-2N0 Union for International Cancer Control (UICC) stage.[29] On multivariate analysis, a higher 75th percentile K_{21} was associated with a higher tumour response rate, whereas the DCE-MRI quantitative parameters—amplitude and time to peak—were not associated with tumour response. In addition, mucinous tumour morphology was significantly associated with poorer response to treatment. Other quantitative studies demonstrated higher pre-CRT K^{trans} (contrast medium exchange in the Tofts pharmacokinetic model) was predictive of tumour response.[30,31] Intven et al[30] found in a study of

Table 3. Dynamic contrast-enhanced (DCE) MRI studies—assessment of chemoradiotherapy response and prognosis

Study	*n*	MRI time points	MRI variable	Histopathological Physiological variable	Result	Significance
De Vries et al (2014)[38]	83	1.5 T Pre	Semi-quantitative PI	Responders: ypT0-2 Non-responders: ypT3 DFS OS	PI lower in responders than non-responders (7.6 *vs* 9.8) Mean follow-up 71 (±29) months PI-predicted DFS (HR 1.85) PI-predicted OS (HR 1.42)	$p < 0.001$ $p < 0.001$ $p = 0.04$
Martens et al (2014)[33]	30	1.5 T Pre, post	Semi-quantitative Initial slope Initial peak Late slope AUC 60, 90, 120 s	TRG (Mandard): Good responders TRG1–2 Non-responders TRG3–5	Late slope (-0.05×10^{-3} GR *vs* 0.62×10^{-3} PR) Other parameters—no significant difference	$p < 0.001$ NS
Oberholzer et al (2013)[29]	95	1.5 T Pre	Quantitative/ semi-quantitative K_{21} A TTP	Responder—downshift in UICC stage *vs* non-responder—no downshift Good responder ypT0–2N0	K_{21}—higher in responders *vs* non-responders TTP—difference between responders and non-responders A—75th percentile lower in responders K_{21}—high 75th percentile—higher response	$p < 0.001$ $p < 0.025$ $p = 0.016$ $p = 0.019$
Intven et al (2014)[30]	51	3 T Pre, post	Quantitative K^{trans} Median (50th percentile) p25 (25th percentile) p75 (75th percentile)	TRG (Mandard) Good responder TRG1–2 *vs* non-responder TRG3–5	Pre-K^{trans} median (0.48 GR *vs* 0.39 PR) Post-K^{trans} median (0.33 GR *vs* 0.39 PR) ΔK^{trans} (%) (−34 GR *vs* −1.2 PR)	$p = 0.024$ $p = 0.024$ $p < 0.001$
Lim et al (2012)[32]	39	3 T Pre, during, post	Quantitative K^{trans} V_e	TRG good responder TRG1–2 *vs* non-responder TRG3–5 TNM down-staging	K^{trans} no significant correlation with TRG good-responders *vs* non-responders Pre- and early-K^{trans} higher in TNM downstaging V_e—no correlation	NS $p = 0.0215$ NS

A, amplitude; AUC, area under the curve; HR, hazard ratio; NS, not significant; PI, perfusion index; TRG, tumour regression grade; TTP, time-to-peak signal intensity; PR, poor responder; GR, good responder; DFS, disease free survival; K^{trans}, transfer constant between blood plasma and extravascular extracellular space; V_e, extravascular extracellular fractional volume; OS, overall survival.

51 patients that pre-CRT K^{trans} (50th percentile) was significantly higher in patients with good therapeutic response as defined by Mandard TRG 1–2. Similarly, George et al[31] showed that responsive tumours had higher pre-treatment K^{trans} than non-responsive tumours. This result was also demonstrated in Lim et al,[32] although not significant. Results on the predictive value of post-CRT DCE-MRI were less clear, with Intven et al[30] and George et al[31] demonstrating a correlation between a reduction in post-CRT K^{trans} and good response, and Lim et al showing no correlation.

The role of DCE-MRI derived quantitative parameters in the prediction of therapeutic response to CRT was explored in a study of 30 patients by Martens et al.[33] Six semi-quantitative parameters from pre-CRT and post-CRT DCE-MRI were investigated: (i) initial slope, (ii) initial peak, (iii) areas under the first 60, 90 and 120 s of the enhancement curve and (iv) late slope. Only pre-CRT late slope was able to discriminate between good and poor responders to treatment (-0.05×10^{-3} *vs* 0.62×10^{-3}, $p < 0.001$).

There are a variety of quantitative measurements that can be obtained from DCE-MRI that can be obtained through a simple approach or through pharmacokinetic quantification models. A simple analysis uses a curve descriptor to characterize the signal intensity curve.[34] Examples of this include area under curve and initial peak used in the study by Martens et al.[33] Pharmacokinetic modelling includes the Brix and the Tofts models. K_{21} is derived from the original Brix model,[35] a simple linear two-compartment model that does not require T_1 mapping or arterial input function measurements. K_{21} can measure the rate constant between the extravascular extracellular space and plasma, but a major disadvantage is that it does not measure perfusion. There has been a shift towards using the Tofts model, which provides values that more closely represent pathophysiological processes, such as perfusion. K_{ep} in the Tofts model can measure rate constant between extravascular extracellular space and plasma, a value similar to K_{21} in the Brix model. K^{trans} in the Tofts model is the influx volume transfer constant from the plasma into the extravascular extracellular space. The calculation of K^{trans} requires pre-contrast T_1 measurement and takes into account the arterial input factor, hence this parameter more closely represents physiological perfusion.[28,36] At present, K^{trans} appears to be the most promising parameter for the prediction of treatment response in rectal cancer.

Most DCE-MRI studies assess therapeutic response in the primary tumour. There is a lack of studies on the potential of DCE-MRI in nodal therapeutic response assessment. The ability of DCE-MRI to assess nodal response to treatment has been explored only in a study of 55 patients who underwent DCE-MRI following completion of CRT. In this study, Alberda et al[37] assessed the accuracy of qualitative DCE-MRI methods, with histopathological assessment of nodes being the standard reference. They found that early incomplete arterial phase enhancement on DCE-MRI was a significant indicator of malignant nodes.

Prognostic value

The prognostic value of DCE-MRI in rectal cancer has been investigated by DeVries et al.[38] In a prospective study of 83 patients with T3 rectal cancer undergoing neo-adjuvant CRT, DeVries et al investigated the value of the pre-CRT perfusion index, a microcirculatory parameter which integrates information on flow and permeability, in the prediction of therapeutic disease-free survival and overall survival. A lower pre-treatment mean perfusion index was found to be significantly predictive of therapy response (defined as ypT0-2). After a mean follow-up of 71 ± 29 months, the perfusion index significantly predicted disease-free survival (hazard ratio 1.84, $p < 0.001$) and overall survival (hazard ratio 1.42, $p = 0.04$), with patients with lower perfusion index having worse survival.

Biological correlation

DCE-MRI allows functional characterization of biological changes in the tumour microvasculature.[27] The findings from studies correlating DCE-MRI with tumour biological characteristics are summarized in Table 4. Clinical DCE-MRI studies in rectal cancer have shown correlation of DCE-MRI parameters with variables of tumour angiogenesis relevant to radiation response.[29,31,39–41] A prospective study[39] of 17 patients showed that DCE-MRI can be used to assess radiation-induced changes in tumour vasculature. Radiotherapy inhibits tumour angiogenesis, which correlated with a significantly lower microvessel density (MVD) compared with non-irradiated tumours. Similarly, K_{ps}, the endothelial transfer coefficient, was found to be 77% ($p = 0.03$) lower in the radiotherapy-treated group. Hong et al[42] also showed that DCE-MRI could be used to assess tumour angiogenesis. The semi-quantitative parameter of E_{max} (maximal enhancement) had a significant correlation with microvessel count.

Yeo et al[40] performed a retrospective analysis of pre-operative DCE-MRI in 31 patients undergoing surgery alone, and pre-CRT and post-CRT DCE-MRI in 15 patients undergoing CRT. Quantitative parameters including K^{trans}, K_e, K^{trans}, K_{ep}, extravascular extracellular fractional volume (V_e), and initial area under the concentration curve in 60 seconds (iAUC) were correlated with histological markers of tumour aggressiveness [expression of epidermal growth factor receptor (EGFR) and *KRAS* gene mutations], tumour angiogenesis (MVD) and vascular endothelial growth factor (VEGF). They found that a higher mean K^{trans} and K_{ep} correlated with increased tumour aggressiveness, as indicated by the presence of an EGFR mutation. Furthermore, the mean K_{ep} from the high K^{trans} area had a significant positive correlation with MVD (higher mean K_{ep}, correlated with greater MVD). However, there was no correlation between DCE-MRI and VEGF in this study. In another study by George et al,[31] there was a correlation in K^{trans} and VEGF before treatment; however, this correlation was no longer present after the commencement of treatment.

MR SPECTROSCOPY: METABOLIC IMAGING

MRS is an important tool for studying cancer metabolism. It can improve the understanding of the altered metabolic pathways in cancer and identify new metabolic biomarkers for treatment response prediction, prognosis and novel therapeutic targets. The discovery of an activated choline metabolic pathway in cancer was mostly due to MRS studies of tumours in the 1980s.[43] This pathway is characterized by increased choline-containing compounds, such as phosphocholine, glycerophosphocholine and total choline-containing compounds, which are detectable by

Table 4. Dynamic contrast-enhanced (DCE) MRI studies—correlation with biological characteristics

Study	*n*	MRI time points	MRI variable	Histopathological Physiological variable	Result	Significance
de Lussanet et al (2005)[39]	17	1.5 T	K_{ps}	Micro-vessel density (MVD) (scored by CD31, CD34) Tumour cell and endothelial cell proliferation (scored by expression of Ki67 protein) Treated ($n = 7$)—long radiotherapy Non-treated ($n = 10$)—short radiotherapy or no radiotherapy	K_{ps} 77% lower in treated than non-treated MVD 37% lower in treated than non-treated Tumour cell proliferation reduced in both long RT and short RT	$p = 0.03$ $p = 0.03$
George et al (2001)[31]	31	1.5 T	ln K^{trans}	Serum VEGF Radiological (MR) response by World Health Organization criteria: CR *vs* partial response (50% decrease in size), progressive disease (increase ≥25% size or new lesions, stable disease	Pre-ln K^{trans} correlated with VEGF Post-ln K^{trans} no correlation with VEGF Pre-ln K^{trans} higher in responders than non-responders (−0.46 *vs* −0.72) Post-ln K^{trans}—significant reduction in responders compared with pre-ln K^{trans} (−0.86)	$p = 0.01$ NS $p = 0.03$ $p = 0.04$
Zhang et al (2008)[41]	38	3 T	ER_{peak} T_{peak} $T_{first\text{-}enhance}$ Uptake rate	Histopathological MVD, VEGF	Rectal cancer *vs* normal rectum: ER_{peak} higher Uptake rate higher T_{peak} earlier T_{peak} negative correlation with MVD T_{peak} earlier for VEGF+ ER_{peak}, uptake rate and T_{peak} no correlation with MVD/VEGF	$p < 0.001$ $p < 0.001$ $p = 0.027$ $p = 0.01$ $p = 0.021$ NS

CR, complete response; ER_{peak}, peak enhancement ratio; NS, not significant; T_{peak}, time to peak enhancement; RT, radiotherapy; $T_{first\text{-}enhance}$, first enhancement time; VEGF, vascular endothelial growth factor; K_{ps}, endothelial transfer co-efficient; K^{trans}, volume transfer constant between blood plasma and extravascular extracellular space.

non-invasive MRS. The increased choline compounds are caused by malignant transformation and the hypoxic and acidic micro-environment of tumour. Enzymes of choline metabolism, such as choline kinase, may provide novel therapeutic targets.

MRS is a unique technique that can be applied with high resolution, as an initial *ex vivo* metabolic screening tool on tissue samples, and then translated into lower resolution *in vivo* MRS protocols for clinical use. MRS can identify the "spectral fingerprint" of cancer subtypes due to its ability to detect multiple tissue-specific metabolites in a single experiment. It is a quantitative technique with the intensities of MRS signal being directly related to metabolite concentration.[44] Analysis of intact tissue using standard MRS techniques result in spectra with lower resolution than normal liquid-state spectra. The resolution of tissue spectra can be improved by applying high-resolution magic angle spinning, a specialized MRS technique that detects high-resolution spectra by spinning solid tissue samples at the angle of 54.7°. High-resolution magic angle spinning profiling of intact tissue produces spectra that approach the resolution of solution-state samples.[45] An advantage of this technique is that tissue sample preparation results in minimal tissue destruction, allowing for correlation with histological and molecular analysis of the same tissue sample post MR analysis.

Ex vivo MR spectroscopy: prediction of disease behaviour and survival

A small number of studies have highlighted the potential of metabolic profile derived from *ex vivo* MRS tissue analysis in predicting disease behaviour[46] and survival outcomes.[47,48] The key findings are summarized in Table 5. Pacholczyki-Sienicka et al[47] analysed frozen tissue samples from surgical specimens of 52 patients with colorectal cancer who underwent surgery using 700.33-MHz (16.4-T) proton (^{1}H) MRS. Survival time was defined as the interval from surgery until death or the end of a 5.5-year observation period. They found that long-term survival in patients was characterized by lower levels of choline

compounds (choline, phosphocholine, glycerylphosphorylcholine), glycine, lactate and myo-inositol compared with non-survivors. Choline compounds have been found to be associated with malignant transformation, therefore this finding of a lower level of choline compounds in long-term survivors is biologically plausible. In addition, they found that an increase in taurine/glycine (sensitivity 64% and specificity 96%) and taurine/myo-inositol (sensitivity 65% and specificity 100%) ratios were significantly higher in survivors than in non-survivors.

Jimenez et al[48] also found that metabolic profiling of colorectal cancer samples was predictive of 5-year survival. However, this study found that metabolic changes predictive of survival occurred in tumour-adjacent macroscopically normal colon, 5–10 cm from the tumour. The authors suggest this represents a "field cancerization" effect, whereby the tumour affects the metabolism of its surroundings. However, the metabolic profile within the tumour specimens was not predictive of survival.

MRS metabolic profiling can potentially predict patients at risk of tumour recurrence.[46] Minicozzi et al proposed a marker of malignancy (MRS-tm) based on the metabolic findings in a study by Chan et al.[49] MRS-tm was defined as the ratio between the sum of the amplitudes of increasing components with peak between 3.0 and 3.8 ppm (including choline-containing compounds, taurine, scyllo-inositol and glycine) to the amplitude of the lipid methylene at 1.3 ppm. 4.7-T ^{1}H-MRS was performed on 29 surgical specimens. The MRS-tm was calculated for each specimen for tumour compared with healthy mucosa. The results demonstrated that MRS-tm was higher in tumour than normal mucosa. At 5-year follow-up, they reported statistical significance for tumour MRS-tm when patients were discriminated according to disease progression (either local recurrence or distant metastases). 5 of 6 patients with disease progression had a tumour MRS-tm <0.1, whereas 16 of 18 disease-free patients had MRS-tm >0.1 ($p = 0.04$).

Clinical MR spectroscopy

One published study[50] assessed the use of MRS *in vivo* in patients with rectal cancer. Kim et al[50] performed ^{1}H-MRS at 3 T with a 6-channel phased-array pelvic coil in 34 patients with rectal cancer at diagnosis and after neoadjuvant CRT. They found that the choline peak at 3.2 ppm was characteristic of rectal cancer at diagnosis. Following CRT, the choline peak disappeared and only the lipid peaks remained in 33 patients post CRT. The study identified three spectral types and found that 67% of patients with a post-CRT spectral type containing lipid peaks at 1.3 and 0.9 ppm at both long and short echo times

Table 5. *Ex vivo* high-field MR spectroscopy (MRS) studies of colorectal cancer

Study	*n*	Methods	Tumour findings (relative to normal bowel)/prognostic findings	Other findings
Minicozzi et al (2013)[46]	29 CRC (29 colon, 2 RC, 5 RC after neoadjuvant chemoradiotherapy)	^{1}H HR-MAS NMRS 4.7 T Surgical specimens Proposed marker of malignancy (MRS-tm)—ratio between sum of amplitudes of increasing components and amplitude of lipid methylene	↑ MRS-tm 5-year follow-up: Local recurrence or distant disease progression: MRS-tm <0.1 in 5/6 cases Disease-free patients: MRS-tm ≥0.1 in 16/18 cases	
Pacholczyk-Sienicka et al (2014)[47]	52 CRC (22 survivors, 30 non-survivors)	^{1}H HR-MAS NMRS 16.4T Frozen tissue samples	Long-term survival: ↓ glycine, choline, phosphocholine, myo-inositol, lactate, glycerophosphocholine ↑ creatine, glucose, aspargine Long-term survival: ↑ taurine/glycine, and taurine/myo-inositol ratio	Rectal cancer: ↑ formate
Jimenez et al (2013)[48]	26 CRC (6 RC, 20 colon)	^{1}H HR-MAS NMRS 400-MHz Frozen tissue samples	↑ isoglutamine, choline, phosphocholine, taurine, lactate, tyrosine, phenylalanine ↓ lipids, triglycerides	Lymph node positive disease: ↑creatine, scyllo-inositol, ↓triglycerides "Field cancerization" Tumour-adjacent mucosa had unique metabolic field changes distinguishing TN stage, 5-year survival prediction

CRC, colorectal cancer; RC, rectal cancer; ^{1}H HR-MAS, proton high-resolution magic angle spinning; NMRS, nuclear MRS.

had histological residual tumour. The lipid in normal controls was present at short echo time only, and the authors hypothesized that the nature of the lipid was different in patients with residual tumour compared with normal controls. This study showed that MRS metabolic profiling *in vivo* may have the potential to identify tumour at diagnosis and changes in the metabolic profile post CRT may be used to assess treatment response.

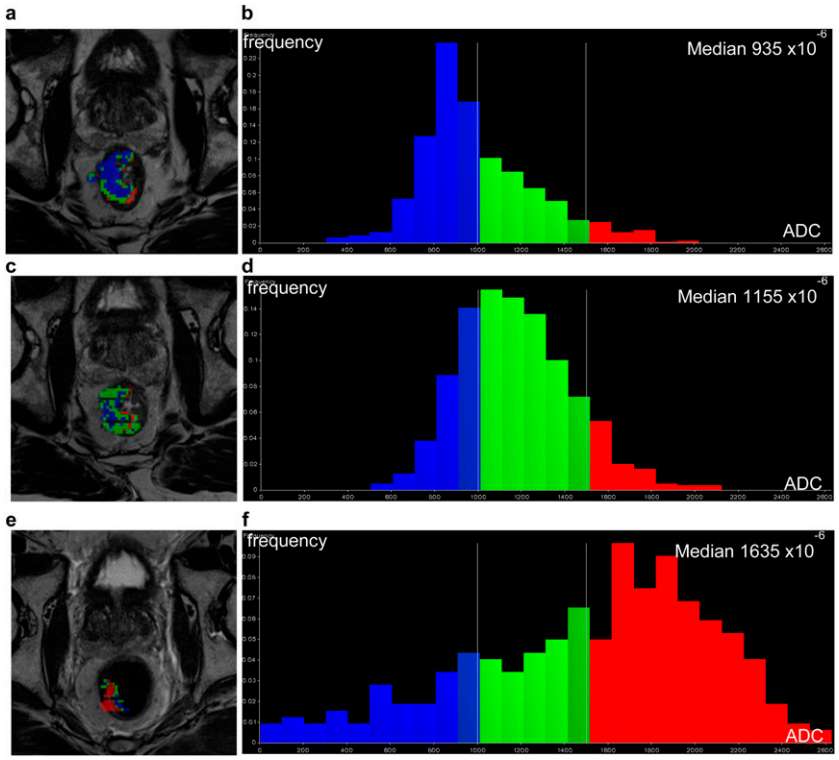
Translation of *ex vivo* to clinical MR spectroscopy

Choline is currently the most investigated metabolite in *ex vivo* and clinical studies. *Ex vivo* studies have shown higher levels of choline-containing compounds in colorectal tumour.[46,49] *Ex vivo* studies have demonstrated lower levels of choline-containing compounds and glycine, and higher taurine ratios in surgical specimens were associated with longer survival in patients with colorectal cancer.[47] The clinical MRS study found choline to be a potential biomarker of treatment response, with results showing a choline peak characteristic of rectal cancer at diagnosis and disappearance of this peak post CRT.[50] However, further clinical studies are required to translate high field *ex vivo* findings to clinical protocols and to assess whether choline would be a useful biomarker in the assessment of response to CRT (good *vs* poor response) and prediction of survival. Clinical studies are also required to assess whether taurine and glycine are useful clinical biomarkers in rectal cancer treatment response. Furthermore, there are some challenges in the translation of *ex vivo* MRS findings to MRI protocols to current clinical strengths (1.5–3 T) that need to be overcome. Localization of metabolite, weak metabolite signal relative to the larger water signal, *in vivo* can present challenges to clinical translation.[44]

FUTURE DIRECTIONS: MULTIPARAMETRIC MRI AND HISTOGRAM ANALYSIS

Although the evidence for DWI and DCE-MRI is promising, results from either of these techniques alone currently lack sufficient accuracy and standardization to be routinely used to alter clinical patient management. There is a wide variation reported in the performance of functional MRI in response prediction. Parameter thresholds obtained from studies require prospective validation of *post hoc* values in larger prospective studies. Most published studies have measured single parameter values from either diffusion or perfusion MRI. Single-parameter measurements, such as mean ADC or K^{trans} of pixels in the ROI, do not reflect tumour heterogeneity.

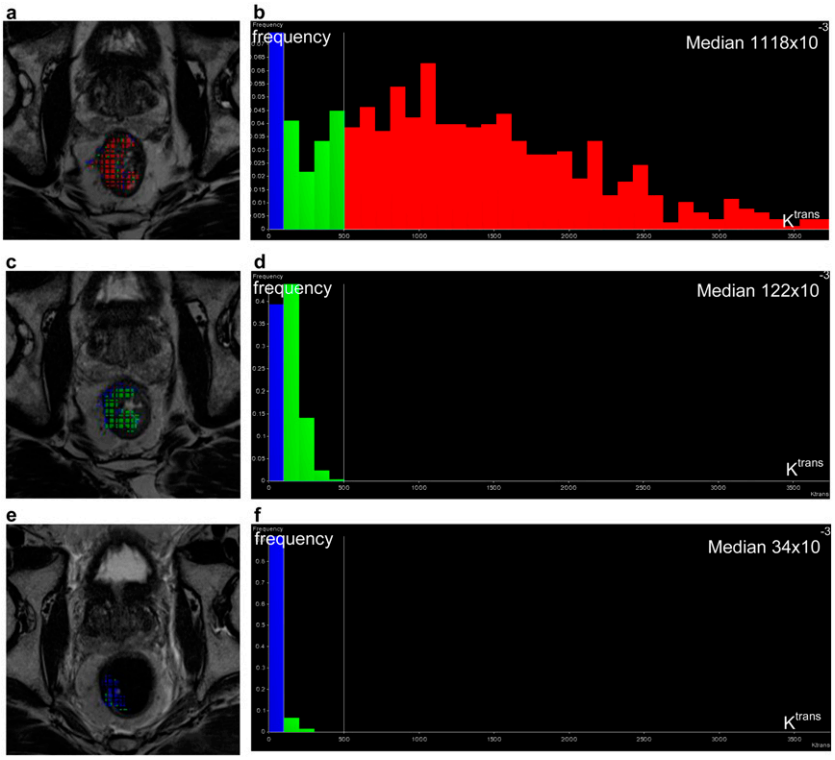
Figure 1. Diffusion-weighted imaging (DWI) MRI histogram analysis—apparent diffusion coefficient (ADC) colour-coded maps and histograms of a patient with good response following neoadjuvant chemoradiotherapy for rectal cancer. Example of ADC histogram analysis using Siemens OncoTreat (WIP), Erlangen, Germany. This patient had histological American Joint Committee on Cancer (7th edition) tumour regression grade 1 (moderate response, single cells or small groups of cancer cells). A voxel-by-voxel technique was used to assess changes in the entire region of interest. (a, c, e) Representative axial colour-coded ADC maps for MRI pre-chemoradiotherapy (CRT), Week 3 of CRT and post CRT, respectively. (b, d, f) Colour-coded histograms for every voxel within the segmented region of interest for DWI-MRI pre-CRT, Week 3 of CRT and post-CRT, respectively. Colour code: blue voxels, ADC values $<1000\times10^{-6}$; green voxels, ADC values $1000-1500\times10^{-6}$; red voxels, ADC values $>1500\times10^{-6}$. The histograms demonstrate an increase in the absolute ADC values of voxels over the time points.



Multiparametric MRI combining multiple functional parameters can provide a more complete physiological assessment of tumour, which may improve accuracy in response prediction. A combined analysis of DWI and DCE-MRI would provide complementary information on tumour microarchitecture and cellularity (DWI) and angiogenesis and perfusion (DCE-MRI).[51] For example, in the case of pCR, the presence of radiation-induced fibrosis may cause restricted diffusion resulting in low ADC and inability to detect pCR on DWI. The addition of DCE-MRI would allow assessment of perfusion within this region; the absence of perfusion or low K^{trans} would indicate the absence of residual tumour, which may improve the detection of pCR. Multiparametric functional MRI for response prediction is an emerging area, and currently, there are limited studies assessing the role of DWI and DCE-MRI in combination in rectal cancer CRT response prediction. Intven et al demonstrated that good responders to CRT had a larger decrease in median K^{trans} and larger increase in median ADC post CRT than non-responders. However, the addition of DCE-MRI did not improve the detection of good responders compared with the use of DWI alone.[52] A histogram analysis that incorporates a voxel-by-voxel technique to calculate MRI functional parameters for every voxel within the ROI would provide a more accurate representation of intratumour heterogeneity.[53] A histogram technique can be sensitive to a shift in distribution of ADC or K^{trans} values of all voxels within the entire tumour region over the time points, thereby providing information on any heterogeneity in tumour response to treatment. Other information such as mean, mode, skewness and percentile distributions can also be extracted. Overall trends from histogram studies have shown that following treatment, DWI (ADC) histograms demonstrate a shift to the right with decreased skewness and kurtosis, and DCE-MRI histograms shift to the left with narrower and increased peak height.[53] A new analysis software is being investigated that allows this analysis to be conducted for multiple parameters, allowing for multiparametric histogram analysis. An example of voxel-by-voxel histogram analysis is shown in Figures 1 and 2. Furthermore, semi-automated segmentation tools can also reduce interobserver variability in defining a three-dimensional ROI and lead to more reproducible results.

The fundamental aspect to multiparametric imaging is achieving a standardized imaging protocol that provides consistent and reliable data sets for quantification. For voxel-wise analysis of multiple functional MRI sequences, selection of imaging parameters that minimize distortion is essential to ensure geometrically accurate data. For DWI, RESOLVE is one method to minimize distortion.[54] For DCE-MRI, short temporal resolution of between 5 and 10 s would enable adequate sampling of the rapid "washin" of contrast into tumour, and

Figure 2. Dynamic contrast-enhanced (DCE) MRI histogram analysis—K^{trans} colour-coded maps and histogram of the same patient with good response following neoadjuvant chemoradiotherapy for rectal cancer. Example of K^{trans} histogram analysis using Siemens OncoTreat (WIP) for the same patient as in Figure 1. A voxel-by-voxel technique was used. (a, c, e) Representative axial colour-coded K^{trans} maps for MRI pre-chemoradiotherapy (CRT), Week 3 of CRT and post CRT, respectively. (b, d, f) Colour-coded histograms for every voxel within the segmented region of interest for DCE-MRI pre CRT, Week 3 of CRT and post CRT, respectively. Colour code: blue voxels, K^{trans} values $<100\times10^{-3}$; green voxels, K^{trans} values $100-500\times10^{-3}$; red voxels, K^{trans} values $>500\times10^{-3}$. The histograms demonstrate a marked reduction in the absolute K^{trans} values of voxels over the time points.



acquisition of pre-contrast T_1 flip angle scans would enable calculation of native T_1, allowing for more accurate assessment of perfusion using the Tofts two-compartment model. Although multiple parameters can be obtained from DWI and DCE-MRI, at present, ADC and K^{trans} appear to be the most promising parameters for clinical prediction of radiotherapy response in rectal cancer. Standardization of imaging protocols and analysis methods is essential in order to establish optimal thresholds of ADC and K^{trans} and to permit the clinical role of multiparametric MRI for treatment prediction to be properly evaluated.

CONCLUSION

Functional MRI is emerging as an important and promising tool for assessment of physiological characteristics of the tumour microenvironment. MRI has the benefit of sampling the whole tumour and can be repeated on multiple occasions unlike biopsies.

DWI has been shown to be superior to morphological MRI in the assessment of therapeutic response of the primary tumour in rectal cancer. In general, studies have demonstrated that lower primary tumour pre-CRT ADC value and greater percentage increase in ADC during CRT are predictors for good response. There is limited prospective data on the use of DWI for identification of patients with pCR, an important subgroup of patients in whom early MRI detection of CR would enable optimization of the surgical approach and reduction in surgical related sphincter morbidity. DCE studies have demonstrated that high tumour K^{trans} pre-CRT is a predictor for response. The role of MRS in the clinical management of rectal cancer remains yet to be defined. Although published *ex vivo* studies have identified colorectal cancer-specific metabolic profiles that can aid with prediction of disease behaviour, there is only one clinical rectal MRS study which identified choline as a potential biomarker of CRT response.

Multiparametric MRI assessing diffusion and perfusion in combination using a histogram analysis technique can assess tumour heterogeneity and its response to treatment. This strategy has the potential to improve the accuracy of therapeutic response prediction and facilitate individualized management for patients with rectal cancer.

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