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FULL PAPER

Changes in dynamic contrast-enhanced pharmacokinetic and diffusion-weighted imaging parameters reflect response to anti-TNF therapy in Crohn's disease

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Objective: To investigate the effect of tumour necrosis factor (TNF)- α antagonists on MRI dynamic contrast-enhanced (DCE) and diffusion-weighted imaging (DWI) parameters in Crohn's disease (CD).

Methods: 42 patients with CD (median age 24 years; 22 females) commencing anti-TNF- α therapy with baseline and follow-up (median 51 weeks) 1.5-T MR enterography (MRE) were retrospectively identified. MRE included DCE ($n = 20$) and/or multi- b -value DWI ($n = 17$). Slope of enhancement (SoE), maximum enhancement (ME), area under the time-intensity curve (AUC), K_{trans} (transfer constant), v_e (fractional volume of the extravascular-extracellular space), apparent diffusion coefficient (ADC) and $ADC_{fast/slow}$ were derived from the most inflamed bowel segments. A physician global assessment of disease activity (remission, mild, moderate and severe) at the time of MRE was assigned, and the cohort was divided into responders and non-responders. Data were compared using Mann-Whitney U test and analysis of variance.

Results: Follow-up K_{trans} , ME, SoE, AUC and ADC_{ME} changed significantly in clinical responders but not in non-responders,

baseline {[median [interquartile range (IQR)]: 0.42 (0.38), 1.24 (0.52), 0.18 (0.17), 17.68 (4.70) and $1.56 \text{ mm}^2 \text{ s}^{-1}$ ($0.39 \text{ mm}^2 \text{ s}^{-1}$) vs follow-up [median (IQR): 0.15 (0.22), 0.50 (0.54), 0.07 (0.1), 14.73 (2.06) and $2.14 \text{ mm}^2 \text{ s}^{-1}$ ($0.62 \text{ mm}^2 \text{ s}^{-1}$), for responders, respectively, $p = 0.006$ to $p = 0.037$]. SoE was higher and ME and AUC lower for patients in remission than for those with severe activity [mean (standard deviation): 0.55 (0.46), 0.49 (0.28), 14.32 (1.32)] vs [0.32 (0.37), 2.21 (2.43) and 23.05 (13.66), respectively $p = 0.017$ to 0.033]. ADC was significantly higher for patients in remission [$2.34 \text{ mm}^2 \text{ s}^{-1}$ ($0.67 \text{ mm}^2 \text{ s}^{-1}$)] than for those with moderate [$1.59 \text{ mm}^2 \text{ s}^{-1}$ ($0.26 \text{ mm}^2 \text{ s}^{-1}$)] ($p = 0.005$) and severe disease [$1.63 \text{ mm}^2 \text{ s}^{-1}$ ($0.21 \text{ mm}^2 \text{ s}^{-1}$)] ($p = 0.038$).

Conclusion: DCE and DWI parameters change significantly in responders to TNF- α antagonists and are significantly different according to clinically defined disease activity status.

Advances in knowledge: DCE and DWI parameters change significantly in responders to TNF- α antagonists in CD, suggesting an effect on bowel wall vascularity.

INTRODUCTION

The advent of tumour necrosis factor α antagonists (TNF- α antagonists) represents a significant advance for the treatment of Crohn's disease (CD), improving symptoms and long-term outcomes and reducing hospitalization and major abdominal surgery.^{1,2} TNF- α antagonists are now recommended to treat adults with severe active CD refractory to conventional therapy.³ However, between 10% and 40% of patients do not respond to TNF- α antagonists. Of those that do a further 20–50% more lose response by 12 months, which is problematic because TNF- α antagonists are costly, have side effects and are inconvenient for patients. It is recommended that patients taking these medications undergo clinical assessment at least every 12 months to determine whether treatment should be continued.³

Assessing therapeutic response to TNF- α antagonists in CD is challenging. Clinical indices such as Harvey–Bradshaw index are subjective, time consuming and difficult to employ. Serum and faecal markers such as C-reactive protein (CRP) and faecal calprotectin are sensitive but non-specific. Colonoscopy is arguably the gold standard but is invasive and unsuitable for frequent repeated assessment. Cross-sectional imaging plays an increasing role for management of CD. MR enterography (MRE) in particular not only identifies disease and stages it accurately,^{4–6} but may also have potential to measure treatment response.^{6–8} MRI scores of CD activity based on morphological observations such as wall thickness, mural T_2 signal etc. are increasingly validated against clinical and endoscopic standards of reference.^{8–10}

Functional parameters derived *via* dynamic contrast-enhanced (DCE) sequences and diffusion-weighted imaging (DWI) MRI may potentially provide more reproducible quantitative indices than subjective scores and could act as independent biomarkers of disease activity.^{10–12} Florie et al,¹¹ for example, reported good correlation between DCE parameters and clinical activity indices, and restricted diffusion has been associated with increased disease activity in several studies.^{10–12}

To date, however, there are little data that investigate changes in DCE and DWI MRE parameters with treatment, and, if present, whether changes reflect treatment response or whether they can help differentiate responders from non-responders. We aimed to investigate the effect of TNF- α antagonists on DCE and DWI parameters in patients with CD according to clinical treatment response. The secondary aim was to compare parameters across clinically defined disease activity groups.

METHODS AND MATERIALS

Patients

Informed consent was waived for this retrospective study following institutional review board approval (The National Hospital for Neurology and Neurosurgery, and Institute of Neurology Joint Research Ethics Committee). Patients were identified from the CD patient databases compiled as part of the UK Royal College of Physicians/British Society of Gastroenterology National Biologics Audit¹³ at a single tertiary referral hospital (University College London Hospital). The database was interrogated for all patients (age ≥ 14 years) commencing TNF- α antagonists [either infliximab (IFX; Remicade, Titusville, NJ) or adalimumab (ADA; Humira, AbbVie, Maidenhead, UK)] to treat active small bowel or ileocolonic CD between March 2007 and June 2013 inclusive.

Retrieved patients were cross-referenced against the hospital's radiology information system to identify those who had both undergone MRE within 1 month of starting TNF- α antagonists (baseline) and at least one further MRE examination performed 3 months or longer following the initiation of TNF- α antagonists (follow-up). To be eligible, both baseline and follow-up MRE examinations had to include either DCE and/or DWI (multiple b -values) sequences (see "MR enterography protocol" section below) and to have been performed on the same 1.5-T MRI machine. Sufficient clinical information had to be available for an experienced gastroenterologist to confidently define patient treatment response (see "Clinical disease activity" section). Part of this cohort has been described in Plumb et al¹⁴ and Prezzi et al.¹⁵

MR enterography protocol

Patients were fasted for 4 h prior to ingesting 1000 ml 2% mannitol over 45 min preceding MRI scanning. All patients underwent a standardized clinical protocol in the prone position on a 1.5-T magnet (Siemens Avanto; Siemens Healthcare, Erlangen, Germany) using the manufacturer's body and spine array coils.

Diffusion-weighted imaging

20 mg of spasmolytic (Buscopan®; Boehringer Ingelheim, Ingelheim, Germany) was administered 3 min prior to acquiring DWI sequences. Axial echoplanar DWI images were acquired (from the inferior third of the liver to the inferior extent of the small bowel,

usually by scanning in three separate blocks) at five b -values = 0, 50, 100, 300 and 600 s mm⁻² during free breathing.

Dynamic contrast-enhanced parameter

The DCE sequence was acquired 5 min following Buscopan administration. A breath-hold pre-contrast T_1 volume-interpolated breath-hold sequence (VIBE) with fat saturation was performed in the coronal plane (encompassing the majority of the small bowel; 14-cm volume coverage per acquisition, centred on the terminal ileum). A single (18 ml) dose of intravenous gadopentetate dimeglumine (Magnevist®; Berlex Laboratories, Wayne, NJ) was injected into an arm vein at 3 ml s⁻¹ by a power injector (Sonic Shot GX; Nemoto Kyorindo Co., Ltd, Tokyo, Japan). As the injection commenced, the patient was asked to breath-hold for 30 s followed by gentle breathing for the scan duration. The same VIBE coronal sequence block acquired at injection onset (time point = 0) was repeated for 23 measurements at 7-s intervals thereafter. Total DCE acquisition lasted 180 s. Standard axial and coronal T_2 half-Fourier-acquired single-shot turbo spin echo and True fast imaging with steady-state precession sequences were also acquired. MRI parameters are detailed in Table 1.

Image analysis

MRE images were reviewed using OsiriX (64-bit) imaging software (Pixmeo, Geneva, Switzerland), an open-source picture archiving and communication system workstation and digital imaging and communications in medicine viewer. A radiologist with 4 years' experience of small bowel MRI (blind to all clinical, demographic and follow-up information) reviewed the baseline MRE and identified the most inflamed small bowel or colonic segment based on conventional morphological parameters of increased wall thickness and increased mural/perimural T_2 signal.¹⁶ If the patient had more than one discrete segment of disease (separated by at least 5 cm of normal bowel), the most inflamed segment (in the reader's judgement) was chosen for analysis.

Once the abnormal segment was identified on the T_2 half-Fourier-acquired single-shot turbo spin echo sequence, the reader co-located the same segment on the DCE coronal and DWI axial sequences.

For DCE analysis, the post-contrast VIBE sequence block acquired at 30 s post injection (when mural conspicuity was likely to be greatest) was selected. The reader drew a freehand region of interest (ROI) over the chosen segment on the slice that contained the maximum longitudinal extent of abnormality. The ROI was drawn to encompass the whole bowel wall while simultaneously excluding luminal and extra mural tissues. It included all abnormal bowel visible within the segment on the selected slice. The full bowel wall thickness was included in the ROI regardless of its contrast enhancement pattern. The observer then used OsiriX software to propagate this ROI on each of the pre- and post-contrast acquisitions over the complete time series. The propagated ROIs were checked and adjusted manually to correct for any respiratory motion artefact. The mean signal intensity (SI) in each ROI was recorded.

For DWI, the reader drew a freehand ROI on the selected segment on the B600 images that contained the maximum longitudinal extent of abnormality as for the DCE ROI. This was propagated automatically throughout the b -values and again corrected manually as required. Mean SI was recorded.

Table 1. Detailed MRI parameters (1.5 T MRI)

MRI parameter	Coronal/axial SSTSE	Coronal/axial true FISP with and without fat saturation	Baseline volume-interpolated gradient ECHO	Dynamic contrast-enhanced VIBE	Diffusion-weighted MRI
Field of view (mm)	Variable	Variable	Variable	Variable	Variable
Number of slices	20/26	25/34	40	40	26
Stacks	1/3	1/3	1	1	3
Repetition time (ms)	1200/800	3.98/4.25	3.07	2.73	2500
Echo time (ms)	86/86	1.72/2.13	1.08	0.9	85
Image matrix	256/256	256/256	256	256	156 × 192
Slice thickness (mm)	4/4	4/4	3.5	3.5	5/5.25
Averages	1	1	1	1	4
Flip angle (°)			15	15	90
<i>b</i> -values					0, 50, 100, 300, 600

FISP, fast imaging with steady-state precession; SSTSE, single-shot turbo spin-echo; VIBE, volume-interpolated breath-hold sequence.

Dynamic contrast-enhanced and diffusion-weighted imaging analyses

Time signal intensity curves were generated using in-house software developed in MATLAB® (The Mathworks® Inc., Natick, MA) (Figure 1). Initial slope of enhancement (SoE), maximum

enhancement (ME), curve type (E-type) (Type 1: signal enhancement (SI) continues to increase with time; Type 2: SI levels following ME to lie within 10% ($\pm$) of ME; Type 3: SI decreases following ME to <10% of ME), onset time and total area under the time-intensity curve (AUC) were calculated using standard definitions as

Figure 1. Demonstration of an MRI (VIBE coronal) post-contrast image with region of interest positioned in inflamed segment and subsequent dynamic contrast-enhanced curve parameters estimated from $C_{TIC} = (\text{Signal} - \text{Baseline Signal}) / (r1 \times \text{Baseline Signal})$.

described previously.¹⁷ Furthermore, pharmacokinetic parameters of blood plasma volume (v_p), the transfer constant between plasma and interstitial space (K_{trans}) and interstitial space volume (v_e) based on the Tofts model were derived. A pharmacokinetic analysis was performed by fitting the extended Toft model:

$$C(t) = v_p \cdot C_p(t) + K_{trans} \cdot \int_0^t C_p(\tau - t_0) \times e^{\left(-\frac{K_{trans}}{v_e} \cdot (t - \tau)\right)} d\tau \quad (1)$$

to the time–intensity curve.

$$C_{TIC}(t) = \frac{1}{r_1} \left(\frac{S(t) - S_0}{S_0} \right)$$

where r_1 is the *in vivo* relaxivity ($4.5 \text{ mmol}^{-1} \text{ s}^{-1}$), $S(t)$ is the image signal, as a function of time, S_0 is the average of the acquired images before the injection of the contrast agent, C_p is a population arterial input function (in mmol l^{-1})¹⁸ and t_0 is the arrival time of the bolus at the tissue (in seconds).

For quantification of DWI parameters, monoexponential apparent diffusion coefficient (ADC) parameters were generated using all five b -values, and fits of low ($0, 50$ and 100 s mm^{-2}) and high (300 and 600 s mm^{-2}) were performed to separate fast (ADC_{fast}) and slow (ADC_{slow}) diffusion components. To account for multi-exponential DWI signal decay due to microcapillary perfusion, the stretched exponential model¹⁷ was employed where the ADC (ADC_{SE}) and heterogeneity index (α) were quantified. The heterogeneity index indicates the level of correlation between a monoexponential and multiexponential decay and relates to microcapillary perfusion occurring at early b -values.

Clinical data

For each patient, the following clinical parameters were collated: age, sex, Montreal classification, medication and surgical history.

Clinical disease activity

A gastroenterologist (RV) with 15 years' experience of managing CD and blind to DCE/DWI data (but not blind to the clinical MRI report) used the electronic patient record to review all available clinical data both at the time of baseline MRI and again at the time of follow-up MRI. Specifically, the gastroenterologist reviewed all out-patient clinic letters documenting symptoms and progress, biochemistry results including CRP, imaging, endoscopy and histopathology reports in order to define a composite "physician global assessment" (PGA) of disease activity with which to define treatment response.⁷ The physician employed standardized criteria in line with the second European evidence-based consensus on diagnosis and management of CD to stratify patients into four disease categories: "remission"—lack GI symptoms and normal CRP; "mild"—ambulatory patient, eating and drinking, no significant weight loss, lack of fever, obstruction, mass or tenderness and CRP increased above the upper limit of normal; "moderate"—intermittent vomiting or weight loss, ineffective treatment for mild disease, tenderness or mass but no overt obstruction and raised CRP; "severe"—severe weight loss or obstruction or abscess, persistence of symptoms despite intensive treatment and increased CRP at time points corresponding to each MRE.¹⁹ A treatment response was defined as a reduction in disease

severity by at least one category. Patients were automatically classified as non-responders if between the two MRIs they required dose escalation of their TNF- α antagonists, treatment with an alternative TNF- α antagonists or surgery. If the gastroenterologist (RV) did not feel confident in their disease classification at either time point owing to insufficient available clinical information, the patient was excluded.

Statistical analyses

Data were collated using Microsoft® Excel® 2011 for Mac (Microsoft Corp., Redmond, WA) and analysed using SPSS® Statistics v. 22 (IBM Corp., New York, NY; formerly SPSS Inc., Chicago, IL). DCE and DWI data were not normally distributed, and therefore, baseline and follow-up data were compared between clinical responders and non-responders using the Mann–Whitney U test. Comparison of MRI parameters between disease activity states (remission, mild, moderate and severe) was performed using one-way analysis of variance (ANOVA) with *post hoc* analysis using Bonferroni's method. A p -value of <0.05 was taken to represent statistical significance.

RESULTS

Baseline clinical data

A total of 42 patients with CD initiated on TNF- α antagonist therapy had undergone MRI scanning as specified. Of these patients, 27 had DCE assessment and 23 had DWI assessment for both baseline and follow-up studies, of whom 12 had both DCE and DWI. 7 patients were excluded from DCE assessment (technical problems with 4 studies and 3 patients underwent follow-up MRI on a different machine) leaving a total of 20 patients (median age 23 years, 12 females). 6 patients were excluded from DWI assessment (technical problems with 3 studies and 3 patients underwent follow-up MRI on a different machine) leaving a total of 17 patients (median age 25 years, 10 females) for the assessment of DWI changes pre/post treatment.

Of the 20 patients undergoing DCE, 5 were judged to have severe disease, 12 to have moderate disease and 3 to have mild disease at the time of baseline MRI. There was a median 8.5-day difference between the initiation of TNF- α antagonists and baseline MRE. Follow-up MRE was performed a median of 76 weeks following the initiation of TNF- α antagonists. At this time, 1 patient had severe, 7 had moderate and 2 had mild disease, and 10 patients were in remission. Patient characteristics and scan time data are given in Table 2.

Of the 17 patients undergoing DWI, 2 were judged to have severe disease, 12 to have moderate disease and 3 to have mild disease at the time of baseline scanning. There was a median 2-day difference between the initiation of TNF- α antagonists and baseline MRE. Follow-up MRE was performed at a median 49 weeks following the initiation TNF- α antagonists (Table 2). At this time, two patients had severe, three had moderate and three had mild disease, and nine patients were in remission.

DISEASE RESPONSE

Dynamic contrast-enhanced parameter

PGA defined response in 14 of 20 (70%) patients following the initiation of TNF- α antagonists. Median improvement was by

one disease activity category. Baseline DCE parameters were not significantly different between subsequent responders and non-responders (Table 3).

Following treatment with TNF- α antagonists, K_{trans} , ME, SoE and AUC fell significantly in responders but were not significantly different in non-responders (Table 3). There was however reasonable overlap in parameter changes between non-responders and responders (Figures 2–5). There were no other significant DCE parameter differences between responders and non-responders (Table 3).

Diffusion-weighted imaging

PGA defined response in 12 of 17 patients (71%) following the initiation of TNF- α antagonists. Median improvement was by one disease activity category.

Following treatment with TNF- α antagonists, ADC_{ME} increased significantly in responders but was not significantly different in non-responders (Table 3, Figure 6). There were no other significant DWI parameter differences between responders and non-responders (Table 3).

PARAMETERS ACCORDING TO DISEASE ACTIVITY

Dynamic contrast-enhanced parameter

One-way ANOVA demonstrated significant differences in SoE ($p = 0.02$), ME ($p = 0.02$) and AUC ($p = 0.05$) between clinical disease categories (remission, mild, moderate and severe) (Table 4). After *post hoc* analysis, significant differences remained in SoE between patients with severe disease [0.32 (0.37)] and those in remission [0.55 (0.46)] ($p = 0.02$). ME was significantly lower for those in remission [0.49 (0.28)] than for those with severe disease [2.21 (2.43)] ($p = 0.03$). AUC was significantly lower for those in remission [14.32 (1.32)] than for those with severe disease [23.05 (13.66)] ($p = 0.03$). There were no other significant DCE parameter differences between the different clinical disease states (Table 4).

Diffusion-weighted imaging

One-way ANOVA demonstrated significant differences in ADC_{ME} ($p = 0.01$) and ADC_{fast} ($p = 0.04$) between clinical disease categories (remission, mild, moderate and severe) (Table 4). After *post hoc* analysis, significant differences remained in ADC_{ME} between patients in remission [$2.34 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ (0.67)] and those with moderate [$1.59 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ (0.26)] ($p = 0.01$) and severe disease [$1.63 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ (0.21)] ($p = 0.04$). There were no other significant DWI parameter differences between the different clinical disease states (Table 4).

DISCUSSION

This study demonstrates significant changes in post-treatment DCE- and DWI-derived MRI parameters in patients who respond to TNF- α antagonists but not in those who do not. We also found evidence that some parameters differ significantly according to the disease activity category ascribed using a PGA.

DCE-MRI parameters such as K_{trans} , SoE, AUC and ME reflect the concentration of contrast media in the extracellular space, in part owing to increased vascular permeability or tissue blood flow. K_{trans} in general reflects the balance between flow and permeability within a tissue.

Table 2. Baseline characteristics of patients

Patient details	DCE	DWI
Total number	20	17
Male/female, <i>n</i> (%)	8/12 (40/60)	7/10 (41/59)
Median age at inclusion (years)	23	25
Previous intestinal resection, <i>n</i> (%)	10 (45)	9 (50)
Crohn's disease phenotype (Montreal classification), <i>n</i> (%)		
A1	12 (57)	5 (29)
A2	9 (43)	12 (71)
A3	0 (0)	0 (0)
L1	2 (9.5)	1 (6)
L2	2 (9.5)	0 (0)
L3	17 (81)	16 (94)
L4	0 (0)	0 (0)
B1	8 (38)	6 (35)
B2	5 (24)	4 (24)
B3	5 (24)	5 (29)
P	3 (14)	2 (12)
Medication, <i>n</i> (%)		
Infliximab	5 (24)	6 (35)
Adalimumab	4 (19)	4 (24)
Both	12 (57)	7 (41)
Days between start of medication and baseline scan [median (IQR)]	8.5 (28)	2 (33)
Response to treatment, <i>n</i> (%)		
Yes	14 (70)	12 (71)
No	6 (30)	5 (29)
Weeks between baseline and follow-up assessments [median (IQR)]	76 (73)	49 (40)

A, age at diagnosis; A1, below 16 years; A2, between 17 and 40 years; A3, above 40 years; B, behaviour; B1, non-stricturing non-penetrating; B2, stricturing; B3, penetrating; DCE, dynamic contrast-enhanced; DWI, diffusion-weighted imaging; IQR, interquartile range; L, location; L1, ileal; L2, colonic; L3, ileocolonic; L4, isolated upper disease; P, perianal disease.

With regard to CD, studies^{13,14,20,21} have demonstrated that these parameters (amongst others) are deranged in patients with active disease. Florie et al¹¹ demonstrated that enhancement ratio correlated with two validated clinical indices, the CD activity index and van Hees activity index, in 48 patients with clinically suspected relapse. Giusti et al²⁰ found different DCE curve types and higher ME in patients with biopsy-proven active disease vs in those without. Also using biopsy data, Oto et al¹² found significant differences between K_{trans} and the v_e values in 18 patients with inflamed and non-inflamed bowel. In resected specimens from 20 patients, Tielbeek et al²¹ found ME and initial slope of increase correlated significantly with the acute inflammatory score and fibrosis score. In general, our data concur with these findings, *i.e.* DCE parameters tend to be higher in those

Table 3. Dynamic contrast-enhanced (DCE) and diffusion-weighted imaging (DWI) parameters [median (IQR)] at baseline and following treatment with anti-tumour necrosis factor α therapy for Crohn's disease

MRI parameter	Non-responders			Responders		
	Baseline (median)	Follow-up (median)	<i>p</i> -value ^a	Baseline (median)	Follow-up (median)	<i>p</i> -value ^a
DCE parameters						
v_p	1.07×10^{-6} (3.49)	0.00 (0.01)	0.06	7.79×10^{-6} (0.01)	1.36×10^{-6} (0.01)	0.84
K_{trans}	0.28 (0.41)	0.32 (0.19)	0.70	0.42 (0.38)	0.15 (0.22)	0.02 ^b
V_E	0.98 (0.04)	0.65 (0.41)	0.31	0.73 (0.30)	0.68 (0.23)	0.60
OT	18.71 (14.64)	30.50 (22.44)	0.18	19.04 (28.78)	27.00 (24.49)	0.80
SoE	0.18 (0.24)	0.14 (0.13)	0.81	0.18 (0.17)	0.07 (0.10)	0.01 ^b
ME	1.66 (0.46)	1.04 (0.88)	0.59	1.24 (0.52)	0.50 (0.54)	0.00 ^b
E-type	3 (0)	3 (0)		3 (0)	3 (0)	
AUC	16.86 (2.19)	16.56 (2.15)	0.70	17.68 (4.70)	14.73 (2.06)	0.01 ^b
DWI parameters						
ADC _{slow}	1.49 (0.41)	1.60 (0.48)	0.84	1.59 (0.40)	1.72 (0.70)	0.56
ADC _{fast}	2.16 (1.45)	2.00 (0.17)	0.69	2.31 (1.33)	3.05 (1.71)	0.05
ADC _{ME}	1.41 (0.05)	1.74 (0.30)	0.15	1.56 (0.39)	2.14 (0.62)	0.01 ^b
ADC _{SE}	1.32 (0.11)	1.52 (0.51)	0.99	1.50 (0.25)	1.52 (0.84)	0.63
Heterogeneity index	0.85 (0.04)	0.81 (0.16)	0.84	0.71 (0.22)	0.63 (0.24)	0.17

ADC, apparent diffusion coefficient; ADC_{ME}, ADC derived from monoexponential decay model using all five *b*-values (in mm²s⁻¹); ADC_{SE}, ADC derived from stretched exponential decay model using all five *b*-values (in mm²s⁻¹); ADC_{fast}, ADC derived from high *b*-values (300 and 600) (in mm²s⁻¹); ADC_{slow}, ADC derived from low *b*-values (0, 50 and 100) (in mm²s⁻¹); AUC, total area under the time-intensity curve; E-type, curve type (Type 1, signal enhancement (SI) continues to increase with time; Type 2, SI levels following ME to lie within 10% ($\pm$) of maximal enhancement; Type 3, SI decreases following maximal enhancement to <10% of ME); heterogeneity index, the degree to which the SE decay resembles the ME decay; K_{trans} , transfer constant between plasma and interstitial space (in min⁻¹); ME, maximum enhancement; OT, onset time (in seconds); SoE, initial slope of enhancement; v_e , interstitial space volume; v_p , blood plasma volume.

^aMann-Whitney *U* test.

^bStatistically significant.

with clinically active disease. In addition, our data suggest clinical responders to TNF- α antagonists exhibit on average a significant reduction in K_{trans} , SoE, AUC and ME, unlike non-responders, which may reflect normalization of perfusion with treatment.

DWI is also increasingly used to assess CD activity, with data suggesting restriction is related to severity of inflammation. Oto et al¹² in a study of 18 patients reported ADC values (calculated by monoexponential signal decay) were significantly different in inflamed vs non-inflamed terminal ileum. Kovanlikaya et al²² reported on a small cohort of five patients who underwent surgical resection. They demonstrated significant differences in ADC values between strictures and inflamed, non-stenotic segments as well as between normal and diseased bowel segments. Freiman et al²³ evaluated DWI using the intravoxel incoherent motion model of DWI quantification. They compared findings between enhancing and non-enhancing segments of bowel in paediatric patients and concluded that the ADC_{fast} parameter (acquired from the lower *b*-values) was most able to predict active disease. Our data also suggest the rise in ADC after treatment was on average statistically significant in responders, unlike in non-responders. The ADC_{fast} rise was numerically greater in responders, but this failed to reach statistical significance (*p* = 0.05). However, there was some evidence that it differed across clinical disease activity categories as

a group. ADC_{fast} is dependent on microcapillary perfusion and therefore in the current context likely reflects bowel perfusion.²⁴

It should be noted however that there was reasonable overlap between the changes in these DCE and ADC parameters according to

Figure 2. Box-and-Whisker plot (minimum, interquartile range, median and maximum) demonstrating the change in K_{trans} (min⁻¹) in responders compared with non-responders to tumour necrosis factor α antagonists between baseline and follow-up MRI.

Figure 3. Box-and-Whisker plot (minimum, interquartile range, median and maximum) demonstrating lower maximum enhancement (ME) in responders than in non-responders to tumour necrosis factor α antagonists between baseline and follow-up MRI.

treatment response. Our data therefore suggest that cut-off values to differentiate between treatment response cohorts may not be possible on an individual patient basis, limiting the direct clinical impact of our observations. Instead, the findings of the present study are perhaps more useful when considering the underlying pathophysiology of CD and the mechanisms of treatment effects.

A large volume of data support the importance of mural vascularity in the pathophysiology and possible aetiology of CD.^{25–27} Indeed, advances in vascular biology have delineated a key role for the microcirculation in the initiation and perpetuation of inflammation. For example, neoangiogenesis is very well described,^{25,28} although the relationship between new vessel formation and tissue inflammation and hypoxia is complex. The present literature therefore suggests strongly that changes in DCE parameters and to an extent ADC reflect abnormal vascular permeability and vasodilatory microvascular dysfunction^{25,28} found in active CD.

The precise mechanism of action TNF- α antagonists is complex but exceeds simple neutralization of TNF- α itself (a potent pro-inflammatory cytokine pivotal to a variety of inflammatory responses) activity. Bonnin et al,²⁷ for example, demonstrated that a single

Figure 4. Box-and-Whisker plot (minimum, interquartile range, median and maximum) demonstrating lower slope of enhancement in responders than in non-responders to tumour necrosis factor α antagonists between baseline and follow-up MRI.

Figure 5. Box-and-Whisker plot (minimum, interquartile range, median and maximum) demonstrating lower area under the curve (AUC) in responders than in non-responders to tumour necrosis factor α antagonists between baseline and follow-up MRI.

injection of TNF- α antagonist normalized blood flow velocity rapidly in both mesenteric and retrobulbar arteries without affecting blood pressure, suggesting an immediate impact of TNF- α antagonists on inflammatory hyperaemia and microvascular perfusion. Rutella et al²⁶ also demonstrated that infliximab downregulates mucosal angiogenesis and limits vascular endothelial growth factor α in mucosal fibroblasts, proposing that these antiangiogenic effects are at least in part responsible for therapeutic efficacy in CD. Our data, in combination with existing literature, suggest that MRI is a useful tool to assess this antiangiogenic effect.

Our study has limitations. Our cohort was relatively small, with a risk of Type 1 errors given the number of statistical comparisons made. However, we did apply *post hoc* testing with the Bonferroni statistic in an attempt to counter this. Reassuringly, our data did concur with known associations between functional disease parameters and CD activity. As noted above, owing to the overlap in parameter changes between treatment response cohorts, the clinical impact of our observations is limited. However, we feel that based on our data, appropriately powered prospective studies of treatment effects are now indicated.

Figure 6. Box-and-Whisker plot (minimum, interquartile range, median and maximum) demonstrating higher apparent diffusion coefficient (ADC) (in mm^2s^{-1}) in responders than in non-responders to tumour necrosis factor α antagonists between baseline and follow-up MRI.

Table 4. Dynamic contrast-enhanced (DCE) and diffusion-weighted imaging (DWI) parameters [mean (standard deviation)] according to clinical disease state

MRI parameter	Remission	Mild	Moderate	Severe	<i>p</i> -value (ANOVA)
DCE parameters					
v_p	0.00 (0.00)	0.79 (1.77)	0.51 (1.75)	0.01 (0.00)	0.62
K_{trans}	0.13 (0.12)	0.23 (0.25)	0.54 (0.59)	0.61 (0.81)	0.15
v_e	0.68 (0.18)	0.64 (0.28)	0.75 (0.21)	0.80 (0.23)	0.57
OT	29.78 (12.19)	28.86 (13.99)	22.04 (23.03)	32.14 (23.32)	0.63
SoE	0.55 (0.46)	0.22 (0.15)	0.16 (0.87)	0.32 (0.37) ^a	0.02
ME	0.49 (0.28)	1.19 (0.66)	1.66 (0.88)	2.21 (2.43) ^a	0.02
AUC	14.32 (1.32)	17.02 (4.01)	17.99 (3.17)	23.05 (13.66) ^a	0.05
DWI parameters					
ADC _{slow}	2.04 (0.70)	1.68 (0.40)	1.63 (0.38)	1.59 (0.32)	0.20
ADC _{fast}	3.55 (1.43)	3.07 (1.06)	2.43 (0.92)	2.01 (0.65)	0.04
ADC _{ME}	2.34 (0.67)	1.83 (0.55)	1.59 (0.26) ^a	1.63 (0.21) ^a	0.01
ADC _{SE}	1.92 (0.75)	1.57 (0.51)	1.40 (0.46)	1.43 (0.20)	0.15
Heterogeneity index	0.64 (0.22)	0.67 (0.14)	0.80 (0.26)	0.78 (0.11)	0.29

ADC, apparent diffusion coefficient; ADC_{ME}, ADC derived from monoexponential decay model using all five *b*-values (in mm² s⁻¹); ADC_{SE}, ADC derived from stretched exponential decay model using all five *b*-values (in mm² s⁻¹); ADC_{fast}, ADC derived from high *b*-values (300 and 600) (in mm² s⁻¹); ADC_{slow}, ADC derived from low *b*-values (0, 50 and 100) (in mm² s⁻¹); ANOVA, analysis of variance; AUC, total area under the time-intensity curve; K_{trans} , transfer constant between plasma and interstitial space (in min⁻¹); ME, maximum enhancement; OT, onset time (in seconds); SoE, initial slope of enhancement; v_e , interstitial space volume; v_p , blood plasma volume.

^aSignificantly different to remission category on *post hoc* testing.

Given the retrospective nature, we relied on routine clinical practice to dictate timing of follow-up MRI. The median timing was around 1 year which corresponds to international guidance suggesting that response to anti-TNFs should be reviewed 12 months after initiation.³ This temporal interval limits our ability to assess how early DCE and DWI parameters change in response to treatment. If changes occurred within a few weeks of therapy, this would increase clinical utility for functional MRI. Similar to the study by Tielbeek et al,⁷ we used a PGA definition of disease activity response to therapy based on a retrospective case review rather than using a pre-defined fall in Crohn's disease activity (CDAI) or CRP for example. This is because precise definition of treatment response is controversial; all scores such as the CDAI and biochemical markers have known limitations. Indeed, a PGA allows the gastroenterologist to consider all available data in context, including symptoms, biochemistry and endoscopy, and reflects decision-making in normal clinical practice. Because our MRI analysis was limited to one bowel segment, we assumed that any changes in this segment reflected overall PGA, which may not always be true. However, it is interesting that there are strong parallels between our findings and those of other workers who have used alternative reference standards, including histology. Finally, positioning of ROI over bowel wall can be problematic as the

structure is small, particularly if it normalizes with treatment. Nevertheless, this is a widely adopted method, and while ROIs were placed very carefully to minimize errors, this remains a potential limitation of functional MRI techniques for the bowel.

CONCLUSION

DCE and DWI parameters change significantly in responders to TNF- α antagonists and are significantly different according to clinically defined disease activity status. These changes suggest an effect on mural vascularity by TNF- α antagonists in CD.

CONFLICTS OF INTEREST

SA Taylor and S Halligan are National Institute for Health Research Senior Investigators. SA Taylor is a research consultant for Robarts.

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