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**Applications of MRI sequences in perianal and
luminal Crohn's disease.**

Thesis submitted to the University of Nottingham for the degree
of Doctor of Philosophy

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2020

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List of abbreviations

ADC	Apparent Diffusion Coefficient
B ₀	Main Magnetic Field
bFFE	Balanced Fast Field Echo
CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
CDMI	Crohn Disease MRI Index
CI	Confidence Interval
CNR	Contrast-to-Noise Ratio
CoV	Coefficient of Variance
CRP	C-Reactive Protein
CT	Computed Tomography
D	Pure Molecular Diffusion Coefficient
D*	Pseudo-Diffusion Coefficient
DCE	Dynamic Contrast Enhancement
DSS	Dextran Sulphate Sodium
DWI	Diffusion Weighted Imaging
EPI	Echo-planar imaging
ESR	Erythrocyte Sedimentation Rate
FA	Flip Angle
FCP	Faecal Calprotectin
FDI	Free Induction Decay
FIESTA	Fast Imaging Employing Steady-state Acquisition
FLASH	Fast Low Angle Shot
FOV	Field of View
FSE	Fast Spin Echo
Gd	Gadolinium
GE	Gradient Echo
HBI	Harvey Bradshaw Index
IBD	Inflammatory Bowel Disease
ICC	Intraclass Correlation Coefficient
IR	Inversion Recovery
IVIM	Intravoxel Incoherent Motion
IV	Intravenous
M	Magnetisation
M _{xy}	Transverse Magnetisation
M _z	Longitudinal Magnetisation
MaRIA	Magnetic Resonance Index of Activity
ME	Maximum Enhancement
MEGS	Magnetic resonance Enterography Global Score
MFA	Multiple Flip Angle
MRE	Magnetic Resonance Enterography
MRI	Magnetic Resonance Imaging
MT	Magnetisation Transfer
MTR	Magnetisation Transfer Ratio
NMR	Nuclear Magnetic Resonance
pCD	Perianal Crohn's Disease
PDAI	Perianal Disease Activity Index

PF	Perfusion Fraction
R1	Longitudinal Relaxation Rate
RF	Radiofrequency
ROI	Region of Interest
SAR	Specific Absorption Rate
SBCE	Small Bowel Capsule Endoscopy
SE	Spin Echo
SI	Signal Intensity
Sn	Sensitivity
SNR	Signal to Noise Ratio
SoE	Slope of Enhancement
Sp	Specificity
SPGR	Spoiled Gradient Echo
STIR	Short Inversion Time Inversion Recovery
T1	Longitudinal relaxation time constant
T2	Transverse relaxation time constant
TE/TR	Echo Time/ Repetition Time
TFE	Turbo Field Echo
TI	Inversion Time
TIC	Time-Intensity Curve
TNF	Tumour Necrosis Factor
TSE	Turbo Spin Echo
UC	Ulcerative Colitis
Ve	Extracellular-Extravascular Volume Fraction
Vp	Plasma Volume
WI	Weighted Image

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Acknowledgements

This PhD journey has been a memorable and valued personal experience. To begin with, I would like to thank God Almighty for granting me strength and patience to accomplish this work. In fact, without the support and assistance of many people, this work would not have been possible. Deep and honest thank you to my primary and co-supervisors, Dr Gordon Moran and Dr Caroline Hoad for their insight, support, and guidance provided throughout this journey. Thank you for your dedication, kindness, precious motivation and infinite patience throughout the last few years.

I have learnt life-changing skills from your unlimited academic experience you shared with me. With sincere gratitude, I thank you for encouraging and helping me to tackle all faced challenges every time I stepped into your office all over my PhD journey.

A special thanks go to Dr Hannah Williams and Dr Caroline Hoad who wrote the MATLAB codes, and collaborative staff at Manchester University for modelling DCE data.

I would also like to thank my co-supervisors' Dr Khalid Latief, and Dr Sunil Samuel for their provided help and support. Many thanks and appreciation to all people involved in my PhD study who showed an honest desire to help and advise me throughout the completion of this work: Professor Penny Gowland, Dr Phillip Lung, Dr Argyriou Konstantinos, Dr Jonathan White, Dr Khaled Heissam, Natalie Horsepool, Bethany Robinson,

Emma Bradley, the SPMIC team, the GI MRI Research Group, MRI Operators, the PhD students at the NDDC, my friends.

Recruitment would not have been possible without the help of the nurses and specialists of NIHR Nottingham Digestive Diseases Biomedical Research Centre at Nottingham University Hospitals NHS Trust and the University of Nottingham. I am also grateful to the IBD collaborative team at centres in London, Sheffield and Manchester who also contributed with participants.

Thank you to Jazan University for funding and supporting my study. I would like to thank Dr Luca Marciani for reviewing my previous yearly progress and for giving me valuable feedback and advice.

Finally, I would like to thank my family and friends for standing beside me and showing honest support throughout all the ups and downs. To my parents, I will be always indebted and grateful to you for being always there for me.

“Without the inspiration, drive, and support that you have given me, I might not be the person I am today”

Chapter one: Abstract and thesis outline

1.1 Abstract

Magnetic resonance imaging (MRI) is a non-invasive imaging method which was discovered as the magnetic resonance phenomenon or nuclear magnetic resonance by Felix Bloch¹ and Edward Purcell² in 1946. It had long been used to study molecular structure and diffusion in the fields of chemistry and physics. Currently, the primary use of clinical MRI is in imaging anatomy, body pathology, and is used to assess organ function. In combination with the transmission and detection of radio waves, MRI uses a high magnetic field to produce images of the body from MR signals. Also, MRI offers a variety of techniques and measurable physical parameters for sensitising the image. This thesis focuses on using MRI in abdominal imaging and in clinical applications. The focus of this thesis is on the use of MRI in abdominal and peri-anal clinical applications in Crohn's disease in particular. Many studies have been conducted on human or animal abdominal organs in various areas of research, both in basic experimental and in clinical applications. This thesis focuses on the performance of MRI sequences in areas, such as, magnetisation transfer (MT), T2 relaxometry, diffusion-weighted imaging (DWI), and dynamic contrast enhancement (DCE), in evaluating treatment response in Crohn's disease (CD) and its complications (perianal disease location). Several aspects have been examined: the possibility of assessing therapy response, the correlation among these sequences, also with clinical indexes, and the repeatability of different MRI features.

DWI is a modality of imaging that enables tissue microenvironment characterisation through the measurement of variations in water molecules' mobility. These variations are typically the result of several factors, including water molecule compartments (intracellular or extracellular spaces, intravascular), cell membranes, and depend on changes in the tissue structure that alter under certain inflammatory processes. DCE visualises the weighted intensity of the T1 signal over time following an intravenous contrast injection. These curves of time-intensity can provide various quantitative measurements of disease activity ^{3, 4}, and assessments of treatment response in CD⁵. In a quantitative assessment, T1 and T2 relaxation times can also provide estimates of total water content in the tissue. The tissue's properties, and the amount of water within may dramatically change with injury and disease. Pathology, such as inflammation, is associated with accumulation of water in the intra-extracellular spaces, thereby affecting T1 and T2 relaxation times. MT suggests the possibility of quantifying intestinal fibrosis through non-invasive determination of tissue collagen in CD.

1.2 Thesis Overview

Work in this thesis focuses on developmental work to validate the role of quantitative MRI sequences in measuring disease activity in inflammatory Bowel Disease (IBD), mainly in CD and perianal CD (pCD). Moreover, specific focus has been given to the role of these sequences (MT, DWI and relaxometry) in assessing treatment responsiveness to these diseases with

respect to looking to separate out inflammatory effects from fibrotic tissue changes.

This thesis has eight chapters. The **first chapter** contains an overview of this thesis. The **second chapter** provides a general overview of MRI physics and use of MRI sequences (DCE, DWI, MT, and relaxometry). The **third chapter** outlines a general overview about IBD and pCD including definitions, aetiology, diagnosis, clinical and MRI assessment of disease severity, and management of these conditions. The **fourth chapter** offers a brief overview of the use of MR Enterography detailing preparation aspects, diagnostic features, and technical aspects. The section involves a review of recent literature using Quantitative MRI sequences (DCE, DWI, MT, T2, T1, relaxometry) in CD and perianal pCD.

In a MAP study discussed in **chapter 5**, DWI, DCE, MT, and T2 sequences were evaluated for quantitative assessment of pCD activity based on clinical indicators. In addition, these sequences were correlated before and after 12 weeks of biological treatment. In addition, changes in clinical indicators after biological therapy were considered. In **chapter 6** the repeatability and inter-observer variability of T2, T1, IVIM-DWI, and MT small bowel wall measurements, following bowel preparation and administration of an anti-spasmodic agent in healthy volunteers were assessed.

In the study, reported in **chapter 7**, the values of MT, DWI, and T2 were determined as a baseline for predicting the response to biological

therapies in an active CD cohort at 6 months. This study was a local sub-study of the National Institute for Health Research (NIHR) - Efficacy and Mechanism Evaluation (NIHR EME) funded study MOTILITY. The final **chapter 8** offers a brief summary, and details of the main findings in the work, the possible future directions for the project, and suggestions for applications in various clinical settings have also been presented.

Chapter Two: Concepts of Magnetic Resonance Imaging

This chapter presents an overview of NMR and the MRI methods necessary to understand the work presented in this thesis. First, the basic theory of NMR and MRI and the interaction and relaxation of spins with magnetic field are described. Following this, pulse sequences, in general, are then explained. Finally, newer MRI sequences used in this thesis to assess IBD activity using DWI, DCE, MT, and relaxometry techniques are then described and subsequently applied in chapters 4-8.

2.1 Basic principles of MRI

MRI is based on the principles of NMR, a phenomenon based on the properties of the nucleus of an atom. The atom is a fundamental unit of matter that contains a nucleus having neutrons and protons, surrounded by clouds of negatively charged electrons. Spin is a basic property of many atomic nuclei. The nuclear mass consists of a mixture of electrically neutral neutrons and positively charged protons. Not all nuclei have a spin angular momentum (I) or net nuclear spin. Atomic nuclei that possess an even number of neutrons and protons have no spin angular momentum or no net spin (I), thus no magnetic moment while atomic nuclei that have an odd number of neutrons or protons or both possess I .

For example, carbon-12 ($^{12}_6\text{C}$) has an even number of both six neutrons and protons, thus no spin, resulting in no magnetic moment. Carbon-13 ($^{13}_6\text{C}$) has an even number of six protons and an odd number of seven neutrons, and hence they have spin resulting in a magnetic moment.

This can be defined as a charged sphere spinning around its own axis, which leads to creating a current loop which produces a magnetic dipole moment, μ ^{6, 7}. The equation below describes the relationship between magnetic moment μ and nuclear spin (I).

$$\mu = \gamma \cdot I \quad (1)$$

Where γ is the gyromagnetic ratio, it can be negative or positive, with μ and I aligned parallel to each other if γ is positive or anti-parallel if γ is negative.

Hydrogen atoms are the most often used to generate signals for MRI as they are abundant in human tissue. These atoms have a relatively high gyromagnetic ratio and large magnetic dipole moment as they possess one proton each.

In the absence of an external magnetic field, the nuclear spin directions of all atoms are random and cancel each other out. If the protons are placed in a strong magnetic field B_0 , some tend to align along the magnetic field direction and others tend to line up in the opposite direction (Figure 1). As a result, the magnetic fields of several protons will be cancelled, but a small excess of the protons will be aligned with the main magnetic field, resulting in a 'net magnetisation' aligned in the direction of the main magnetic field. The nuclei will "rotate" about the axis of the magnetic field. This is known as precession (Figure 1).

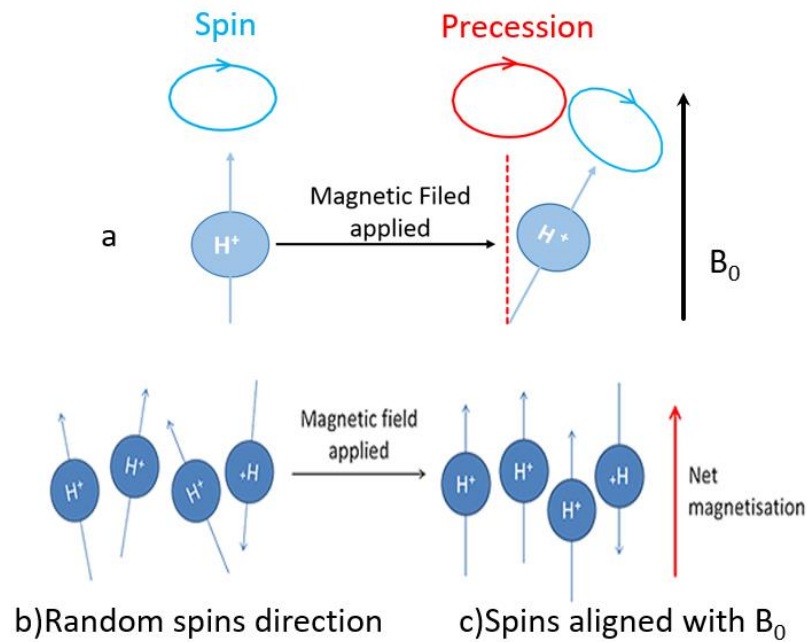


Figure 1: Protons are represented as blue ball possessing a positive charge and are constantly spinning around their axes (a) and the precession (red dotted line) induced by using a magnetic field (conventionally called B_0 in the z-axis. Alignment of protons with the B_0 field (b,c). In the absence of a magnetic field(b), the precession of the spins is oriented randomly. In the presence of an external magnetic field B_0 , a spinning particle precesses around B_0 direction at the Larmor frequency and a net magnetisation will be generated parallel to the main magnetic field (c).

The frequency of precession, called the Larmor frequency, is related to the magnetic field through the gyromagnetic ratio γ in the following equation:

$$\omega_0 = \gamma \cdot B_0 \quad (2)$$

Equation 2: It is known as the Larmor relationship where γ is the “gyromagnetic ratio” and is nuclei dependent. The Larmor frequency depends on the strength of B_0 and is directly proportional to the external magnetic field strength.

Coordinate system

To understand the principles of MRI, coordinate systems for the magnetic field and nuclei can be defined. The direction of the main magnetic field is the longitudinal direction that can also be called the z-direction. The plane perpendicular to this direction is known as x y plane or the transverse plane as seen in Figure 2.

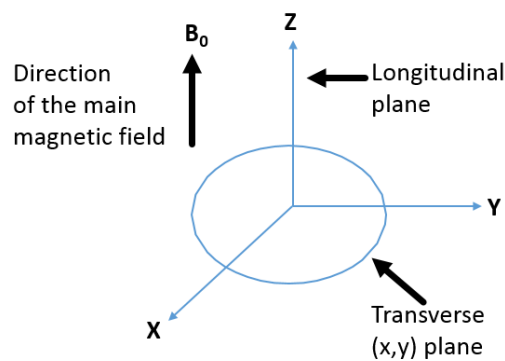


Figure 2: The coordinate system. For a typical MRI scanner, the longitudinal direction is parallel to the main magnetic field (Z-axis); the plane perpendicular to this is known as the x-y plane.

The nuclei precess around the z-axis along the applied magnetic field as the main magnetic field (B_0) is applied. As stated previously more spins are aligned along the magnetic field compared with those anti-parallel to it creating a net longitudinal magnetisation (M_z) in the direction of the z-axis⁶, and M_0 refers to the equilibrium M_z magnetisation.

Application of a radiofrequency pulse (RF) (B_1) rotating synchronously with the precessing spins, results in a net magnetisation rotating away from its equilibrium position into the transverse (x-y) plane (moves from aligned with B_0 (z-axis) to perpendicular to B_0 into the transverse plane (x-y

plane). The RF pulse is applied at the same frequency as the spin precession for a certain period of time. Magnetic resonance receivers can only detect the transverse component of the magnetisation vector. The angle of the magnetisation (also referred to as the flip angle) depends on the power of the RF pulse and the duration of the application

2.2 Mechanism of T1 and T2 Relaxation

An external RF pulse is applied to flip the magnetisation vector of the spins 90° into the x y plane. The magnetisation vector in the x y plane is known as M_{xy} .

After exciting the protons with a 90° RF pulse, all spins are in phase, and the spin populations are equated between the low energy spin-up and high energy spin-down states, creating a net transverse magnetisation (large M_{xy}). The transverse magnetisation begins to decrease when the RF is turned off and the nuclei relax back to the resting state of net longitudinal magnetisation (large M_z , and $M_{xy} = 0$). This mechanism is called relaxation. The longitudinal magnetisation is restored by the recovery process called T1 recovery and the transverse magnetisation is decayed by the process T2 decay (Figure 3 a&b)

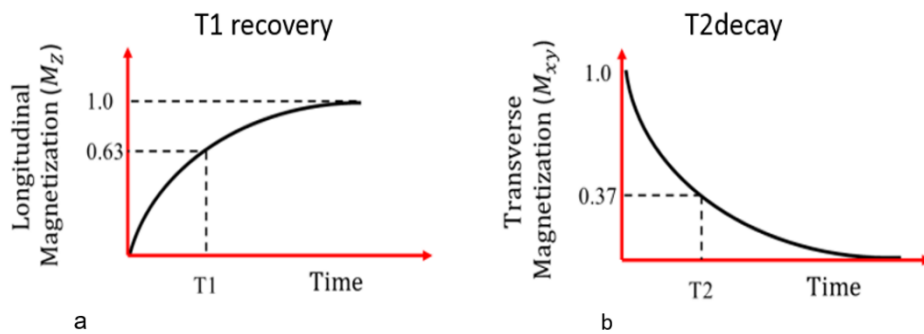


Figure 3: Illustrates the relaxation times. The T1 relaxation time is defined as the time needed to achieve 63% of the original longitudinal magnetisation (a) while T2 shows the time interval required for the transverse magnetization to drop to 37% of its original value.

T1 Recovery

T1 spin-lattice (longitudinal recovery) relaxation time is the decay constant for the recovery of the longitudinal magnetisation towards its thermal equilibrium value (along the Z (B_0) axis). This happens as the hydrogen nuclei transfer their energy to their surrounding lattice. T1 is dependent on the magnetic field strength, and the inherent characteristics of the tissue (described in more detail below).

T2 Decay

T2 spin-spin (transverse decay) relaxation time is the interaction between spins and causes the spins to exchange energy with neighbouring spins causing dephasing, and, hence, the decay of transverse magnetisation. T2 also depends on the inherent tissue properties and the strength of the magnetic field. The time needed for the transverse magnetisation to fall to approximately 37% ($1/e$) of its initial value, is referred to as the T2 relaxation time.

The loss of order in the x-y plane may be increased by the presence of local magnetic field disturbances (inhomogeneity) and this known as T2* relaxation.

The T1 and T2 characteristics of each biological tissue type are different, which are influenced by the nature of the tissues. For example, freely moving fluids, such as synovial fluid, have long T1 times, whereas more solid tissues such as muscles or liver have considerably shorter T1 times due to the higher level of structure of the molecules. Typically, T2 is faster than T1 relaxation, for example in the kidney T1 is about 1000 ms at 3T, whereas T2 is about 70 ms.

Many intrinsic (tissue properties) and extrinsic (environmental) factors can affect the relaxation times T1 and T2. Some main factors affecting the relaxation times are temperature, viscosity, water content, and chemical and magnetic environment. In the human body, T1 and T2 times are different in different tissues as shown in Table1.

Table 1: Typical T1 and T2 values for various body tissue at 1.5T. Adapted from⁸.

9

Tissue	T1 (ms)	T2 (ms)
White matter	1441	290
Skeletal Muscle	1008	44
Kidney	690	55
Grey matter	1124	95

NMR signal Generation

To detect the MRI signal, the bulk magnetisation (M_0) must first be perturbed from the equilibrium configuration that is along the z-axis to the transverse plane (x-y) by using a RF pulse (B_1) and resulting in some of the magnetic moment precessing in the transverse plane as shown in Figure 4¹⁰. This precessing magnetisation can induce a voltage in a receiver coil, which creates the MR signals. The signal magnitude is related to the magnetisation in the x-y plane and the signal frequency is the Larmor frequency.

As mentioned earlier, the precessing magnetisation can be detected to provide a signal oscillating at the Larmor frequency (free induction signal). This signal eventually decays due to the action of relaxation; hence the signal is often referred as the free induction decay or FID

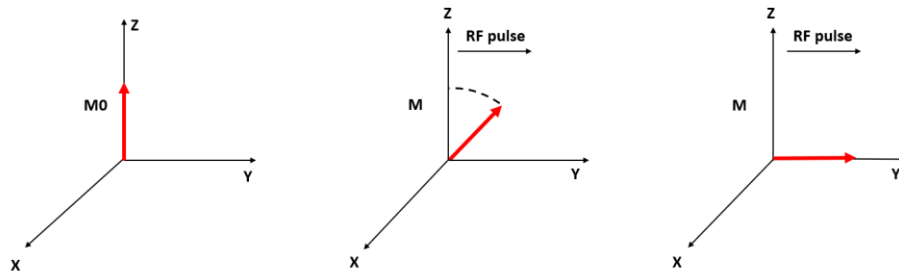


Figure 4: Before an RF pulse, the net magnetisation (red arrow) is aligned parallel to the main magnetic field and the z-axis (left). The RF pulse at the Larmor frequency allows the protons to absorb energy that results in a net magnetisation rotating away from the z-axis.

Generating a spin-echo

In MRI, there are two types of rephasing approaches used routinely to produce echoes; spin echoes (SE) are formed using refocusing pulses and gradient echoes (GE) are formed by using a linear gradient. Hahn was the first to study echoes in 1950, and observed that after applying two 90° consecutive RF pulses, constructive interference of the spins recreates the FID (i.e. the spins refocuses).

Conventional spin-echo imaging, uses a 90°RF pulse to excite the magnetisation, and one or more 180° pulses (applied along the y-axis at a time τ) to refocus the spins in echo time (TE) to generate signal echoes when the spins are again in-phase (Figure 5). TE is defined as the time between the application of the RF pulse and the peak of the signal induced in the coil. The initial 90°RF excitation rotates the magnetisation into the x-y plane, the individual magnetic moments dephase due to inhomogeneities in B_0 (T_2^*), and spin-spin interactions (T_2). A 180° refocusing pulse is then applied to reverse the effects of inhomogeneities, and, after a time delay, equal to TE/2, thus a signal is able to be obtained. The refocusing pulse is applied at exactly half the (TE/2).

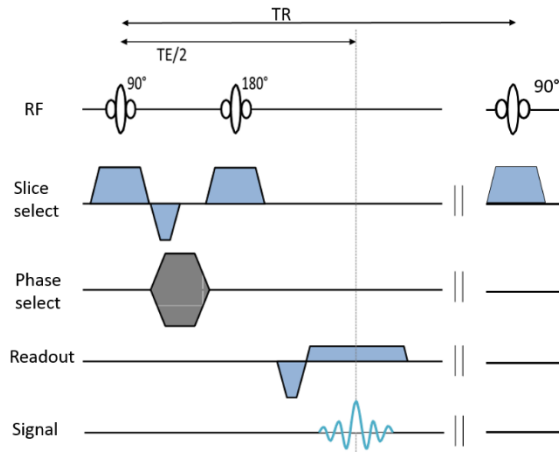


Figure 5: Scheme of a spin-echo (SE) sequence.

Spatial localisation of MR signals and the gradient-echo.

To create an image in MR, the signals from the tissue being investigated need to be localised. There are three principal approaches relating to spatial discrimination, which are all combined with magnetic field gradients in a sequence of pulses. The methods are phase and frequency encoding, and slice selection. They all work on the principle that, by applying a magnetic field gradient, the B_0 Larmor frequency will be spatially distinct across the tissue under investigation.

Slice selection

Slice selection is the technique through which radio frequency excitation, and, thus, the signals, are confined to a selected slice within the sample¹¹. That is achieved through the simultaneous application of the excitation pulse with a magnetic field gradient perpendicular to the desired slice. The RF pulse contains a range of frequencies (bandwidth), and, when combined with the gradient, allows only a selection of the tissue to become

excited (slice thickness). The slice thickness can be changed in two ways: by changing the steepness of the gradient field or by changing the bandwidth of the RF pulse.

A stronger gradient is required to generate thinner slices whereas a weaker (low) gradient is used to generate a thicker slice (Figure 6).

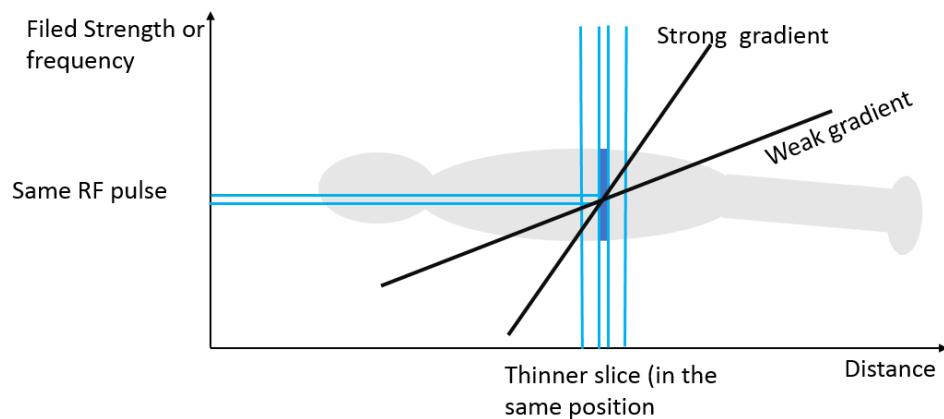


Figure 6: The strength of the slice select gradient determines the slice thickness. The radio frequency pulse of a given frequency bandwidth generates a thick slice if the gradient is weak or a thin slice if the gradient is strong.

Phase and frequency encoding

A phase encoding gradient G_y is implemented following slice selection, and before the positive frequency encoding G_x . When a phase-encoding gradient along the y-axis is turned on, it results in a phase shift between the spins, as it causes some of the protons to precess faster than others depending on their position within the gradient. When the gradient is switched off, all the spins return to the same frequency, but they are now linearly out of phase.

A readout gradient also called the frequency encoding, is applied following and perpendicular (in space) to both the phase encoding, and slice selection gradients. Following an RF pulse, each proton precesses at the same frequency in the excited volume (slice). During the detection of the echo, a readout gradient is applied, causing a variation in the frequencies for the protons generating the echo signals.

Two additional gradient pulses are used, one directly after the slice selection gradient, and the other just before the frequency-encoding gradient. These pulses counter any dephasing of transverse magnetisation induced by the slice and frequency encoding gradients to ensure the maximum echo signal is acquired. It is important to emphasise, that all protons are simultaneously excited by RF pulses and that one echo signal from the whole slice is recorded for a single phase-encoding step. Therefore, the pulse sequence (which consists of the slice selection, frequency encoding, and phase encoding) is repeated many times to reach the sufficient phase encoding information to assign the signal to each location within the slice. The same frequency, and slice section encoding are performed during each repetition, but the strength of the phase encoding gradient is increased by equal increments. Each repetition of the phase-encoding process produces a signal echo digitized and stored within the "k-space" raw data matrix. K-space data points represent an MRI content of spatial frequencies. The data in k-space is transformed into a picture via a Fourier transform (mathematical tool).

Gradient Echo

A Gradient echo sequence starts with a partial flip, and bi-polar magnetic field gradients to create an echo. Low flip angles ($\alpha < 90^\circ$) are used to reduce imaging times, therefore, the TE, and repetition time (TR) can be reduced as it produces faster recovery of longitudinal magnetisation (Figure 7).

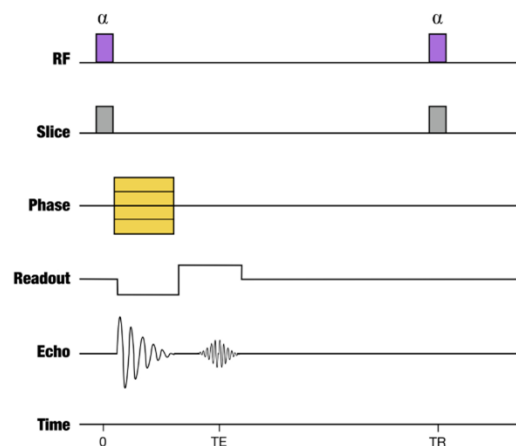


Figure 7: Illustration of conventional gradient echo pulse sequence.

2.3 Measurement of T1 and T2 (Termed Relaxometry)

Relaxometry is the method for the quantitative analysis of MR images that derives numerical values for spin relaxation.

T2 Relaxometry

T2 relaxometry is commonly based on a multiple spin-echo sequence. The series utilises multiple equidistant phase-shifted 180° refocussing pulses, each of which produces an echo. The decay of the MR signals (M_{xy}) can be described in Figure 8. T2 maps can be generated from images acquired from different echo times provided that all other imaging

parameters remain the same. T2 mapping aims to provide an image where every pixel value represents the average tissue T2 of that voxel quantitatively. The decay of the MR signal (M_{xy}) can be written as in equation 3:

$$M_{xy} = M_0 \left(\exp\left(\frac{-TE}{T_2}\right) \right) \quad (3)$$

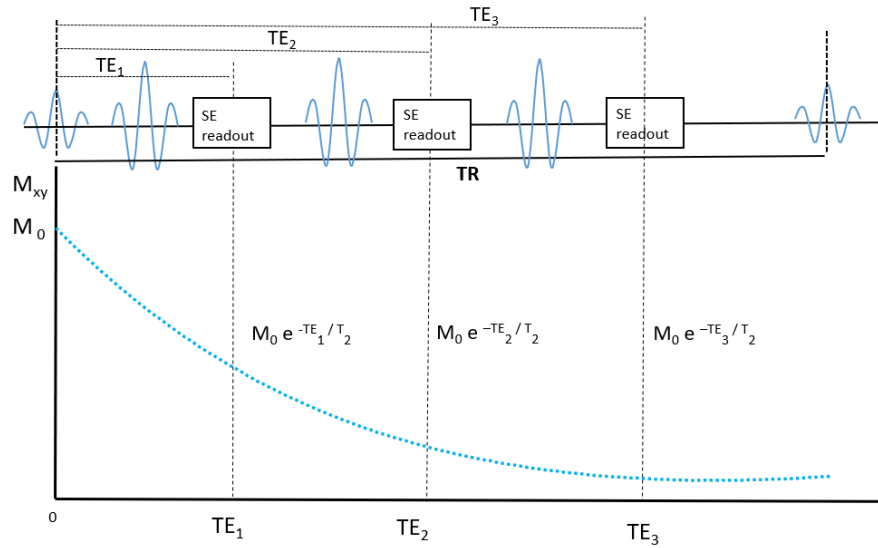


Figure 8: Exponential decay of transverse magnetisation M_{xy} .

T1 relaxometry

The simplest and most frequently used pulse sequence is the inversion recovery (IR) scheme to accurately quantify T1 values. This sequence represents the longitudinal magnetisation (M_0), existing after the application of a 180° RF pulse that flips the M_0 from z-direction to the -z-direction. Then after a delay, termed the inversion time (TI), a further 90° RF pulse is applied to recover all of the z magnetisation into the transverse plane, and allows a signal to be recorded. The amplitude of the

magnetisation rotated into the x-y plane depends on the amplitude of the M_0 recovered during the TI period⁶.

For quantitative T1 mapping, several IR images are collected from the same location with a different TI, and with all other imaging parameters being similar. A long TR must be used to avoid signal saturation, and to ensure full relaxation before each inversion pulse, which results in long overall measurement times. To sample the recovery curve, and to estimate T1 using the equation below (4) for the data for the case following a 180° inversion pulse.

$$S(TI) = S_0(1 - 2 \exp(\frac{-TI}{T_1})) \quad (4)$$

where S_0 is the signal that would be obtained from the equilibrium longitudinal magnetisation, and $S(TI)$ is the signal measured at time TI.

Maps for relaxation can be created either through gradient echo or spin-echo sequences. A relaxation rate map ($R1=1/T1$, $R2=1/T2$) or relaxation time (T1 or T2) requires at least two images. The sensitivity relating to this method relies on the sequence used, the TE or TI times acquired, TR, and the model adopted for fitting the experimental data as well as the number of images obtained by using different TE or TI.

MR image contrast

MRI contrast is a complex combination of pulse sequence parameters, and tissue parameters. The two most important sequence parameters are

the TE, and the TR. The TE is the time between the initial RF pulse and the peak time of the echo signal, which is measured in milliseconds. The decay time for transverse magnetisation determines the amount of T2 relaxation is specified by TE.

The TR is the time between consecutive RF pulses and is measured in milliseconds (Figure9). It also defines the time of relaxation between the end of the first RF pulse, and the following one that decides the amount of relaxation of T1.

The most common pulse sequences are the T1 weighted and T2 weighted sequences. T1 weighted images (T1WI) are produced by using short TE and TR times. T2 weighted images (T2WI) are produced by using longer TE and TR times.

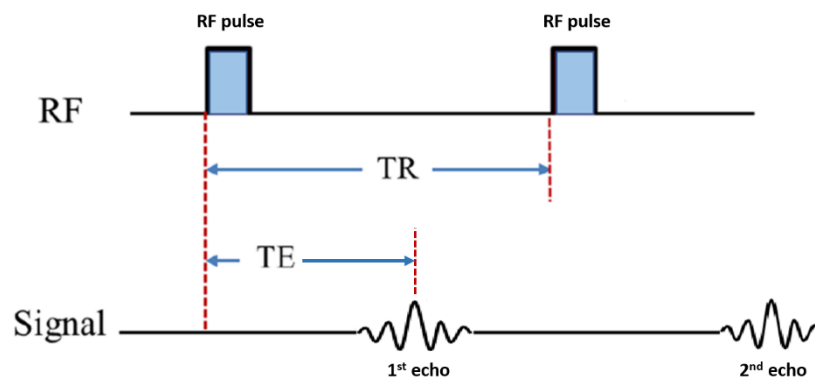


Figure 9: Illustrating the RF sequence and the acquired echo signals.

In T1WI, the contrast depends on the T1 of tissues, that have been scanned. For a short TR, the only tissue that can recover quickly is a tissue with a short T1 (bright on image) before the second RF pulse is applied. The

tissue with a long T1 will partially recover, and, therefore, will have a weaker signal (dark on image).

For T2WI, TE controls the amount of T2 decay that occurs before receiving a signal. In T2WI, a long TE is required to allow tissues with short T2s to lose most of their signal, thus, leaving only long T2 tissues as bright in the images.

Fast Spin Echo (FSE) or Turbo Spin echo (TSE)

These are SE sequences that use multiple 180° refocusing pulses after just one 90° excitation pulse to produce several echoes (Figure10). The number of echoes acquired during the TR is called the turbo factor or the echo train length (ETL). For each echo, a different phase encoding value is used (in a conventional multi-echo sequence all echoes are collected with the same phase encoding), allowing multiple lines of K-space within a given TR to be acquired, thus, reducing the overall scan time¹².

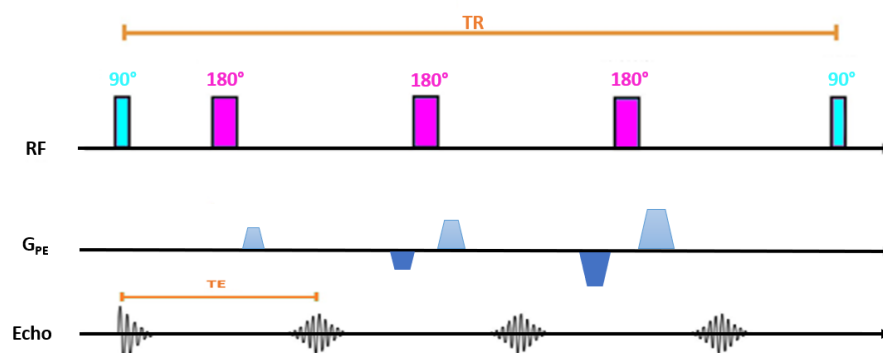


Figure 10: Scheme of FSE sequence.

Spoiled Gradient Echo (SPGR) or FLASH (Fast Low Angle Shot)

Only the signal, generated through the action of the RF pulse on longitudinal magnetisation, is used in SPGR. The residual transverse magnetisation remaining after each excitation cycle is destroyed by RF spoiling or gradient spoiling (Figure 11). This sequence has a very low flip angle, and a short TR, which leads to poor contrast, but is one of the quickest sequences to acquire.

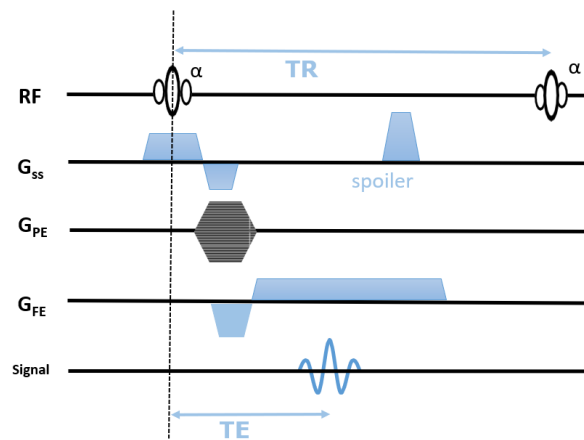


Figure 11: Spoiled Gradient Echo Sequence. Before starting the following pulse, the spoiling gradient in the slice selection direction is responsible for dephasing any residual transverse magnetisation.

Balance Turbo Field Echo (bTFE) sequences

bTFE is a sequence with balanced gradients rewinding in all three directions (Figure 12), termed FIESTA (Fast Imaging Employing Steady-state Acquisition) by GE, and True-FISP (true-fast imaging with steady precession) on Siemens systems. This sequence provides good SNR in the body compared to other fast gradient-echo techniques and maintains a short acquisition time due to very short TR and TE values. To achieve very short TE, this sequence requires high-performance gradient technology, and a homogeneous static magnetic field. Images may be degraded with

banding artefacts that are inversely proportional to their field inhomogeneity if this is not achieved^{10, 13}.

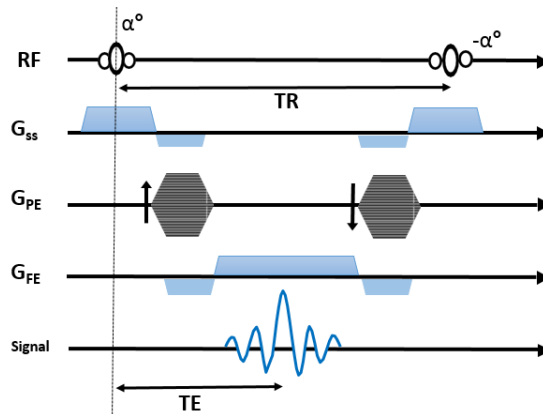


Figure 12: Balanced Fast Field Echo pulse sequence (bFFE). The application of $+\alpha -\alpha$ RF pulses are used to keep the spins in a steady-state, and will, therefore, produce enough echoes to fill k space.

Echo Planar Imaging (EPI)

EPI was first proposed in the 1970s by Mansfield¹⁴. EPI is a very fast MRI sequence that can obtain an entire image in only a fraction of a second (<100ms typically). EPI is accomplished by using a pulse sequence in which a series of echoes from different phase steps are acquired by using rephasing gradients instead of repeated 180° RF pulses following the $90^\circ/180^\circ$ in a SE sequence, or without repeating the initial excitation pulse in a GE sequence.

It was created as the fastest way of collecting data across k-space. This was achieved by utilising rapidly switching gradients to obtain the whole of k-space within one RF excitation pulse (GE or SE). It can be achieved by a blipped or non-blipped method. In both methods, k-space will be filled from left to right and back, with each echo. In the blipped EPI, the

phase encoding gradient is modified in the ky direction. In this method, a phase-encoding gradient (G_y) is applied throughout the time that the readout gradient (G_x) is zero (Figure 13a). The filling of k-space is rectangular in this technique (Figure 13 b).

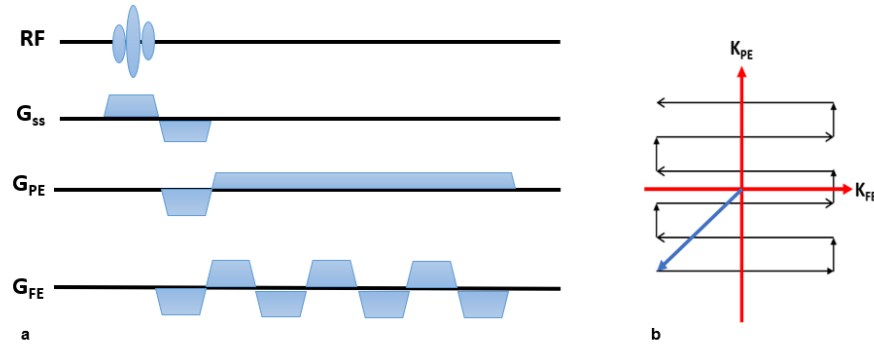


Figure 13: A simple echo planar imaging sequence (a). Signal is acquired with every echo caused by the alternating lobes of the frequency gradient but the tiny 'blips' on the y-gradient phase encode each echo and EPI k-space coverage (b).

Different image contrast is achieved by beginning the sequence either with 90° and 180° RF pulses termed SE-EPI or with a variable RF excitation pulse termed GE-EPI as explained in more detail in the next section.

Spin Echo and Gradient Echo EPI

SE-EPI uses a pair of 90° excitation and 180° re-phasing pulses followed by an EPI sequence readout to generate echoes (Figure 14, a). SS-EPI uses a single TR to acquire all phase encoding steps while in multiple-shot EPI, all of the phase encoding steps are obtained across several TRs. In gradient Echo EPI, an RF excitation pulse of any flip angle is used and is followed by the EPI readout of gradient echoes (Figure 14, b). There are a few drawbacks when using EPI, such as low spatial

resolution, sensitivity to susceptibility artefacts, chemical shift issues and requirement of high-performance gradients.

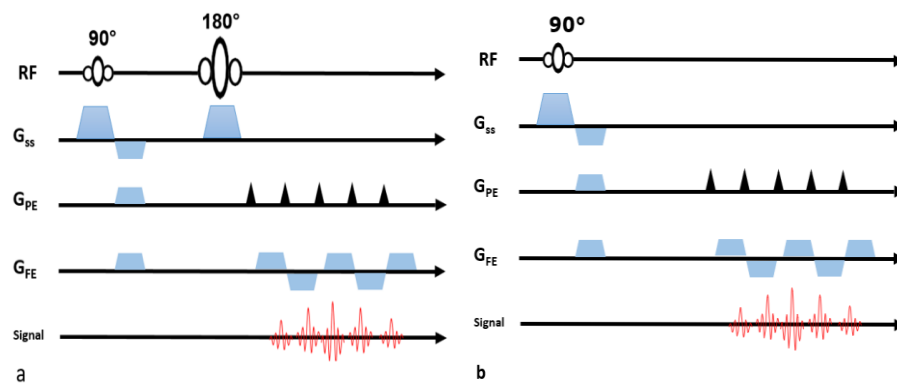


Figure 14: Pulse sequence diagrams for SE-EPI (a) and GE-EPI (b)

Short Inversion Time Inversion Recovery – STIR

STIR is an IR-SE pulse sequence that uses a specific TI to suppress the signal from fat tissue. The action of the initial RF pulse is to invert the starting magnetisation, which then returns to its equilibrium value according to T1 relaxation (Figure 15, a). The null point is when the longitudinal magnetisation has returned to zero, and, hence, there will be no signal in the transverse plane at this time if a 90° pulse were to be applied. The timing of the STIR sequence TI is set so that the fat tissue is at the null point (which passes through first when compared with other tissue) when the subsequent 90° imaging pulse is applied (Figure 15, b).

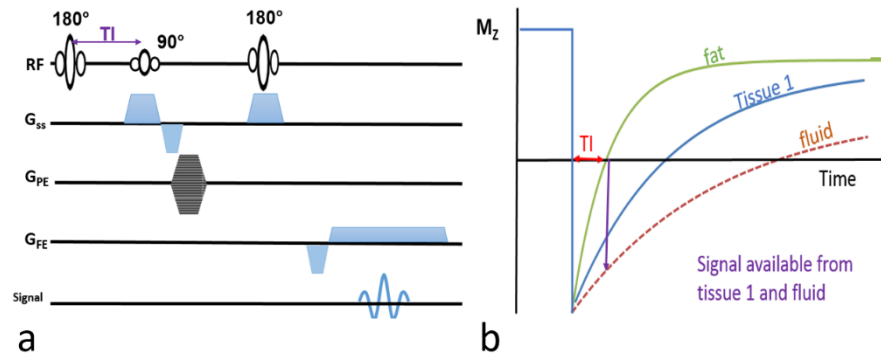


Figure 15: An inversion recovery pulse sequence (a) and M_z recovery in STIR (b).

2.4 Principle of Newer MRE sequences

2.4.1 Magnetisation Transfer (MT)

In standard MR pulse sequences, image contrast can be manipulated by using the TR and TE of the sequences by using the intrinsic T1, T2 and proton density of the images of the tissue. This tends to be from freely moving hydrogen within the tissue. However, some tissues contain both free hydrogen pools, and immobile hydrogen bound to macromolecular lipids and proteins. The direct observation of the restricted-hydrogen pool is usually not possible because its T2 relaxation time is very short (< 200 microseconds). The central resonance frequency in both pools is roughly the same and the only distinction is the greater spread of resonance frequencies of the restricted protons (Figure16).

By adding off-resonance radio-frequency pulses to the sequence before image acquisition to saturate the magnetisation of the immobile pool, the signals from the mobile pool are reduced because of the transfer of the magnetisation from the immobile to mobile protons, Figure16^{15 16}.

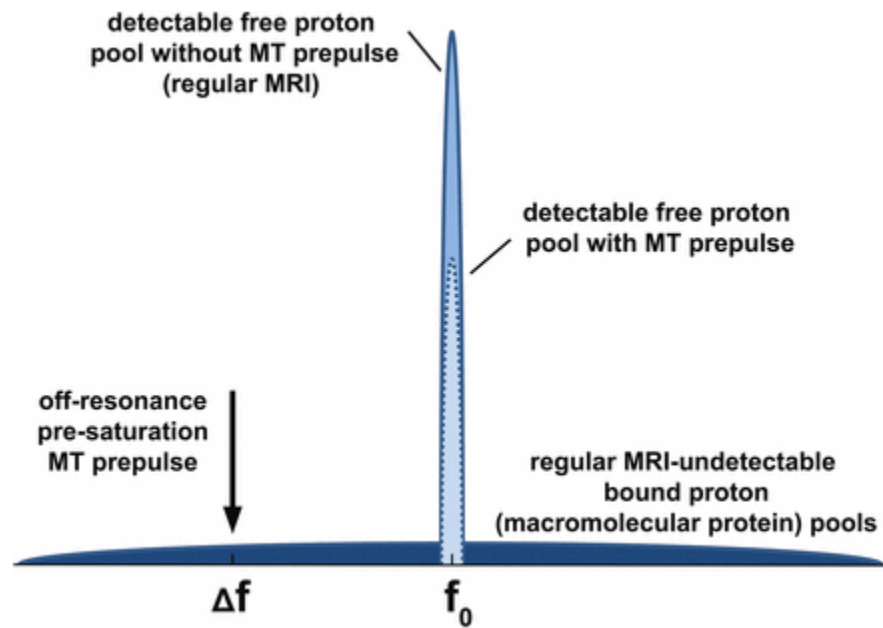


Figure 16: Schematic diagram of the basic principles of MT imaging. MT between free and bound protons. The spectral lines of the ‘bound pool’ and the ‘free pool’ are both centred at the same Larmor frequency f_0 . The bound protons have a short T_2 and the free protons have a long T_2 . The bound protons are not normally ‘MR visible’ in the image due to its signals not being directly detectable (decay quickly to noise levels before data acquisition) because of the very short T_2 . This pool has a broad resonance, and, thus, can be excited by an RF pulse at a frequency many kilohertz away from the free water frequency, which, therefore, has no impact on the free protons. In the exchange of protons between the free and restricted pools, saturated magnetisation from the bound pool (invisible) will move into the free pool, and will reduce the level of the MR signal detected¹⁷.

MT generates contrast that is determined by the fraction of large macromolecules and the immobilised phospholipid cell membranes in tissues, hence, rigid body elements such as fibrotic or muscle tissues have a high MT effect¹⁸. The MT Ratio (MTR) defined as the contrast between images with and without the off-resonance saturation pre-pulse that indirectly measures the bound protons accordingly, informs the biophysical integrity of macromolecular protein pools, and their local micro-environments¹⁷. So, MTR indirectly reflects the concentration of

macromolecules in tissue. There will be no change in the signal in the absence of macromolecules, and the MTR remains low. The MTR will be high in tissues with a mainly macromolecular structure, such as fibrosis. Therefore, it can be used to discriminate between fibrotic tissues and non-fibrotic tissues. MTR is also expressed as percentage units, and is measured in the equation below^{19, 20}.

$$\text{MTR} = 100 \cdot \frac{\text{SI off} - \text{SI on}}{\text{SI off}} \quad (5)$$

where S on and SI off are the measured signal intensities with and without the MT saturation pulse, respectively.

By interleaving sequences with and without off-resonance prepulses, interleaved spin-echo and two and three-dimensional gradient-echo sequences have been effectively used in the determination of MTR²¹.

2.4.2 Diffusion-weighted imaging (DWI)

DWI-MRI is a technique which acquires its image contrast from the Brownian motion of water molecules within tissues^{22, 23}. This is achieved by applying two equal magnetic gradients on either side of the refocusing pulse in conventional spin-echo sequences usually with an EPI readout. Protons in tissues without any Brownian motion are, first, de-phased by the initial gradient, and then re-phased by the second gradient, leading to no loss of signal. Protons free to diffuse around are not completely re-phased as they experience unequal gradients, hence, resulting in reduced signal intensity.

The amount of signal loss is a marker of the diffusion of water in that tissue and allows an indirect evaluation of tissue microstructures.

This sequence is sensitive to water molecules presenting with restricted diffusion. A factor called the 'b-value' is used to determine the weighting of the signal to the diffusion of the tissue. This factor determines the strength and duration of the diffusion gradients. Low b-values are achieved by employing small gradients, and results in a small loss of signal from the tissue due to diffusion. Once the b-value is high, there is a lower contribution from non-restricted water molecules to the signal (as it has not been rephased), and, hence, the signals remaining in the image reflect tissues with restricted diffusion. To calculate the apparent diffusion coefficient (ADC), at least 2b-value images should be obtained: one acquired without using the diffusion gradient (similar to the T2-weighted image (WI) in its appearance) and one acquired with diffusion gradients^{24, 25}.

A variety of techniques can be applied with DWI such as FSE, conventional spin-echo and EPI. These sequences have a major role in DWI image quality. The sequence pulses used in diffusion should, typically, be able to acquire the diffusion signal before its complete attenuation, which can be very rapid in the case of highly diffusing protons. Therefore, the sequences used tend to produce images over a very short period. which also enables faster acquisition of multiple diffusion images in minimal time, and prevent phase accumulation errors, and other artefacts.

Intravoxel Incoherent Motion (IVIM-DWI) imaging, is a novel sequence based on DWI, which provides separation of diffusion and perfusion effects using bi-exponential analysis²⁶. This model is characterised by the perfusion fraction (PF) which reflects the fraction of flowing blood that can be distinguished in a voxel, the pseudo-diffusion coefficient (D^*) that reflects the velocity of capillary blood and perfusion-related pseudo-diffusion, and the pure molecular diffusion coefficient (D).

Le Bihan *et al.* were the first to introduce and develop this method to quantitatively evaluate all the microscopic translational motions that could contribute to the signals acquired with DWI. Together with the foundation of diffusion MRI whose concept was introduced in 1986, it was realised that blood flow in capillaries (perfusion) would imitate a diffusion process and MRI measurements of a diffusion effect. Soon after, it was understood how perfusion and diffusion effects in diffusion MRI could be distinguished, leading to separate maps of molecular diffusion and perfusion measurements²³. According to this technique, both perfusion and diffusion could be quantified by using a multi-b-values DWI acquisition. For this, it is important to acquire several examples of both low and high b-values. The high b values ($>200 \text{ s/mm}^2$) are used to study the signal information from outside the capillary network that is entirely de-phased, acquiring only signals from the extra-vascular space. Conversely, for the low b values ($<200 \text{ s/mm}^2$), the fast water movement is related to the blood flowing through the capillary network in a random manner²⁷ as seen in figure 17. Since the capillary flow is considerably faster than water diffusion in tissues,

signal drop per unit increase in b value is also greater, meaning that signal loss from most perfusing water molecules arises at relatively low b values.

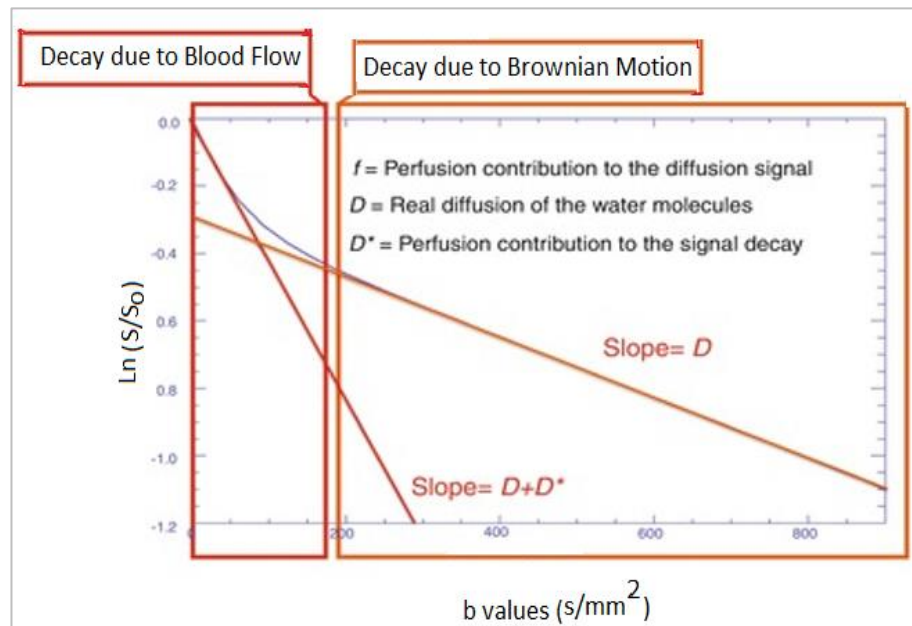


Figure 17: Represents the IVIM model. The first section of the diffusion-related decay curve represents rapid signal decay associated with the blood flowing via the capillary network randomly ($<200 \text{ s/mm}^2$), while the second section of the curve shows the signal drop due to the normal diffusion of the tissues²⁷.

Diffusion has been largely used for the assessment of central nervous system lesions, mainly demyelinating disease, intracranial tumours and acute ischaemic stroke²⁵. This is because the brain is a static organ with DWI acquisitions taking more time to acquire compared to standard sequences due to the application of the additional diffusion gradients, which need to be applied in multiple directions. Recently, the range of DWI application has been increased to include the body, and it has become a promising sequence in abdominal imaging. However, there are more issues when using diffusion in the body due to the motion of respiration, organ movement, and susceptibility artefacts from EPI acquisitions²⁸.

Two methods can be used to model IVIM-DWI signal decays to quantitatively characterize the diffusivity of tissues from DWI mono or bi-exponential models. Mono-exponential decay of the signal with increasing b-values assumes the simplest and most commonly used model for analysis of DW images as in the following equation²³.

$$S = S_0 \exp(-ADC \cdot b) \quad (6)$$

where S is the mean signal intensity with diffusion gradient b, and S₀ is the mean signal intensity without a diffusion gradient. However, this model does not consider the influence of the microcirculation of blood in capillaries, which may lead to an inaccurate description of the diffusion. The parameters of perfusion and diffusion can be taken into account separately through modelling signal decay by using a bi-exponential function from the following equation^{23, 29}.

$$SI/SI_0 = (1 - PF) \cdot \exp(-b \cdot D) + PF \exp(-b \cdot D^*) \quad (7)$$

This is the IVIM model. Where SI₀ and SI are the signal intensity at b=0 s/mm² and given b-values, respectively; D* is the perfusion coefficient; D is the diffusion coefficient and PF is the fractional perfusion and b is the b-factor.

Respiratory motion is one of the main issues when using diffusion for clinical applications in the body. Breath-hold, free breathing acquisition, and respiratory-triggered strategies have been used to acquire DWI in the body, to overcome this issue³⁰⁻³². The most typical DWI sequence is a single shot-EPI (SS-EPI with parallel imaging). Parallel imaging is an important method

that allows for reduced specific absorption rates, increased spatial and temporal resolution, for shorter examination times, for quicker acquisition of imaging data, and in some cases, enhanced image quality.

SS-EPI sequence with a breath-hold acquisition is commonly used in clinical practice due to its availability and shorter image acquisition time, which reduces the motion artefact in the image. However, this sequence has low resolution, and signal-to-noise ratio (SNR), and is prone to EPI related artefacts such as chemical shift and nyquist ghosting artefacts. Free-breathing or respiratory-triggered DWI techniques provide improved SNR, and reduce image noise allowing for a higher spatial resolution, and the use of multiple and high b-values²². However, the time needed to acquire full image datasets in this strategy is long, and dependent on the coverage and number of b-values utilised.

2.4.3 Dynamic Contrast Enhancement (DCE)

DCE-MR imaging is a technique utilized to evaluate tissue perfusion where T1-weighted images are acquired dynamically in the region of interest (ROI) following the intravenous injection of a gadolinium-based contrast agent. Once the contrast reaches the tissue, it reduces the tissue's native T1 as a function of the distribution and concentration of the contrast agent ³³. It is sensitive to changes in extravascular, extracellular, vascular permeability, and blood flow, and vascular volumes. This is a dynamic imaging technique (meaning multiple images of the same section of the body are acquired many times during the experiment), which is used to

monitor the time variation signal change in the tissue. The signal obtained is used for the generation of a time-intensity curve (TIC) for the tissue, reflecting the response of the tissue to the arrival of contrast agent in enhancement values. The curve shows the intensity of the signal at every time point, before, during and after the injection. By analysing this curve, certain physiological properties can be derived, such as tissue volume fraction and vessel permeability, which are related to microvascular blood flow.

To analyse signal changes as a function of time, specific software can be used that provides information on semi-quantitative or quantitative parameters, which use equations to model the image signal changes, and the exchange in the contrast agent between the blood and tissue or studying time-intensity curves from a qualitative viewpoint.

Semi-quantitative parameters can be used to describe the enhancement curve (signal vs. time). Semi-quantitative analysis of the data includes calculation of parameters, such as, maximum enhancement or ratio (ME), time to peak enhancement, the initial slope of enhancement (SoE), and the initial area under the curve (AUC). SoE is defined as the gradient of the signal intensity (SI) time curve between the last point of the baseline signal intensity, and the inflexion point following initial fast enhancement, and ME as $(\text{peak enhancement SI} - \text{baseline SI}) / \text{baseline SI}$ as seen in Figure 18³⁴. These parameters can be calculated in a time efficient manner.

However, one of the challenges is the fact that these parameters do not necessarily have any obvious physiological correlations. The degree to which each of these physiological parameters contributes to MRI-signal remains unknown because they represent a combination of microcirculatory and tissue properties. Another limitation is that most extraction methods of model-free parameters are sensitive to variations between different acquisition protocols. As a result, comparisons of semi-quantitative studies at best are difficult.

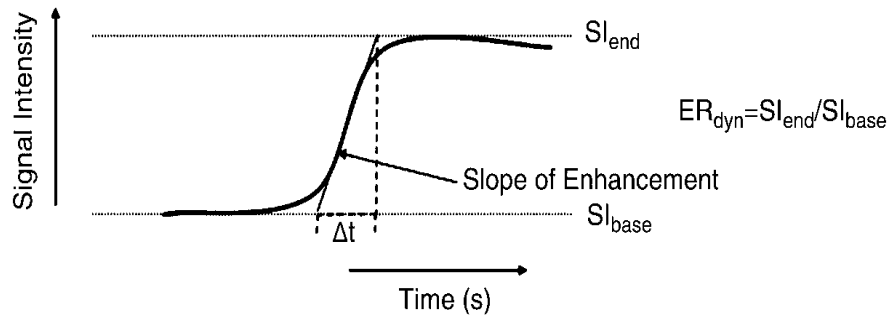


Figure 18: DCE-MRI semi-quantitative parameters derived from SI and time curve data³⁴.

For quantitative analysis, the kinetics of contrast media uptake and washout is used to assess tissue perfusion, extracellular leakage space, and the micro-vessel permeability of the surface area. Pharmacokinetic models are used to convert the SI to tissue concentration of gadolinium, and to calculate the parameters that reflect the tissue perfusion. Several models have been proposed for quantitative parameters including the Tofts- and extended Tofts models^{35, 36}. These models include the calculation of parameters such as volume transfer coefficient (K^{trans}), leakage space (V_e), and the volume fraction of the plasma space (V_p). V_e represents the volume of the extra-vascular extra-cellular space per unit volume in tissues. K^{trans}

shows the transfer constant between the intra-vascular and the extra-vascular extra-cellular space ²⁴. The quantitative approach has been a method used for monitoring the effects of antiangiogenic cancer drugs³⁷.

Dynamic image intensity of DCE-MRI images, however, cannot be directly used for fitting the kinetic model as it does not reflect a proportional concentration of contrast agents in the tissues. Pre-contrast T1 mapping, therefore, is an essential step in converting the intensity of the image into the concentration of the contrast agent in plasma and tissues.

Many T1 measurements have been applied widely for DCE-MRI T1 mapping such as Look-Locker³⁸⁻⁴⁰, inversion recovery⁴¹, and multiple flip angles (MFA)^{42, 43}. A 3D T1-weighted Spoiled Gradient Echo (3D SPGR) acquisition with MFA is the easiest way to estimate the baseline T1 value in tissue, through maintaining equal all other acquisition parameters. Depending on the expected T1 value, an optimised selection of the flip angles may be used to estimate T1 values. From equation 8 below the final estimate of the T1 is achieved by fitting the signal intensity of the images acquired with many angles to this equation. The steady-state SPGR equation is used to generate the pre-contrast T1 map:

$$S = M_0 \cdot \frac{\sin a (1 - E1) \cdot E2}{1 - E1 \cdot \cos a} \quad (8)$$

Where S is signal intensity, $E2 = \exp(-TE/T2)$, $E1 = \exp(-TR/T1)$, a is the flip angle, and M_0 represents the T1 fully relaxed signal that considers T2 effects.

Keeping TE and TR constant, and measuring the signal induced by different FAs, equation 9 can be rewritten in a linear form and by plotting $S/\sin(a)$ against $S/\tan(a)$ one can find a straight line described by slope $m = E1$, and intercept $c = M_0 \cdot (1 - E1) \cdot E2$, and then, by linear regression, T1 is easily calculated as:

$$T1 = \frac{TR}{\ln(m)} \quad (9)$$

2D or 3D gradient-echo sequences represent the most common sequences applied in DCE-MRI due to the fast acquisition required for good temporal resolution. The earliest methods used a 2D sequence with limited slices, to reach the temporal resolutions needed to achieve adequate sampling of the uptake curve⁴⁴. However, since the introduction of parallel imaging, and acceleration systems for fast 3D imaging, the current practice using 2D multi-slice is gradually being replaced by 3D methods. Also, the 3D approach is less sensitive to B₁ artefacts, when examining decreasing slice profile error of the 2D approach⁴⁵. An example protocol used for DCE imaging can be seen in Figure19.

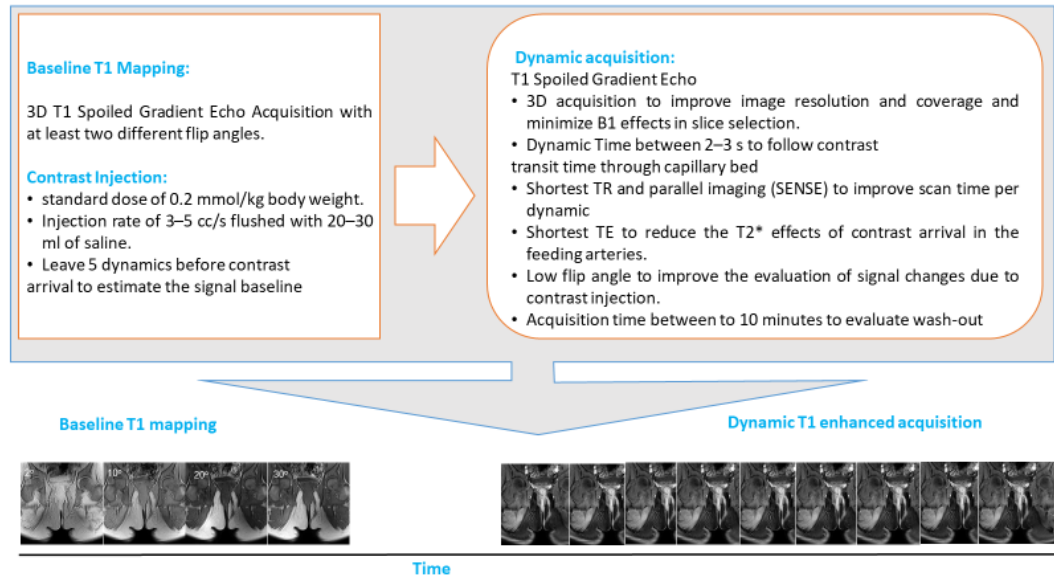


Figure 19: Schematic illustration of a DCE acquisition containing the baseline T1 mapping, the injection protocol, and the dynamic acquisition.

In this chapter, the basics of NMR and MRI have been described. Furthermore, the standard T1 and T2-weighted sequences used in IBD imaging, in conjunction with newer more quantitative sequences, have been discussed. The following chapters of this thesis will explain these sequences to determine their applications in CD and pCD .

Chapter three: Inflammatory Bowel Disease

This chapter addresses the pathogenesis and epidemiology of IBD and explains different treatment strategies. It describes methods available for the diagnosis and evaluation of disease, including MRI, and clinical scores, and describes the lack of an ideal approach to IBD diagnosis and prognosis. This chapter will be focusing more on CD.

3.1 Background to IBD

IBD includes two kinds of chronic, idiopathic intestinal disease that are distinguished by their disease location, confluence of inflammation, histological characterisation, perianal involvement and the presence of fibrotic or penetrating complications. CD is a life-long IBD of an uncertain aetiology. It may have a panenteric distribution leading to various intestinal (perianal abscesses, intestinal strictures, external and internal fistulas), and extra-intestinal complications⁴⁶. Ulcerative colitis (UC) is characterised by diffuse colonic mucosal inflammation, and only affects the colon. Although it most commonly influences the rectum (proctitis), it can reach beyond the sigmoid (distal ulcerative colitis), into the sigmoid (proctosigmoiditis), or to include the other locations within the colon (pancolitis). IBD disease behaviour is characterized by relapsing periods with active symptomatic disease, and disease remission periods^{46, 47}. The main differences between UC and CD are given in Table 2.

IBD leads to impaired quality of life⁴⁸, significant morbidity, and health care expenditure⁴⁹, while increasing mortality when related to age-matched

controls^{50, 51}. The mortality rate of CD patients (50%) was higher than in UC patients(20%) ⁵², when compared with age-matched cohorts.

The severity of mucosal inflammation in CD patients can lead to the development of fibrosis which may lead to stenosis and to obstructing symptoms. CD may include any parts of the gut; involvement can be patchy and affect several, discontinuous parts. Disease location may vary with approximately 45% of the cases involving both the ileum and colon, 20% the colon, and 33% are to be found purely in the small bowel location⁵³. Pharmacological intervention is used to maintain remission, induce mucosal healing, and reduce the incidence of recurrence, and the exacerbation of diseases.

3.2 Epidemiology

IBD is most commonly diagnosed in late adolescence or early adulthood, but the diagnosis can occur at all ages. IBD prevalence is steadily increasing in high incidence regions worldwide, such as, New Zealand, Australia, North America and Northern Europe, while the incidence is rising faster in low-incidence rates in regions, such as, Asia, South Europe and most other developing countries⁵⁴.

The most recent United Kingdom IBD prevalence estimates for CD are 9–144/100 000, and UC 66–389/100 000 ⁵⁵. More than 1.5 million people in North America and 2 million in Europe are suffering from IBD⁵⁶. The increasing incidence of this condition combined with its incurability has

significant cost implications. The cost of treating IBD is estimated at more than £1 billion in 2010 with the annual cost per CD patient being up to £6156⁵⁷.

3.3 Aetiology

The aetiology of IBD is still unclear, however, several implicated factors may lead to this condition such as environmental, immunological and genetic factors⁵⁸. The growth of diseases may be a consequence of complicated interactions of all three factors; however, further research is needed to comprehend completely the complications of these interactions, and is beyond the scope of this thesis.

Table 2: The main differences between CD and UC.

Feature	CD	UC
Location of disease	Anywhere from mouth to anus. Commonly affects terminal ileum (28%) and colon (48%) ⁴⁷	Large intestine only
Pattern of inflammation	Patchy, discontinuous (skip lesion) in inflammation	Superficial and continuous
Histology	Transmural inflammation, granulomas may be present	Mucosal inflammation, no granulomas
Complications	Abscesses, perineal disease	Toxic megacolon
Presenting symptoms	Fever, abdominal pain, diarrhoea, weight loss and fatigue	Abdominal pain, diarrhoea, rectal bleeding

3.4 Clinical presentations and complications of IBD

3.4.1 Crohn's Disease

3.4.1.1 Clinical Manifestations of CD

CD patients suffer from a variety of symptoms including chronic diarrhoea, abdominal pain, bloating, and weight loss⁵⁹. Specific symptoms of CD may vary between patients, but this tends to broadly depend on the predominant disease location. Patients with a predominant colonic distribution tend to present mainly with chronic diarrhoea and rectal bleeding, while patients with small bowel disease can present with a change in bowel habits, abdominal pain, and predominant obstructive symptoms⁶⁰.

Diarrhoea can be caused by impaired fluid absorption, and excessive fluid secretion, overgrowth of bacteria, and malabsorption of bile salt. Abdominal pain is often linked to fibrosis and stricture formation, and may be related to bowel obstruction.

Fatigue, weight loss and fever comprise systemic symptoms of CD. Weight loss may be related to altered eating behaviour⁶¹ and intestinal motility⁶², together with a catabolic metabolism⁶³ and sepsis.

Mild-to-moderate CD patients are ambulatory, and have a normal Quality of Life. Moderate-to-severe CD patients exhibit more debilitating symptoms, such as, vomiting, nausea, abdominal pain and tenderness, weight loss and fever.

Approximately 33% of CD patients develop perianal disease. Symptoms and signs involve anal perirectal abscesses, fissures, drainage and perianal pain from large skin tags⁵⁹.

3.4.1.2 Complication of CD

CD can comprise both local and extra-intestinal complications. Local complications such as acute perforation, severe haemorrhage, intestinal obstruction, and the development of abscesses and fistulae. CD patients may have extra-intestinal complications associated with IBD activity such as joint disease, skin and eye disorders.

3.4.2 Ulcerative Colitis

3.4.2.1 Clinical manifestations of UC

The severity of UC symptoms is generally correlated with the extent and severity of colonic involvement. The initial manifestation often involves an insidious symptom onset, often preceded by a history of self-limited, continuous rectal bleeding though may present acutely. Weight loss, fever and abdominal tenderness are other physical disorders. Patients with UC may have mild, moderate or severe disease, but each of these categories has slightly different symptoms and signs⁶⁴.

3.4.2.2 Complications of UC

UC is associated with both local and extra-intestinal complications. Development of colon cancer, toxic megacolon, fulminant colitis, and massive haemorrhage are the most common local complications of UC.

Toxic megacolon in severe patients may occur when the colonic lumen is dilated due to an atonic colonic, thereby increasing the risk of imminent intestinal perforation, sepsis and mortality ⁵⁹. Joint, eye and skin disorders are an example of extra-intestinal complications.

3.5 Diagnosis and investigations

There is no single test which may diagnose reliably all cases of IBD, and many patients require more than one test, which can delay the diagnosis. IBD is diagnosed by a combination of history and clinical evaluations by employing, endoscopic laboratory, histological and radiological investigations. A historical investigation should involve: type, duration, onset, including severity of symptoms; in addition to family history, past-medical intervention, and drugs taken. Abdominal examination (particularly for tenderness and right iliac fossae masses), body mass index, rectal and perineal examinations, as well as evaluation for extraintestinal manifestations that should be included in a physical examination.

3.5.1 Appropriate tests in people with suspected IBD

3.5.1.1 Laboratory tests

Laboratory tests should comprise stool and blood examinations to rule out other causes of inflammation and diarrhoea. In general, laboratory tests are helpful in diagnosing IBD, evaluating the activity of the disease, monitoring the response to therapy, and in identifying complications. Initial testing often involves measurement of: the hemoglobin, liver enzymes, creatinine, blood urea nitrogen, hematocrit, platelet count, white blood cell

count, and the erythrocyte sedimentation rate(ESR), and C-reactive protein (CRP). Further subsequent testing to monitor complications can involve measurement of iron, vitamin D, calcium, prealbumin, albumin, and total iron-binding capacity.

- Inflammatory markers: ESR and CRP tests may help confirm suspected inflammatory activity full blood count, but their correlation to disease activity and intestinal inflammation is poor, meaning that they have a poor negative predictive value ⁶⁵.

Investigation of blood biomarkers is clinically useful as results are rapid, inexpensive and usually accessible at the point of care. These modalities are hampered by a lack of sensitivity. In fact, studies are suggesting that serum CRP could serve as a pharmacodynamic/response biomarker. The pooled sensitivity (Sn) for CRP is 0.49 (95% confidence interval (CI) 0.34–0.64) with a high specificity (Sp) of 0.92 (95% CI 0.72–0.96) ⁶⁶ and hence cannot be reliably used to measure or exclude disease activity in CD. In terms of predicting response to biological therapy, CRP may be used for this purpose although its reliability is debatable. CRP is a serum biomarker of systemic inflammation. It is related to an interleukin response and is, thus, one of the key non-invasive disease measurements used in IBD monitoring. A high CRP predicts active luminal disease. A patient with high CRP values at baseline is more likely to respond to biological treatment than those with normal values⁶⁷. Baseline CRP levels were found to be significantly higher for responders than for non-responders

(16.8 mg/L (5–160), vs 9.6 mg/L (5–143); $P=0.02$), with CRP levels being high in 73.2% of responders, and 26.8% in non-responders at week 4. A study conducted by Reinisch *et al.* also showed that those with higher CRP before treatment were more likely to respond to treatment. High baseline CRP (> 0.7 mg / dL) predicted an increased response to infliximab therapy on week 14, and a remission of 1 year in the ACCENT 1 study⁶⁸. Not all patients are considered to have high CRP for their diseases, and, therefore, this test in all patients is not accurate^{69, 70}.

- In active disease, the level of albumin is decreased due to a protein-losing enteropathy with extremely low levels predictive of UC treatment failure ⁷¹.
- Faecal calprotectin (FCP): is a granulocyte protein shed into faeces by the inflamed bowel has been used in recent years as a marker of disease activity in IBD. It is a highly sensitive, non-invasive marker of intestinal inflammation⁷². In order to differentiate irritable bowel syndrome from organic bowel pathologies, FCPs were used initially. More recently, FCP can be used for the therapeutic monitoring of IBD patients to determine if the current level of disease activity, risk of relapse and/or response to current type and dose of treatment merited further intervention. Like CRP, there are studies published on the utility of FCP in IBD, indicating it could be useful as a diagnostic, predictive, and/or response/ pharmacodynamic biomarker. For example, a high FCP value is predictive of disease

relapse when evaluating disease activity in a patient on anti-TNF, and may prevent a clinician from withdrawing anti-TNF.^{73, 74} Meta-analyses estimate it has 80-87% Sn and 68-82% Sp for clinical⁷⁵, and endoscopic⁶⁶ CD activity, and 78% Sn, and 73% Sp to predict a relapse⁷⁶. Meta-analyses estimate FCP in IBD had pooled Sn of 85%, and Sp of 75% in diagnosing active disease⁷⁷. An analysis by a subgroup shows that FCP obtained better results in UC than in CD (87.3% versus 82.4% pooled Sn, 77.1%, versus 72.1% pooled Sp⁷⁷. Moreover, FCP may perform inferiorly in small bowel disease in comparison with large bowel but conflicting data exist. In a recent systematic review⁷⁸, the sensitivities and specificities of FCP were too diverse to accurately reflect disease activity in CD at different intestinal sites to generate any sound conclusions. They found that Sn of FCP in small bowel ranged from 42.9% to 100%, while those in the large bowel ranged from 66.7% to 100%.

3.5.2 Further investigations in suspected IBD

3.5.2.1 Endoscopy

Ileocolonoscopy with radiology and histology are all used to establish the diagnosis of IBD, and to evaluate its severity and extent. Ileocolonoscopy is the gold standard examination for diagnosing, and for the assessment of disease activity⁷⁹. Features of endoscopic findings include superficial aphthous ulcerations, deep ulcerations, cobblestoning, skip lesions, stricturing and penetrating complications. Ileocolonoscopy has some disadvantages. Endoscopic procedures are time-consuming

(especially now, in the post-COVID19 era), invasive, and have associated complications such as risk of bowel perforation and bleeding. Moreover, endoscopy does not allow a transmural assessment of the disease and is not able to characterise any extra-luminal extension of the inflammatory process. Hence, in the absence of luminal inflammation or obvious stricturing disease, endoscopy is unable to characterise, precisely, the disease burden.

3.5.2.2 Capsule endoscopy

Small bowel capsule endoscopy (SBCE) is a useful method for assessing small-bowel involvement in CD, its extent and severity. It has a high sensitivity for detecting small bowel disease, but the effectiveness of this modality is limited by inter-observer variability, and limited availability within medical institutions. Moreover, it is not possible for SBCE to assess the extraluminal disease extent. Most importantly, in patients with bowel strictures, the risk of capsule retention, and intestinal obstruction necessitating intestinal surgery is considerable. Patients with known bowel strictures are therefore not eligible for this investigative modality⁸⁰.

3.5.2.3 Imaging

Imaging modalities play a significant role in the evaluation of patients with IBD. Imaging has been used for a long time to determine portions of the digestive tract that are not accessible to conventional endoscopy. An initial assessment may be undertaken by a standard x-ray. Contrast studies like a small bowel imaging with fluoroscopic barium small bowel follow-

through can help with identification of internal fistulas as well as confirmation of the anatomic location of the disease⁸¹.

The role of imaging in IBD has been greatly extended to include the mucosal, transmural, extra-luminal and extra-intestinal manifestations of these diseases through advances in cross-sectional imaging techniques such as ultrasound, computed tomography, and MRI have recently come to the fore and changed practices. Imaging now not only helps diagnose IBD, including discrimination between CD and UC, but also plays a critical role in determining active versus remitting disease. Gastroenterologists are increasingly using IBD imaging as a biomarker of the therapeutic response/resistance for treatment planning.

Ultrasound is one of these techniques extensively used in IBD imaging. There are many advantages of this technique, such as, real-time imaging capability, lack of ionizing radiation, and low relative cost. However, there are some disadvantages of ultrasound, including the difficulty of tracing long bowel segments, operator dependence, which requires a highly qualified ultrasonographer, and, further, an inability to visualise portions of rectum and sigmoid. Although the colon and ileocecal region are often visualised easily, it is difficult to see additional portions of the bowel due to overlying bowel loops⁸²⁻⁸⁴. Moreover, an ultrasound scan is poorer at demonstrating the extent of abnormalities compared with MRE, and had a lower Sn and Sp compared with MRE as well⁸⁵. A recent multicentre trial conducted with 284 newly diagnosed, and suspected relapsed CD patients,

indicated that MRE had a significantly higher Sn and Sp (presence and location) to the extent of bowel disease compared with ultrasound (80% versus 70%: 95% versus 81% respectively). The Sn of ultrasound for active small bowel disease is 90% significantly lower than that of MRE 96% for active small bowel disease. Also, for detecting the presence of small bowel disease irrespective of location, MRE Sn was 97%, compared with 92% for the ultrasound⁸⁵. The usefulness of an ultrasound scan in evaluating disease activity is disputed^{82, 83}.

These cross-sectional imaging is now the mainstay imaging modality to assess a disease's extent and severity in IBD, and to assess for perforating complications⁸⁶. Computed tomography (CT) and MRI have a high diagnostic sensitivity in detecting small bowel involvement and extra-luminal complications of IBD, involving intraabdominal abscess and internal fistulae. Both modalities are useful in evaluating disease activity and extent, with the aid of intravenous contrast agents. Both are non-invasive methods, but CT is more widely available, less costly and less time consuming, but exposes patients to ionising radiation which hampers its serial use to measure disease activity⁸⁷. For these reasons, MRI is considered the standard imaging modality for assessing patients with IBD who require many follow-up examinations ⁵⁹.

To summarise, these investigative tests have been used for different purposes. To explain, Ileocolonoscopy, histology, and MRE are used for diagnosis while CRP, FCP, colonoscopy and MRE have been used for

monitoring. In addition, prognostic tests do not exist in the real world, and within the remit of this thesis . A drop in CRP or FCP is predictive of response to any drug, and not specific to biological therapy.

3.5.3 Clinical and MRI assessment of disease severity

Several symptom-based scoring systems have been developed for IBD activity monitoring in both research and in clinical practice. For CD, the main clinical indices are Harvey-Bradshaw Index (HBI) ⁸⁸, the Crohn's Disease Activity Index (CDAI) ⁸⁹, and the Perianal Crohn's Disease Activity Index (PDAI) ⁹⁰ and MRI scores such as magnetic resonance Index of Activity (MaRIA) ⁹¹⁻⁹³, Crohn's disease magnetic resonance imaging index (CDMI) ^{94, 95}, and the magnetic resonance enterography global score (MEGS)^{96, 97}, and Van Assche MRI-Based score for the severity of pCD^{98, 99} and biomarkers such as FCP and CRP, although this list is not meant to be exclusive. For UC, the main clinical indices are the Simple Clinical Colitis Activity Index (SCCAI), the Mayo Index and the Truelove and Witts' Severity Index TWC (for acute severe colitis ASUC), and radiological indices such as the segmental magnetic resonance score (MR-score-S), the magnetic resonance colonography simplified index (MRC-S), and the Nancy-DWI score^{98, 100}. Summary strengths and weaknesses of most useable clinical and MRI indices in IBD are depicted in Tables 3 &4.

Table 3: In clinical practice, CD and pCD activity evaluation indices ^{98, 99}

Name of index	Advantages	Disadvantages	ref.
clinical index			
CDAI	- used widely	- high variability - low contribution to the pCD total score. - complex calculation with a diary of 7 days	89
HBI	- well correlated to CDAI - simpler	- low contribution to the pCD total score - subjective score	88
PDAI	- use easily	- severity of fistulas not included - for perianal disease only	90
Biomarkers			
FCP	- useful for monitoring disease activity - well correlated to (Crohn's disease endoscopic index of severity) (CDEIS)	- lacks Sn and Sp for endoscopy disease activity (particularly ileal disease) - wide range of cutoff values for determining active versus inactive disease	74, 98

CRP	- helpful for monitoring disease activity	- modest correlation with endoscopic disease activity - lack of validated thresholds for active disease and remission	98
MRI index			
MaRIA is based on T1/T2.	- validated - good concordance between inter-observer and intra-observer - well correlated to endoscopy - index response - assess segmental and global diseases	- can best be used in clinical trials instead of clinical practice - Complex calculation - does not assess the whole small intestine - Need IV contrast injection	91-93
CDMI is based on T2.	- good interobserver variability - well correlated to CDEIS endoscopy score - Responsive index - easy to use	- does not assess the whole small intestine	94, 95

MEGS is based on T2 and T1 post-contrast sequences.	- validated against endoscopic score, CRP, FCP. - good agreement between interobservers - Responsive index - incorporates extra-intestinal damage - assesses the entire gut tract	- several assessed features	96, 97
Van Assche MRI-based score for severity of pCD	- good agreement between interobservers - simple to calculate - partially validated (against PDAI)	- utility may be limited in clinical practice because of complexity and does not correlate with clinical scores and does not change with treatment. - not fully validated (conflicting data between studies) - lack of validation of changes on T2WIs	101, 102
Magnetic resonance novel index for fistula imaging in CD (MAGNIFI-CD)	- internally validated against VAS	- no correlation between changes in the MR indices and changes in clinical measures of disease activity	103

Table 4: In clinical practice, UC assessment disease indices⁹⁸

Name of index	Advantages	Disadvantages	Ref
Clinical Indices			
SCCAI	- can be calculated by patient - feasible, responsive and reliable	- not validated	104
Partial Mayo Index	- used widely - discriminates remission from active disease	- relies on subjectivity physician global assessment	105, 106
TWC	- objective criteria for ASUC. - helpful for prognosis	- limited used	107
Biomarkers			
FCP	- useful for monitoring disease activity	- wide range of cut-off values for determining active versus inactive disease	98
CRP	- widely available - predictive of outcomes in acute severe colitis	- poor correlation with endoscopic disease activity - less useful in mild disease	98
MRI's score			
MR-score-S	- well correlated to endoscopy score - no bowel preparation necessary - Good reproducibility and interobserver agreement	- MRI cost and availability	100
MRC-S	- Easy to use	- MRI cost and availability	108

	- correlated to endoscopy disease activity	- Need for bowel preparation	
Nancy score	- No need bowel preparation or fasting for the assessment of colonic disease - easy to use grid scoring - responsive to change	- needs intravenous contrast injection	100

The disease activity score indices used in this thesis (HBI, CDMI for CD), Van Assche score and PDAI for pCD scoring system will be described in the following chapters.

3.6 Management

3.6.1 Goals of therapy and outcomes

The main aim of treating IBD patients is to achieve long term clinical remission, and mucosal healing¹⁰⁹, thus improving patient-related outcomes with respect to hospitalisation, surgery, and quality of life. The approach to therapy is initially similar in UC and CD, with surgery having a role in both diseases.

3.6.1.1 Medical Management

Several therapies can be used for the treatment of IBD and various factors should be considered when choosing the appropriate therapy such as, severity, the location of the disease, and the goal of therapy (maintenance or induction of remission)¹¹⁰. Determining the disease

category allows the clinician to decide on the appropriate therapy. Also, the purpose of treatment is not only to relieve IBD symptoms, but also to heal the mucosa, decrease complications, and to improve long-term outcomes. There are various categories of treatment to control the inflammation in order to allow the gut to heal such as aminosalicylates (5-ASA), corticosteroids, immunomodulators, biologic agents including anti-TNFs such as (adalimumab, infliximab, and golimumab), the Janus Kinase inhibitor (tofacitinib), and ustekinumab that is an antibody to the p40 subunit of IL12/23, and vedolizumab that is an antibody to alpha4 beta7 integrin, and exclusive enteral nutrition¹¹⁰.

Corticosteroids (steroids) can be beneficial for patients with moderate-to-severe IBD symptoms, and can help to reduce the inflammatory burden, and associated symptoms. Corticosteroids may be given orally and are, normally, administered for disease relapses. Corticosteroids should be used only short term to avoid secondary complications from the medication if used long term.

5-aminosalicylates drugs are more effective in UC than in CD, and are the cornerstone of remission in UC¹¹¹. This first-line treatment is usually used to cure mild-to-moderate colonic IBD symptoms. Patients with mild IBD may be treated and maintained with oral 5-aminosalicylates formulations such as mesalamine and sulfasalazine⁸⁶.

Maintenance therapy

Long-term maintenance is the standard care for continuing remission both in UC and CD and, therefore, adherence and on-going patient education to long-term therapy are recommended for all but the mildest of cases.

Immunomodulatory drugs, such as thiopurines, and methotrexate are nowadays effectively considered as first-line therapies in the management of CD. Patients who are intolerant or refractory to immunomodulatory agents can be treated with biological agents like anti-TNF therapy (Infliximab, NAPP pharmaceuticals, UK or Adalimumab, Abbvie, USA), and anti-integrin therapy (Vedolizumab, Takeda, Japan), and the recently licenced antibody to the p40 subunit of cytokines IL-12/23, Ustekinumab (Janssen Pharmaceuticals, Johnson and Johnson, USA). It is best practice to undertake a clinical assessment of disease activity using one or several the above-mentioned modalities in a timely manner, and whenever patients have symptoms¹¹².

CD with perianal involvement is predictive of a poorer disease outcome. Hence it is usually advocated to adopt a top-down approach in the management of this CD complication with an earlier use of surgery, and biological thereapy before instituting any medical therapy¹¹³.

3.6.1.2 Surgical management

For patients with refractory disease with an inflammatory phenotype that has failed to respond to medical therapy or with a complicated disease (stenosis or internal fistulae), a multidisciplinary discussion with the surgical team is usually advised to consult regarding the appropriateness of intestinal resection. Surgery may provide temporary relief of symptoms; however, recurrence rates are high and multiple surgeries are related to a risk of short-bowel syndrome, a condition characterised by significantly shorter gut, resulting in fluids, and nutrient malabsorption. Over half (50.8%) of CD patients experience a stricturing or penetrating disease complication after 20 years ¹¹⁴, with an associated 10-year surgical risk of 46.6% ¹¹⁵. In UC patients, 7.5% undergo a colectomy within five years of diagnosis; the majority among them within the first 2 years following diagnosis ¹¹⁶.

3.7 Perianal Crohn's disease (pCD)

pCD is a common complication in CD with up to 38% of patients affected¹¹⁷, and 30% of those experiencing recurring disease symptoms. Durable clinical remission rate for complex fistulas is only 37% at the end of a 10-year follow-up. Presence of fistula can lead to major morbidity due to cutaneous perianal abscess formation or drainage¹¹⁸. The impact of multiple surgical interventions, and costly medication on scarce healthcare resources is high.

3.7.1 Classification of pCD

Classification of pCD is essential in order to decide on an appropriate management approach. Fistulas can be divided into high or low fistulas depending on the relative position with the dentate line¹¹⁹. There are various other more detailed clinical classifications for perianal fistulas, but it is not the intention of this thesis to discuss these, extensively. The main classifications will be briefly discussed.

3.7.1.1 Parks Classification

The Parks Classification system describes perianal fistulas based on the relationship between a primary fistula tract to the anal sphincter complex. There are five types of perianal fistula based on relationship of the fistula to the sphincter muscles: superficial, inter-sphincteric, trans-sphincteric, supra-sphincteric, and extra-sphincteric, as seen in Figure 20

120.

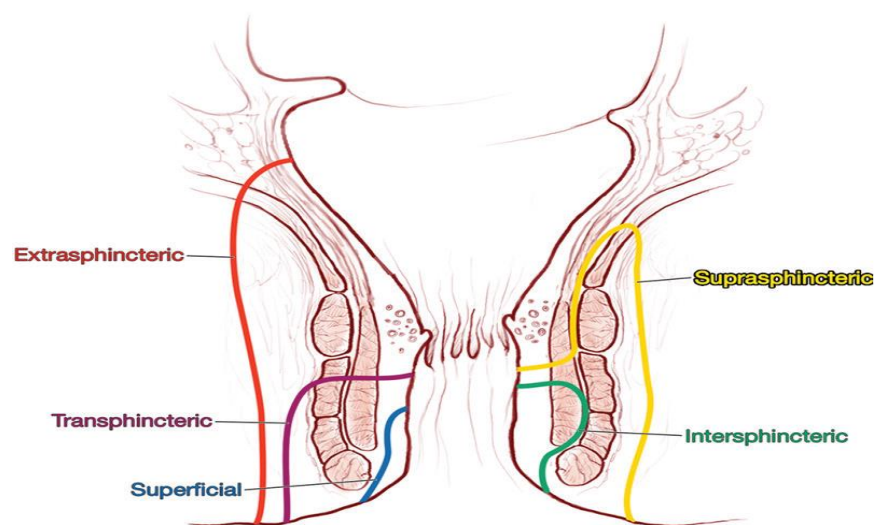


Figure 20: Summary of Parks classification perianal types¹²⁰.

The American Gastroenterological Association (AGA)¹²¹ in 2003, classified perianal fistula with CD into two types that are complex and simple fistula.

Complex fistula passes through or above a significant amount of muscle (either high extra or supra-sphincteric, high inter-sphincteric, or high trans-sphincteric) may have many external openings. It is also associated with an anorectal stricture, complicating abscess, that involves the urogenital tract. In contrast, simple fistulas are low (low intra-sphincteric, low inter-sphincteric, superficial), does not have an associated abscess and has a single opening in the skin ¹²².

3.7.2 Treatment of perianal fistulas

Perianal fistula management requires the availability of expert imaging techniques and a thorough understanding of anatomical and pathological factors. Since the introduction of biologicals, treatment of perianal fistula has changed to more non-surgical therapy. The complete fistula tract closure can be achieved by medical treatment or by surgical approach. The treatment of perianal fistulas is difficult and always multidisciplinary. The European guidelines recommend combined surgical and medical treatment with biological drugs to achieve best results.

Medical treatment

Medical therapy administration in pCD is intended to reduce symptoms, induce fistula closure, treat or prevent complications, and improve the quality of life. Several medical options are available. Symptom

reduction can be achieved through medical therapy, but definite fistula closure is rare, and the overall rate of recurrence, unfortunately, high. With anti-TNF agents (adalimumab and infliximab), pCD treatment has been changed from almost entirely surgical to a much wider focus using medical therapy.

Surgical treatment

The majority of pCD patients require surgical therapy during their disease. The main aims of this treatment are abscess drainage, and recurring abscess prevention via the remaining patency of the tract. Fistula closure and continuity preservation are much more important in the long term. Depending on the type of fistula, and its characteristics several surgical approaches are available. Examples of surgical technique include seton and advancement flaps. Surgical treatment should be considered for patients with low, simple, fistulas refractory to medical treatment and for those with disabling or severe symptoms.

Chapter four: The application of MT, DWI and DCE in pCD and CD

This section will present a review of the literature relating to MRI used in CD and pCD, and the use of MT, DWI and DCE. First, an overview of MRE will be briefly discussed.

4.1 Magnetic Resonance Enterography

MRE has become the primary imaging approach in the evaluation of IBD in both children and adults. This modality combines large volume oral contrast distention of the bowel with T2-WI, SSFP, and multi-phase T1-WI fat-suppressed contrast sequences, which are used to maximize bowel wall abnormality detection. Preparations for oral contrast differ among various organisations, however, they are usually biphasic.

Biphasic agents have a high intensity on T2W images and low signal intensity on T1W images. The low signal on T1W images allows better appreciation of contrast enhancement, while the high signal on T2W images facilitates the delineation of the bowel wall. Some examples of the most common biphasic oral contrast agents are dilute barium sulphate, mannitol (2.5%) with locust bean gum (0.2%), sorbitol, polyethylene glycol and water¹²³. Water by itself does not provide adequate distension because of its hypo-osmolarity, leading to reabsorption after oral uptake. To prevent reabsorption, ingredients such as mannitol and sorbitol or other polysaccharides should be added to the water in order to prevent reabsorption¹²⁴. There is some evidence that stated that the best bowel distention is provided by a combination of mannitol and locust bean gum¹²⁵,¹²⁶ but all of those agents can cause hyperosmolar diarrhoea.

Various ingestion schemes are outlined, but commonly used volumes range from 1,000 to 2,000 mL, consumed steadily over 40 to 60 minutes before scanning¹²⁷. Increasing volumes over 1500 ml causes a significant increase in side effects from diarrhoea to abdominal cramping with no potential diagnostic benefits^{125, 128}. Sufficient distension of the small bowel is important since collapsed loops may hide or mimic disease or lesions by indicating a thickened bowel wall¹²⁹.

The position of the patient also varies according to the institution, where many institutions favour MRE in the supine position for patient comfort, while others prefer a prone positioning to decrease scan time, and to compress the bowels¹²³.

MRE imaging (3T vs 1.5T).

Recently, using high-field MRI (3T) has been increasingly using in clinical practice with increasing availability in many centres though 1.5T MRI platforms are still the most common available modality in different healthcare systems. Most MRE studies have been performed using 1.5 Tesla. A high field strength imaging method such as 3T provides advantages that are of relevance in bowel scanning. In theory, 3T MRI higher signal-to-noise, (SNR) is double that of the 1.5T¹³⁰ which may translate into reducing the scan time, and improving the spatial resolution by changing other parameters, such as, in the reduction of voxel size. In 3T sequences, this increased SNR can be used to increase temporal resolution. Also, contrast-to-noise ratio (CNR) is increased at 3T compared

with 1.5T, which improves structure and lesions conspicuously, as well as, by using a lower dose of gadolinium²⁴. Feasibility of MRE on 3T with high image quality performance has been demonstrated in several studies^{131, 132} that revealed a strong correlation between MR image findings and endoscopic activity in terminal ileitis¹³³ and ileocolonic disease⁹². However, the installation of a 3T has a high initial cost and maintenance, which can be nearly twice as high as 1.5T.

In 3T MR abdominal imaging challenges results relating to increasing energy deposition and increasing artefacts¹³⁰. Different artefacts can be commonly recognized in 3T, such as, susceptibility, chemical shift and B₁-inhomogeneity (dielectric effect or standing wave artefact). By using receive only surface coils instead of transmit-receive body coils and parallel imaging, some of these challenges can be mitigated, and the use of multi-transmit coils, in addition, can reduce the specific absorption rate (SAR.) For other specific artefacts, there are also specific solutions involving adjusting the sequence parameters and using dielectric pads ²⁴

4.2 MRI Assessment of luminal and pCD activity

4.2.1 Luminal CD

Before instituting medical management in a patient, it is important to evaluate the inflammatory component of the disease. Unlike inflammatory disease behaviour where medical treatment is the most cost-effective first-line therapy, fibrostenotic disease is usually unresponsive to medical therapy and is best treated by intestinal resection or strictureplasty ¹³⁴. The

evaluation of disease activity is essential in patients with proven IBD to assess therapy adequacy and the future need for therapy modifications.

CD can present with intra-abdominal abscesses or phlegmons (inflammatory mass). As abscesses contain air and fluid, it appears as a rim-enhancement lesion. The presence of these lesions is a stark marker of disease severity⁵⁹. The accuracy of contrast-enhanced MR assessment of fistulising disease is high, with an Sn and Sp ranging from 71% to 100%, and from 93% to 100% respectively ¹³⁵.

Progressive deposition of submucosal fibrosis or thickening of the smooth muscle layer¹³⁶ leads to intestinal stricturing disease. Strictures are known as luminal narrowing in the area of CD with immediate upstream bowel dilatation ¹³⁷. Acute severe inflammation of the intestines wall may cause a reduction of the luminal diameter due to inflammatory cell infiltration and mural oedema. MR imaging can detect disease activity within a stricture. Relatively high and early hyper-enhancement after contrast administrations, ulcerations, blurred margins of the wall, mural oedema, presence of inflammatory peri-enteric changes or the 'comb sign', indicate the presence of a predominant inflammatory component. Whereas, delayed and low enhancement indicates the presence of predominant fibrosis as the cause of a stricture ¹³⁸⁻¹⁴⁰. MRI Sp and Sn for detection of stenosis range from 91% to 100% and 75% to 100% respectively¹³⁹.

There have been numerous attempts to develop scoring systems for less invasive but informative quantification of the activity of CD, including MaRIA, CDMI, MEGS, and Clermont scores^{91, 93, 94, 108, 141}. The MaRIA score is presently the only validated radiological CD activity index tested for large populations of patients. Rimola *et al.* developed an MRE-based index for quantification of CD activity. They⁹² studied 50 patients with CD undergoing MRI and a reference standard (ileocolonoscopy), with the main objective of examining disease extension and activity in the treatment of CD. The underlying objective was the determination of the diagnostic accuracy of MRI in the management of CD. In their study, the researchers recruited both clinically active (n=35) and inactive patients (n=15). They used T2WI and pre and post contrast-enhanced T1-WI sequences, to evaluate the endoscopic activity using the Crohn's Disease Index of Severity (CDEIS). Also, they classified the endoscopic lesions for absence, presence and severity of lesions, noting whether they were mild or severe, indicating inflammation without ulcers or presence of ulceration respectively.

By comparing intestinal segments with various degrees of inflammation (severe, mild or absent), the authors found that a progressive and significant ($P < 0.001$) increase in the following MRI parameters: relative contrast enhancement, wall thickness, post-contrast wall intensity, presence of lymph node enlargement, oedema, pseudopolyps, and ulcers. Wall thickness ($P = 0.0007$) together with relative contrast enhancement ($P = 0.001$), presence of ulcers at MR ($P = 0.003$), and presence of oedema ($P = 0.002$). were independent predictors of disease activity as measured

through CDEIS, A significant correlation between the MR index and CDEIS of segments was observed ($P<0.001$, $r=0.82$).

The MaRIA score was used to radiologically quantify disease activity⁹². This score measures wall signal intensity and wall thickening, relative contrast enhancement, presence of ulcers, lymphadenopathy, oedema, and pseudopolyps⁹². The following formula was used to calculate the MaRIA score: = [Wall Thickness (mm)*1.5] + [Relative Contrast Enhancement*0.02] + [Oedema*5] + [Ulcers*10].

The researchers concluded that MRI was accurate in detecting disease activity, and assessing its severity, which was an indication of possible usage as an alternative to endoscopy in the evaluation of ileocolonic CD.

Rimola and colleagues have identified cut-off points for disease severity in external validation of their previous study⁹¹. Severe disease was defined as superficial or deep ulcerations at endoscopy, and active disease was defined as endoscopic lesions of any severity. The cut-off value for the MaRIA score for active and severe disease was (≥ 7 and ≥ 11) respectively⁹¹.

In a prospective study⁹³, the accuracy of MRE in assessing response to therapy and mucosal healing in patients with CD was assessed, with the aim of monitoring response to therapy. In this study, ileocolonoscopy was

used as a standard reference and included 38 patients who had active CD and ulcers in at least one ileocolonic segment. All the patients recruited in the study had undergone MRE and ileocolonoscopy at baseline, and had undergone a further 12 weeks upon completion of the corticosteroids or anti-TNF⁹³. Ulcer healing was determined by MRE and endoscopic remission with an accuracy of 90% and 83% respectively⁹³. At week 12, the mean MaRIA and CDEIS scores significantly changed in segments with ulcer healing (MaRIA at baseline (18.86 ± 9.50) vs at week 12; 8.73 ± 5.88 ; ($p=0.002$) and CDEIS at baseline: 21.28 ± 9.10 against at week 12, 2.73 ± 4.12 ($p<0.001$). The conclusion was that MaRIA was a suitable, reliable and responsive index that assessed response to therapy in patients suffering from CD⁹³.

The MaRIA score has shown a high response to therapy changes, and is considered a reliable instrument for assessing therapy response. In a similar study, Stoppino *et al.*¹⁴², have also assessed MaRIA's accuracy and its correlation with the simple endoscopic activity score for CD (SES-CD) in treatment with anti-TNF therapy. The MaRIA values before and after therapy have changed significantly, and have been significantly correlated with the endoscopic score¹⁴².

Rimola *et al.*¹⁴³ further studied the characterisation of fibrosis and inflammation in CD lesions by MRI. In this study, 41 patients with CD were included, with the researcher's objective being to characterise MR findings associated with the activity of inflammation and fibrosis while relying on

dynamic and conventional sequences. Standard reference was histology analysis, and pathological macroscopic study of surgically resected *ex-vivo* intestinal lesions.

Upon examining the 44 segments, it was apparent that inflammation and fibrosis correlated significantly with different MRI parameters. The pathological intensity of inflammation was correlated with MRI variables: mucosal enhancement, hyper-signal on T2, blurred margins and ulcerations . The histological degree of fibrosis correlated with the presence of stenoses ($P=0.05$), the percentage of enhancement of gain, and the pattern of delayed enhancement at seven minutes. The ability of the MRI to discriminate between severe and mild-moderate fibrosis deposition had a Sn and a Sp 0.94 and 0.89 respectively, although this diagnostic accuracy relies on the percentage of enhancement gain. The MRI's accuracy to detect the presence of severe fibrosis in CD lesions depended on the enhancement pattern¹⁴³. These studies showed a strong agreement relating to CD activity and treatment between MaRIA and endoscopic indices.

The findings from the previous study were revalidated in this multicentre prospective cohort study in which 51 CD patients with intestinal stenosis were evaluated with MRE sequences (T1 with gadolinium, MTR and DWI) for the detection of histological fibrosis prior to intestinal resection. ADC was assessed as an exploratory objective and MTR as a secondary endpoint. It was found that there was no association of inflammation or fibrosis with either MTR or delayed gain of enhancement, which was in

contrast to previously published findings ¹⁴³. There was a good correlation between the MaRIA score and the inflammation score. A negative weak correlation between ADC and fibrosis was observed ($r=-0.36$, $p=0.011$). The MaRIA score was in good agreement with inflammation scoring¹⁴⁴.

A simplified version of the pre-existing MaRIA, referred to as simplified MaRIA (sMaRIA), has recently been developed by Ordás *et al.*¹⁴⁵ to address the practical limitations of the original score. sMaRIA is a categorical 4-variable score : three radiological elements from the original score (mucosal ulcerations, mural edema and mural thickening), and a new element (fat stranding, defined as the loss of the normal sharp interface between mesentery and the intestinal wall, with fluid/ edema in the perienteric fat) was added. In a retrospective study researchers used data from 98 participants using the CDEIS as standard reference. They found that a strong correlation between the original MaRIA and MaRIAs ($r = 0.93$, $p < 0.001$) and between MaRIAs and CDEIS ($r = 0.83$) were observed. Moreover, inter-observer agreement between experienced MRI radiologists for simplified score was excellent (ICC= 0.85; 95% CI: 0.78–0.90; $p < 0.001$).

CDMI is another MRE score used for identifying active disease⁹⁴. In this study, the investigators use postoperative histology as a reference standard. Using a resected segment postoperative MRE, the location of the specimens was assigned to MRE prior to surgery. The MRE was measured by using the following criteria: lymph nodes and lymph node enhancement, mural enhancement pattern, degree of enhancement, peri mural T2 signal,

mural T2 signal and mural thickness. In a multivariable and backward selection model only the mural T2 signal and mural thickness showed correlation, consequently, the final formula included the following parameters: 'CDMI = 1.79 + 1.34 x Mural Thickness Score + 0.94 x Mural T2 Score'. The two additional parameters, perimural T2 signal and contrast enhancement revealed only a univariate correlation to an acute inflammation score⁹⁴. In this study qualitative scoring (0–3) for the peri mural T2 signal, mural enhancement, mural T2 signal and mural thickness were all correlated with disease activity based on a histopathological grading system, for reference. There was a significant correlation between the acute inflammation score and the MR index.

The CDMI was modified in 2014 by Makanyanga *et al.*⁹⁶ to include pathological loop length, colonic haustra loss and the presence of extra-enteric complications, such as, fistulae, abscesses and enlarged lymph nodes. This new score, referred to as MEGS was developed to better reflect the overall disease burden and to quantify disease activity. The final MEGS score divided the gastrointestinal tract into nine segments, and each segment was scored independently. The authors prospectively evaluated and enrolled 71 patients with CD by MRE, by comparing clinical and laboratory biomarkers of activity with MEGS. They found that the MEGS score significantly correlated with CRP ($r = 0.39$, $P = 0.002$) and FCP ($r = 0.46$, $P < 0.001$). However, no significant correlation was found between HBI and MEGS was observed. The MEGS model had an Sn of 65% and a Sp of 78% for the detection of active disease.

The ability of MEGS scores to characterise CD response to anti-TNF- α therapy has also been assessed. Clinical indices were used as a standard reference. At the time of MRE physicians' global assessment was assigned to define disease activity (remission, mild, moderate or severe). A clinical responder was determined when a patient's clinical status improved by at least one rank along the scale of disease activity. Investigators found that MEGS scores changed significantly in clinical responders, but not in non-responders, and a moderate correlation with clinical activities has been observed⁹⁷.

Another validated score is the Clermont score or DWI-MaRIA, which incorporates DWI and ADC measurements, and was developed by Buisson *et al*¹⁴⁶. The only segment to be assessed was the ileum. The Clermont score, represents a DWI-based index for the quantification of disease activity, and was developed by using the MaRIA score as a reference standard to combine conventional MRE parameters derived from the MaRIA index. External validation of the Clermont score was later performed by the same group¹⁴⁷ in a prospective observational study of 130 patients that compared the Clermont score and the MaRIA for CD inflammation assessment. In this study, bowel loops were divided into seven parts. The radiologists evaluated the following findings for each bowel segment in conventional sequences: presence of mucosal ulceration, presence of mucosal oedema, wall thickness, presence of abscesses and fistula, enlarged regional mesenteric lymph nodes, and proliferation of fibrofatty acids. They found that the MaRIA was highly correlated with the Clermont

score in ileal CD ($p=0.99$) but not in colonic CD ($p < 0.8$). Evidence is accumulating on the utility of MRI scores in detecting active inflammation presented in Table 5.

Table 5: Assessing Crohn's disease activity with different MRI scores. Pros=prospective and Retro= Retrospective studies.

Study	No.of patients	Standard Reference	MRE Index	Result
Rimola <i>et al</i> ⁹²	50 Pros.	ileocolonoscopy	MaRIA	Strong correlation between MaRIA and the CDEIS (($r = 0.78$) HBS ($R=0.78$) and CRP($r=0.53$) Sn 81% , Sp 89% , AUC 0.91
Rimola <i>et al</i> ¹⁴⁸	48 Pros.	Ileocolonoscopy	MaRIA	Significant correlation between endoscopic severity index and MRI index $r=0.84$, $p<0.001$ Distinction between mild, moderate and severe Sn 92% and Sp 92%
Ordás <i>et al</i> ¹⁴⁵	98 Retro.	Ileocolonoscopy CDEIS	Simplified MaRIA	Significant correlation between sMaRIA scores with CDEIS scores ($r = 0.83$) and with original MARIA scores (and $r = 0.93$) ($P < .001$) Sn 85% , Sp 92% , AUC 0.94

Steward <i>et al</i> ⁹⁴	16 Retro.	Pathology	CDMI	Significant correlation between the acute inflammation score and the CDM (r=0.80) Sn 81%, Sp 70%, AUC 0.77
Makanyanga <i>et al</i> ⁹⁶	71 Pros.	HBI, CRP, FCP	MEGS	correlations between MEGS and the reference standards were: with the HBS: r = 0.102; with FCP: r = 0.46 with CRP: r = 0.388.
Bussion <i>et al</i> ¹⁴⁹	44 Pros.	Ileocolonoscopy; SES-CD and CDEIS	Clermont and MaRIA scores	Correlation between Clermont and ileal SES-CD; r = 0.58, and CDEIS; r = 0.63. Correlation between ileal segmental MaRIA and SES-CD; r = 0.61, and CDEIS; r = 0.67. A score >18.9 detected ulcerations with a Sn of 79% and Sp of a 73%.

Hordonneau <i>et al</i> ¹⁴⁷	130 Pros.	MaRIA	Clermont	Excellent correlation between Clermont score and MaRIA in distal ileum (r=0.99) Good Sn 86% and Sp 82% of ADC cutoff 1.9* 10 ⁻³ mm ² for differentiating active disease .
Ordas <i>et al</i> ⁹³	48 Pros.	Colonoscopy CDEIS	MaRIA	Total MaRIA < 50; 83% Sn, 84% Sp, AUC 0.864 for mucosal healing. Total MaRIA < 50; 75% Sn, 80% Sp, AUC 0.833 for ulcer healing Significant correlation between MaRIA and CDEIS (r = 0.81, p< 0.001
Prezzi <i>et al</i> ⁹⁷	36 Pros.	Clinical activity	MEGS	MEGS correlated significantly with clinical activity (r = 0.53; P < 0.001).

4.2.2 Perianal Crohn's Disease

To date, it is very difficult to quantify inflammation in patients with pCD due to the absence of reliable disease activity measures. Optimising therapies for pCD is of prime importance, and may have a major impact on quality of life. Lack of validated measures for evaluating disease activity and

therapy response is a barrier to efficient drug development for pCD. Pelvic MRI is a gold standard scan for assessment of perianal disease, providing high contrast resolution, and details of the sphincter, and perianal anatomy to better guide decision-making ¹⁵⁰.

A T2WI with fat-suppression sequence is the currently recommended technique for MR fistula imaging. With the use of T2WI, it is important to recognise the fluid content in fistula tracts while gadolinium enhanced T1W images are useful for the distinction between granulation tissues and pus within the fistula tract and inflammatory masses. Generally, no sedation, oral contrast or bowel preparation is needed, with intravenous (IV) injection or infusion administration, if there is no contraindication¹⁵¹. MRI modality has the ability to demonstrate uncharacterised disease extensions together with abscess formation, which may necessitate surgical intervention¹⁵¹.

Many clinical measurements have been investigated to quantify pCD activity and response to therapy such as PDAI and Fistula Drainage Assessment (FDA). The PDAI cut-off was suggested as > 4 for active pCD, resulting in an accuracy of 87% when using clinical evaluation [signs of local inflammation and/or presence of drainage] as a reference. However, despite ongoing fistula drainage, 10% of patients were falsely classified as having a low PDAI. It is insensitive to change after medical therapy, and its remission cut-off has never been properly determined ¹⁵².

The Van Assche index was originally developed to meet the need for a tool for measuring a pCD response to medical treatment. This score provides the combined information of anatomical fistula description (relation to the sphincters, supralavator extension, complexity), and identifies inflammatory activity (T2 hypersensitivity in MR images, presence of abscesses/cavities and rectal-wall thickening). The total score ranging from 0 to 22, with weighting scores exerted to all parameters, with lower scores assigned to the anatomical parameters compared with the inflammatory parameters as seen in Table 6. The score is insensitive to clinical response, with no significant difference shown in clinical responders to anti-TNF therapy before, and 6 weeks after treatment, nor is there a difference in the score between clinical responders and non-responders¹⁵³.

Van Assche *et al.* used this score in their prospective cohort study. The objective was to assess the behaviour of perianal fistulas in 18 patients before and after anti-TNF (Infliximab), using MRI. The most important parameters of inflammation activity in their scoring were noted to be in the T2 hyperintensity signal.

Table 6: MRI-Based Score of Severity of pCD according to Van Assche *et al.* scoring.

Description	scoring (maximum= 22)
Complexity of fistula tracks	
None	0
Unbranched, single	1
Branched, single	2
Multiple	3
Site	
Inter/extra-sphincteric	1
Trans-sphincteric	2
Supra-sphincteric	3
Extension	
Infralevatoric	1
Supralevatoric	2
Hyper signal intensity on T2-WI	
Absent	0
Mild	4
Pronounced	8
Collections (cavities >3 mm	
Absent	0
Present	4
Rectal wall involvement	
Normal	0
Thickened	2

In this study, fistula tracks were assessed with good inter-observer concordance ($P < 0.001$). The fistula tracks with signs of active inflammation were found at baseline in all 18 patients, and collection in seven. The average MRI score at week 0 in all patients was 14.5 ± 4.6 . The baseline scores in subsequent clinical responders were not significantly different from subsequent non-clinical responders. At the end of the study after 46 weeks, the most sensitive MRI variables that predicted a clinical response were qualitative T2 hyperintensity rather than anatomical variables. In clinical responders, fistula localisation and extension did not change after 46 weeks of therapy while fistula numbers dropped non-significantly (-0.03 ± 0.3). T2 hyperintensity dropped in the majority (8/11) of the responders(-

3.2 $\pm$ 1.9), while cavities (-0.8 $\pm$ 1.9) and rectal wall involvement did not change significantly (-0.8 $\pm$ 1.0). These changes indicate that the inflammatory rather than the anatomical components of the Van Assche score are predictors of a subsequent clinical response.

Savoye-Collet *et al.*¹⁵⁴ aimed to correlate MRI changes to clinical outcomes in pCD after anti-TNF therapy for one year while, simultaneously, validating the Van Assche score. The authors observed remission in 35% of cases and a response in 40% of cases. In patients with response or remission, the Van Assche score varied from 13.8 at baseline to 6.13 after 12 months of therapy ($p < 0.05$). There was a significant decrease in T2 hyper-intensity follow-up value in patients in remission ($P < 0.01$). In clinical remission or response, T2 hyperintensity value decreased or disappeared in 14 out of 15 respondents, as compared with 1 amongst the 5 non-responding patients ($p < 0.01$). The decrease in T2 hyper-intensity value, and in comparison, with the Van Assche score were not significantly different between patients in remission and response. In clinical remission, only one patient showed persistent contrast enhancement on MR, while all other non-remission patients showed a persisting contrast enhancement ($p = 0.002$). The authors concluded that change in T2 hyper-intensity value is the most valuable factor associated with clinical benefits of maintenance anti-TNF- α therapy in pCD.

Another study assessed the response of 12 patients with pCD to three infliximab infusions over six weeks by MRI ¹⁵⁵. MRI was rated unchanged,

worsened or better, and evaluated for fistula tracks and abscesses. All the patients underwent MRI twice, at two weeks before treatment and at 14 weeks after treatment for comparison. Abscesses were detected by pre-treatment MRI in three of the 12 patients (two were not treated). Of the ten patients treated, three had abdominal wall enterocutaneous fistulae and seven had perianal fistulae (two also had rectovaginal fistulae). At week 14 (after treatment) five patients were deemed non-responders, one had responded and four were in remission. All patients' fistula tracks with active inflammation were reported in MRI pre-treatment. MRI was worse in two, unchanged in two, and improved in six patients (three perianal, two abdominal walls, one rectovaginal + perianal). MRI and the clinical outcome correlated in seven of the ten patients while in three (two enterocutaneous and one perianal), discordance was noted. The authors concluded that full resolution of internal fistulous tracks did not happen in response to short-period therapy, despite external orifices being healed¹⁵⁵. Limited conclusions can be gleaned from this study with such subjective endpoints.

Another observational cohort study used clinical assessment and the Van Assche score to evaluate pCD in 59 patients from baseline to post 56 weeks of treatment (infliximab)¹⁵⁶. Patients were assessed by MRI, and clinically at baseline, after which they were treated with Infliximab. Patients were then evaluated clinically and using MR in the short (10–12 weeks from baseline), medium (26–52 weeks) and long term (beyond 56 weeks) to investigate any concordance between clinical findings and MRI examinations. Compared with the MRI at baseline, the short-period MRI

evaluation demonstrated a decrease in the number of fistula tracks in 14% of patients and inflammatory activity in 55% of patients. Mid- and long-term MRI showed a reduced number of fistula and inflammatory activity in 56% and 52% and 15.4% and 31% of patients, respectively. There was an improvement of pCD shown in the MRI findings which concurred with clinical improvement in 55 of patients. In the long-term MRI activity score, no change was shown, whereas a significant decrease was seen in both short-term and mid-term activity. In the final MRI, patients with persisting fistula had a worse therapy outcome ($p=0.01$), rectal wall involvement ($p=0.01$) and collections ($p=0.009$). Patients with collections ($p=0.006$), and branched or single fistulas in their MRI at baseline were more likely to have surgery. The authors concluded that in long-term follow-up, the improvement identified in MRI correlated with the endoscopic and clinical response to infliximab in only 50% of patients.

In a prospective cohort, an interventional study of 34 patients with pCD, Ng *et al.* aimed to monitor deep healing as indicated by MRI and to determine the extent and rate of healing to ascertain treatment therapy. In this study, MRI scans were repeated at different times (at 6, 12, and 18 months) during scheduled anti-TNF therapy, thalidomide($n=8$), adalimumab($n=19$), and infliximab ($n=7$) to investigate fistula tract healing. Clinical remission at one year was reported in 29% of adalimumab-treated patients, and 53% of infliximab-treated patients. The authors reported that MRI healing occurred in 28% of patients who were treated with TNF therapy. The consecutive patterns of initial hyper intensity on T2 were observed, with

an initial attenuation in intensity followed by complete loss of hyperintensity. In some patients, there was complete healing with no more fistula MRI evidence with a Van Assche score of 0. The average score of the baseline MRI was 15 ± 0.9 (n=25). Since most patients responded clinically, the baseline score did not predict clinical improvement. The Van Assche scores at 6,12, and 18 months were 12 ± 1.1 , 10 ± 1.4 , and 10 ± 1.4 , respectively. In their study, the complexity of fistula and length of its history did not affect healing. The clinical benefits of anti-TNF treatment were influenced by proctitis; remission or response was reported in 60% with proctitis as opposed to 89% of those without ($p=0.054$)¹⁵⁷.

In a subsequent small study, Horsthuis *et al.* studied 16 patients, to assess the MRI-based score of disease activity for pCD. Apart from the original Van Assche score, components in the Horsthuis *et al.* study included an assessment to the potential measure of inflammatory activity of T1-WI post gadolinium hyperintensity, and the presence of an infiltrate. The researchers concluded that T1-WI gadolinium-enhanced images did not change significantly before or after treatment. No correlation was found between these MR-based measures of fistula activity and other CRP, but there was a weak correlation between MRI based score and also modified with PDAI scores ($r=0.371$, $P=.036$), ($r=0.394$, $P=0.026$), respectively¹⁰².

Shenoy-Bhangle *et al.*¹⁵⁸ have recently published a study of MRI prediction of treatment response in a paediatric population with pCD, which

identified fistula length as the main predictor of response in the pelvic MR follow-up.

The Van Assche score was recently modified by an expert panel to improve the responsiveness to therapy and inter- and intra-rater reliability¹⁵⁹. Certain items of scoring representing disease activity have been modified, and several items have been added to the initial Van Assche score. They assessed both scores by expert consensus, which were considered essential to determine the activity of perianal fistula. Four radiologists have evaluated identical items in the original index, modified items in the original index, and added new items. The original Van Assche items (in Table 6) were all retained, but they modified some of those items to include the addition of “increased signal intensity” to the rectal wall involvement area, inclusion of extensions in addition to the primary tract in the evaluation of the hyperintensity of T2-WI, added of “horseshoe configuration” to the extension part, and an addition of “submucosal” to the location component, as well as separate categories for inter and extra-sphincteric parts were created. Also, the collections’ descriptor was modified and incorporated into a new descriptor Inflammatory mass. Additional descriptors were added to the Van Assche score: including an evaluation of the dominant feature of the primary tract and extensions; hyper-intensity of the primary tract or extensions on post-contrast fat-saturated T1-weighted images; hyper-intensity of the primary tract or extensions on post-contrast, fat-saturated T1-WIs; presence of an inflammatory mass in conjunction with the assessment of size of collections;

and the presence of an anovaginal/ recto tract. Those nine descriptors were assessed by using a mixed effect approach to choose the descriptors that best represented the severity of the disease. A total of five descriptors to create the modified Van Assche score were selected (Table 7). The final scores were referenced to 100 mm visual analogue scales (VAS), with readers assessing the severity of perianal disease (none to severe active inflammation), complexity (minimum to highly complex), and global assessment, the reference standard for perianal disease.

The authors found that the intra-rater, and interrater reliability between the modified and original versions did not change significantly despite modifications. Moreover, intraclass correlation coefficients (ICCs) were nearly identical in both modified and original indexes (0.67 and 0.66 respectively).

Table 7: Represents the items of Original and Modified Van Assche scores.

Original Van Assche index		Modified Van Assche index	
Description	Score	Item	Score
The complexity of fistula tracks		Extension	
None	0	Absent	0
Unbranched, single	1	Infralevatoric	1
Branched, single	2	Horseshoe configuration	2
Multiple	3	Supralevatoric	3
Hyperintensity on T2-WI		Hyperintensity on T2-WI	
Absent	0	Absent	0
Mild	4	Mild	1
Pronounced	8	Pronounced	2
Rectal wall involvement		Rectal wall involvement	
Normal	0	Normal	0
Thickened	2	Thickened	1
Site		Increased signal intensity	2
Inter/extra-sphincteric	1	Inflammatory mass	
Trans-sphincteric	2	Absent	0
Supra-sphincteric	3	Diffuse	1
Collections		Focal	2
Absent	0	Small collection	3
Present	4	Small collection(3–10 mm)	4
Extension		Median collection(11–20 mm)	5
Infralevatoric	1	Large collection(>20 mm)	
Supralevatoric	2	Predominantly Fibrous	0
		Predominantly Granulation tissue	1
		Predominantly Pus/fluid	2

This modified score was used in a retrospective study to evaluate clinical responses to anti-TNF therapy in 25 patients with pCD¹⁶⁰. They used all nine descriptors that were used in the development of the modified Van Assche index¹⁵⁹(mentioned earlier), and the original Van Assche score to

compare MRI scores in responder and non-responder patients. Clinical response was evaluated at 12 months after the follow-up MRI, and defined as a reduction in external openings and /or reduced or absent fistula drainage. They found that the median-modified Van Assche in the responder group at baseline was 9.6 and 5.8 in the follow-up ($p = 0.008$). In the non-responder group, the median score was 7.7 at baseline, and 8.2 in the follow-up ($p = 0.624$). Clinical responders had a median original Van Assche score of 13 before treatment, and 9.5 after treatment ($p=0.01$). In non-responders ($n = 14$), corresponding scores were 11.5 and 11.5 ($p = 0.32$).

The same team developed a new index known as MAGNIFI-CD to assess MRI data, and to determine pCD activity with improved operating properties in comparison with previous indexes. In this study, researchers assessed 160 MRI scans collected at baseline, and at 24 weeks during a phase 3, randomized, clinical trial of a local injectable stem cell therapy (darvadstrocel [formerly Cx601]) for pCD. Longitudinal validity was, firstly, evaluated by estimating the coefficients of the correlations, and their 95% CIs among change scores for the MR indices, the visual analogue scale and other disease activity indices such as CRP, PDAI. They found that the MAGNIFI-CD had improved operating characteristics compared with the original and modified Van Assche score. Estimation of ICC for MAGNIFI-CD, the Van Assche score and the modified Van Assche score were 0.85 (95% confidence interval [CI], 0.77–0.90), 0.81 (95% CI, 0.71–0.86), and 0.81 (95% CI, 0.74–0.86) for intrarater reliability, and 0.74 (95% CI, 0.63–

0.80), 0.68 (95% CI, 0.56–0.77), and 0.67 (95% CI, 0.55–0.75) for interrater reliability¹⁰³. Table 8 illustrates the most common MRI scores used to determine pCD activity.

In these studies, it has been shown that changes in T2-hypersensitivity rather than in T1 enhancement or anatomical changes seems to be the best predictor of a clinical response with most changes observed in the short to medium term, up to 12 months. The latter phenomenon might be more attributable to the refractory nature of pCD rather than to a lack of sensitivity of MR to measure disease activity at certain time points. Indeed, another limitation of these studies is the fact that clinical response has been used as a blunt endpoint in studies investigating the predictive values of MR measures however, these are more sensitive instruments. pCD study endpoints should be MR based with radiological improvement of disease linked to patient-reported outcome measures and important long-term outcomes in downstream clinical utility studies.

Table 8: Shows the most common MRI score to determine pCD disease activity.

Study	No. of patient	Characterisation/	Index Name	Reference Standard	Result
Van Assche <i>et al</i> ¹⁰¹	18	MRI for monitoring and grading pCD. Severity and therapeutic response	Van Assche	Inter-rater agreement	Response (decrease in the VAI) more closely parallels change in inflammatory than anatomic features (eg, loss of fistula T2 hyperintensity)
Samaan <i>et al</i> ¹⁵⁹	50	Attempt to improve the original Van Assche score	Modified Van Assche	VAS	Modified score had higher ICCs for intra-and inter-rater reliability compared to the VAS and original score.
Hindryckx <i>et al</i> ¹⁰³	160	Fistulizing CD activity and response	MANIFI-CD	Activity: ICC MR indices, VAS; Response: VAS, PDAI, CDAI, CRP	The MAGNIFI-CD improved operating characteristics compared with the original and modified versions with intra-rater reliability nearly perfect at 0.85 vs 0.68 and 0.67 and substantial inter-rater reliability at 0.75 vs 0.68 and 0.67, respectively. Index scores correlated strongly with MR indices and VAS changes ($r \geq 0.60$) for responsiveness when assessing longitudinal validity, but less so relative to clinical inflammatory indicators: PDAI, CRP, CDAI.

4.3 Application of MT, DWI and DCE sequences in CD and pCD

4.3.1 Application in luminal CD

Animal models

The treatment of CD-stricturing patients may include medical, endoscopic, or surgical therapy, depending on whether the nature of the stricture is prevalently fibrotic or inflammatory. Many recent imaging modalities offer at best an indirect evaluation of fibrosis with the recognition that there is a substantial overlap between inflammation and fibrosis when scanning¹⁶¹.

In general, T2-weighted imaging reflects a greater Sn and Sp of fluid within the pathological wall than other imaging methods. A decrease in T2 signal in the bowel wall has been shown to correlate significantly with histological fibrosis. Specifically, a low T2 signal from the bowel wall has been shown to correlate with fibrosis in the submucosal and muscularis propria layers^{162, 163}. Based on the combined evaluation of the degree of T2 signal intensity, and wall enhancement MRI had 95.8% Sn, 100% Sp, and 97.9% accuracy in the diagnosis of fibrotic stenosis¹⁶⁴.

Recent work has highlighted the use of MT-MRI techniques in quantifying inflammation, and directly differentiating it from fibrosis¹⁶⁵. Particularly, when combined with T2 measurements, MT may be helpful in differentiating and quantifying inflammation by the relative absence of collagen, and in differentiating inflamed parts that need treatment, from

other chronic fibrotic and irreversible regions that would not be responsive to medical treatment ¹⁶².

With increasing amounts of macromolecules (including collagen), an MT signal is stronger and should, in theory, be able to differentiate between highly fibrotic tissues. Adler *et al.* aimed to determine the efficacy of MT in the quantification and identification of intestinal fibrosis in an animal model of CD. In this study two groups of Lewis rats were used: 13 control animals were injected with the human serum albumin (HSA), and 25 Lewis rats were injected with peptidoglycan-polysaccharide (PG-PS). PG-PS is a component of bacterial cell walls that, perhaps, possesses an immunogenic property when given to mice and may lead to activation of T-cell-mediated immune response. The model of PG-PS is one of the only models that closely mimics CD with chronic fibrosis, crypt, abscesses, granulomas, and chronic ileocolonic inflammation. Consequently, this model is eminently relevant to CD because muramyl dipeptide, a component of PG-PS, is the ligand for NOD2. All rats developed an acute (<24 hours) intestinal inflammatory reaction, whereas only test rats developed bowel fibrosis in the late phase (> 14 days). In the test group, in a series weekly MRI scans, the MTR increased over time in line with progressive intestinal fibrosis while there was no significant change in the MTR over time in control rats. MTR correlated well with histological fibrosis grade ($r = 0.61$, $p = 0.0001$), and with collagen tissue content ($r = 0.74$, $p = 0.0003$). Also, the researcher found that MTRs were significantly higher in rats with fibrotic bowel compared with controls in late-phase examinations. It was concluded that

MTR had good discriminatory ability for fibrosis (AUC of 0.67 for fibrosis score and 0.88 for collagen content)¹⁶⁵. Another animal study confirmed Adler *et al.*'s findings but showed that the correlation between MTR and collagen might not be linear ¹⁶⁶. In this study, ten rats received multiple enemas of trinitrobenzenesulfonic acid enema to induce colonic inflammation and fibrosis, while 10 additional rats received a single dose of the same acid to induce acute colonic inflammation. In the area of greatest colonic thickening, MTR and T2 wall signal intensity ratios were calculated. The MTR difference between the two rat groups was significantly different, although, MTR correlation to histological collagen content was weak. This work hypothesises that the best discrimination between fibrotic and inflammatory tissue segments is the MTR (which detects fibrosis) and T2 wall signal intensity (which measures inflammatory activity) ratio with an area under the ROC curve of 0.97¹⁶⁶.

Application of MT in luminal CD

The clinical use of MT has also been assessed in human studies ^{20, 167}. A prospective study assessing 31 patients, reported that imaging of the small bowel by using the MT technique is feasible with sufficient image quality, and may help to distinguish acute inflammatory stenosis from fibrotic strictures of the bowel in patients with CD. In this study, the MTRs were intermediate in normal bowel wall segments with $25.4 \% \pm 3.4\%$. The MT ratios in segments with acute inflammation were lower than normal while the MT ratios in bowel wall segments with fibrotic scarring were increased significantly compared with the normal. ²⁰.

A recent study involving 12 patients assessed the diagnostic accuracy of MT in predicting luminal inflammation and fibrosis in patients with stricturing CD by correlating MT measures with histological grades of inflammation/fibrosis, as well as by quantifying type I collagen and vessel density. Xuehua Li *et al.* concluded that MT had a strong correlation with the histological degree of fibrosis ($r=0.711$) and a moderate correlation with collagen type I ($r=0.362$), but was not correlated with vessel densities ($r=0.184$) or inflammation scores ($r=0.177$)¹⁶⁷.

A recent prospective study of 31 CD patients with small-bowel strictures, compared MT, DWI and contrast-enhanced MRE imaging in detecting different degrees of fibrosis, by using surgical histopathologic analysis as a reference standard. The MTR did not correlate with the inflammatory score ($r=-0.03$; $p=0.740$), but correlated strongly and positively with the fibrosis score ($r=0.77$; $p=0.000$). The normalized MTRs differed significantly between non-fibrotic, mild, moderate and severe fibrotic bowel segments ($p=0.001$). MTR had the highest diagnostic accuracy to distinguish between non-fibrosis/mild fibrosis, and moderate-to-severe fibrosis (AUC, 0.92), followed by ADC (AUC, 0.75), and percentage enhancement gain (AUC, 0.59)¹⁶⁸.

These data suggest that MT is non-invasive method to measure bowel fibrosis. The MTR was slightly decreased in the segments with acute inflammation, although it was significantly increased in the bowel wall with fibrotic scarring^{20, 165, 166}.

Application of DWI in luminal CD

The use of this sequence has recently been expanded to enterography assessments of bowel inflammation, and in assessing response to treatment in CD patients. Active inflammation of the CD showed that water molecules in bowel walls have restricted diffusion. Bowel segments with restricted diffusion have high signal intensity on high b-value DWI (b value of at least 500 s/mm²), and low signal intensity on a corresponding ADC map. Many studies have assessed the diagnostic accuracy of DWI in patients with CD ^{146, 169, 170}. A retrospective study of 31 CD patients assessed the performance of the DWI sequence to detect inflammatory activity in luminal small bowel CD by comparing the results of DWI, and conventional MRE involving an MaRIA score calculation, and a T1 post-gadolinium enhancement. Buisson *et al.* found that DWI was correlated with MRI disease activity defined both through quantitative (MaRIA score ≥ 7) ($P = 0.0001$), and qualitative and more subjective ($P = 0.0001$) criteria. The Sn and Sp of DWI to detect inflammation in this study was 100% and 92.9%, respectively. Unfortunately, the data they obtained was not compared with an external reference standard, and the ADC value itself could be variable with respect to an MRI scanner or a different b value. The authors concluded that DWI was an effective method to detect and assess ileal inflammation in CD ¹⁴⁶.

The degree of inflammation in CD strictures can be assessed and quantified by ADC ¹⁷¹. A prospective study of 32 patients with CD, and 18 healthy volunteers investigated CD by DWI and DCE, and compared the

results with serological biomarkers¹⁷². The authors found lower ADC values in the patients with CD than in the healthy volunteers, which indicated a negative correlation with CRP ($r = -0.630$, $p < 0.001$)¹⁷².

DWI can offer reliable quantitative measurements in patients with bowel lesions, and can also be used to investigate bowel inflammation in CD. In a retrospective study, Oto *et al.*¹⁷³ used DWI from 11 patients with CD, and measured their ADC values. The authors found that the bowel segments with inflammation displayed a higher signal when compared with normal segments on DWI. Furthermore, they found that the Sn of 94.7% and Sp of 82.4% for detecting active CD with DWI¹⁷³. A retrospective study was undertaken involving 31 patients with CD to assess a free-breathing technique involving DW images of active CD. This study indicated higher ADC values in inactive disease segments, and also reported the Sn (86%), Sp (81.4%), and accuracy (82.4%) of DWI for active CD¹⁷⁰. In another retrospective study, involving 33 CD patients, a significant correlation, between ADC values in inflammatory segments, and wall thickness, was indicated¹⁷⁴.

In a prospective study involving 18 CD patients, CDMI and endoscopic findings were used as reference standards to assess the diagnostic performance of DWI in quantifying bowel inflammation after medical therapy¹⁷⁵. Accuracy of DWI to measure inflammation or to diagnose complete remission ranged from 76% to 84%. There was a significant negative correlation between changes of CDMI and ADC with correlation coefficients

of -0.461 and -0.438, respectively ($P < 0.05$). They observed that following treatment, a significant increase in the ADC values in patients who showed partial or complete remission. The change between improved or unimproved lesions varied significantly in the ADC values.

Tielbeek *et al.*¹⁷⁶ quantitatively evaluated 20 CD patients with conventional MRI, DWI and DCE techniques by using histopathology of surgical specimens as the reference standard. Fibrotic lesions had hyperintensity on high b-value images and significantly lower ADC values, and reported that an initial slope of increase, and ME correlated significantly with the fibrosis score and acute inflammatory score¹⁷⁶. No significant correlation was demonstrated between histopathological grading of inflammation and ADC values, although the ADC values decreased in affected segments with a higher inflammatory score, thus, proposing that fibrosis and inflammation are not binary processes, but a pathophysiological process of gradation. In sections with active inflammation, a non-significant decrease in ADC values was measured, with low ADC values correlating significantly with fibrosis. Increased fibrotic tissue may result in a reduction in an extracellular area, and may, consequently, restrict water molecules' diffusion in the affected bowel wall¹⁷⁶. In a prospective study that assessed 96 patients with CD, a significant correlation was shown between DWI hyperintensity and endoscopic inflammation in CD¹⁰⁰.

To technically validate a biomarker, it is important to assess its responsiveness to intervention. ADC can be a powerful tool to evaluate and

monitor the response to immunosuppressive treatments. In term of prediction of treatment response, a prospective study¹⁷⁷ in 40 patients with CD showed that combined biochemical and clinical remission after 12 weeks of TNF therapy correlated with a decreased baseline ADC values, with a cut-off of 1.96 (odds ratio 4.87, 95% CI 1.04–22.64).

In 42 CD patients⁵, the anti-TNF therapy was given, and baseline and follow-up MRE with DWI and DCE were performed after a median interval of 51 weeks of therapy in a retrospective study. They acquired axial EPI-DWI with five b values 0, 50, 100, 300 and 600 mm^2 during free breathing. Physician global assessment was used to define treatment response. Response to treatment was defined as the reduction by at least one category in disease severity, such as, moving from severe to moderate. The study concluded that after anti-TNF- α therapy, the measure of DCE parameters and ADC values in the bowel wall significantly changed in clinical responders, but not in non-responders.

Few studies have documented the DWI value for fibrosis characterization. In a paediatric study of 5 CD patients with histological fibrosis, the mean value of ADC with $b=500$ was 0.92 and with $b=1000$ was 0.8¹⁷⁸. The ADC value between the non-inflamed sections, and strictures was significantly different ($p=0.0143$). Preliminary data suggest that transmural fibrosis quantitative ADC measures are lower than the reported inflammation values in CD¹⁷⁸

An IVIM-DWI imaging technique was reported to be able to evaluate CD activity or fibrosis¹⁷⁹⁻¹⁸¹. Freiman *et al.*¹⁸⁰ assessed a DW image by using the IVIM model of DWI quantification. Findings of enhancement, and non-enhancement bowel segments in 24 paediatric patients were compared. The ADC fast parameter (collected from the lower b-values) performed best in predicting active disease. The perfusion fraction as measured by the IVIM-DWI was lower in enhancing abnormal segments¹⁸⁰. Moreover, IVIM offers a better depiction of the DWI signal decay than in the ADC model. A reduced ADC value is associated with alterations in the fast diffusion rather than with alterations in the slow diffusion¹⁸⁰. The researchers found a significantly higher perfusion coefficient, lower fractional perfusion, and no significant difference in the enhanced diffusion coefficient from the non-enhanced bowel wall of paediatric CD patients. Other studies^{181, 182} confirmed a decreased fractional perfusion in bowel segments with CD inflammatory activity. The efficacy of this technique in adults with CD was used to evaluate bowel wall fibrosis by using surgical histopathology as the reference standard compared with traditional DWI, and contrast-enhanced imaging¹⁷⁹. The investigators found that fractional perfusion decreased following increases in fibrosis severity from mild, to moderate, to severe. They also found the ADC ($r = -0.495$, $p < 0.001$), and the fractional perfusion ($r = -0.629$, $p < 0.001$) values were significantly correlated with fibrosis scores. Moreover, contrast-enhanced parameter, diffusion coefficient, and perfusion coefficient were uncorrelated with histological fibrosis¹⁷⁹.

Hectors *et al.*¹⁸¹ conducted a prospective study of 27 CD patients, to assess DCE-MRI and IVIM-DWI metrics in normal and abnormal ileal segments, and to evaluate the correlation between these parameters, and also its correlation with CRP and HBI. They used both model-free (semi-quantitative), and pharmacokinetic (quantitative) models to assess DCE parameters.

The authors reported that abnormal bowel segments had significantly lower ADC and PF than normal segments. The DCE parameters, revealed a significantly increased area under the curve at 60 s, upslope, V_e , K^{trans} and peak concentration (C_{peak}) was found in abnormal versus normal bowel segments. In addition, in terms of correlations between DCE and IVIM-DWI parameters, a negative correlation between ADC and V_p ($r=-0.286$, $p=0.036$), and between PF (measured with IVIM-DWI), and upslope (measured with DCE) ($r=-0.294$, $p=0.031$) were observed. None of the IVIM-DWI and DCE-MRI parameters correlated with clinical indices (CRP and HBI)¹⁸¹.

DWI and DCE were shown in a retrospective study to provide valuable information in differentiating between normal and inflamed small bowel among 18 patients with active CD, especially in the terminal ileum¹⁶⁹. ADC levels were calculated from two DWI with b-value of 0 and 600 s/mm² to distinguish between normal and inflamed ileum segments. In this study, surgery and/or endoscopy were used as a standard reference, with the pathological investigation carried out less than four weeks after the MR

scan. Among conventional MRI findings, increased enhancement and mural thickening were found in all actively inflamed small bowels. The inflamed bowel wall was categorised by lower ADC values, and a brighter signal on DWI.

In summary, the DWI sequence can differentiate between normal intestinal segments from intestinal loops with active inflammation. The intestinal segments with active disease are characterized by restricted diffusion; the ADC value of intestinal loops with active inflammation is significantly lower than that of normal ileal segments. Data from these studies ^{178 176} show that the acutely inflamed segments have a gradual decrease of ADC values compared with normal bowel tissue, and ADC values are further decreased when fibrosis occurs. However, varying degrees of fibrosis and inflammation coexist in the same stricture, and to differentiate pure fibrosis from pure inflammation, and to grade the degree of fibrosis in the intestinal wall remains challenging.

Application of DCE in luminal CD

This imaging sequence is mostly used in oncology scanning, such as, for prostate and breast cancer¹⁸³. Several studies have revealed the possibility of quantitative DCE in accurately discriminating actively normal bowel loop from affected bowel loops in patients with CD ^{3, 34, 169, 172, 184}.

The mean value of K^{trans} reflecting vascular permeability, extracellular volume ratio reflecting vascular permeability (V_e) and K_{ep} (flux rate

constant) in inflamed intestinal tissue in CD were significantly higher than in control group. Also, a good correlation between V_e and K^{trans} with CRP were observed ($r=0.533$, $p=0.002$; $r=0.725$, $p<0.001$). No correlation was observed between K_{ep} and CRP ($P=0.27$)¹⁷².

In a retrospective study of 11 patients³⁴, Taylor *et al.* found that affected bowel segments had significantly higher mean K^{trans} and enhancement ratio, and did not differ in V_e levels. No correlation between DCE derivative parameters and histologic findings of inflammation in any clinical parameters were reported. There was a significant negative correlation between the slope of enhancement and microvascular density ($r=-0.86$; $p<0.001$). CRP levels revealed a negative correlation with a slope of enhancement ($r=-0.77$; $p<0.006$)³⁴. Giusti *et al.*¹⁸⁴ reported that patients with biopsy-proven active disease had different DCE curve types and higher ME compared with inactive disease. Oto *et al.* found significant differences between K^{trans} and V_e levels in eighteen patients with affected and non-inflamed bowels¹⁶⁹, when these MRI readouts were compared with histological data. A prospective study ($n=42$ CD patients) investigating the effect of anti-TNF therapy showed that SoE, ME, K^{trans} , and V_e changed significantly in clinical responders but not in non-responders⁵.

To summarise, it has been shown that, in patients with CD, actively inflamed small bowel loops can be distinguished from normal segments based on quantitative DCE-MRI parameters. The intestinal wall with active

inflammation has increased perfusion (indicated by increased V_e and K^{trans} values).

4.3.2 Application in pCD.

Application of MT in pCD

The most recent contribution to the assessment of the degree of pCD activity was the use of MT, in the field of non-routine MRI sequences. In a study involving 29 patients, aimed to analyse the feasibility of MT to evaluate pCD and to assess its efficacy in evaluating disease activity¹⁸⁵, the average Van Assche score for this cohort was 11.7 (range 4-17). Twenty-two patients had an active fistula, and seven had a non-active fistula. Those with active fistula (n=22) had high signal intensity on T2 and DWI. In the two groups, the Van Assche average score was 14 and 4.1 respectively ($p < 0.005$). The mean MTR for all patients was 47.2 (range 12–68) at 800Hz (Hertz) and 34.3 (range 11–57) at 1200Hz, respectively. At 800 Hz, the MTR was significantly higher in active (mean 51, range 24–68) than in non-active fistula (mean 34, range 12–55) (lower MTR) ($P < 0.02$). There was no significant difference in MTR at 1200Hz between the two groups. Pinson *et al.* concluded that MTR was a feasible method, and offered quantitative measurements for assessing pCD¹⁸⁵.

Application of DWI in pCD

DWI has many benefits that make it easier to include in a routine MR protocol for perianal fistula assessment, such as, a shorter sequence period, involving no need for additional equipment and administration of a

contrast agent when compared with DCE. To date, DWI has only been reported in retrospective studies ¹⁸⁶⁻¹⁸⁹.

In a small retrospective study reporting on 13 patients with 20 anal fistulas, DWI was deemed a useful diagnostic tool in the assessment of pCD, and, perhaps, a helpful adjunct to T2W images ¹⁸⁷. Eighteen (90%) out of 20 anal fistulas, were noted on T2-WI only, 19 (95%) were depicted on gadolinium-enhanced and T2-WIs combined, and 19 (95%) were detected on DWI and T2-WIs combined. No statistically significant difference in sensitivity of the sequences was seen. Gd-enhanced and T2-WIs combined confidence scores, and DWI and T2-WIs combined scores were significantly higher than those with T2-WIs alone ($p = 0.014$, and 0.0047 respectively). No differences were observed between the three MR imaging techniques ¹⁸⁷.

In a study assessing 24 patients with 41 lesions (23 active and 19 inactive fistulas respectively) to evaluate the role of DWI sequence in determining disease activity¹⁸⁶, the authors used a composite index, including the requirements for surgery and surgical results as a standard reference, with patients dichotomised into active and non-active groups. They found that the ADC) values were significantly lower in clinically active fistulas. An optimal cut off ADC of $1.109 \times 10^{-3} \text{ mm}^2/\text{s}$ yielded Sn 95.7%, a Sp of 50%, and positive and negative predictive values of 71% and 90% respectively for discriminating between patients with active from those with

inactive CD ¹⁸⁶. The authors reported that DWI might be valuable for assessing perianal activity.

Dohan *et al.* studied 24 patients in order to investigate the role of DWI in evaluating fistula activity, determining fistula-in-ano, and differentiating inflammatory masses or phlegmons from perianal abscess¹⁸⁸. Their study used clinical examination, T2-weighted fat saturation, surgery and follow-up as a standard reference. The ADC (in 10^{-3} mm²/s) levels of the abscesses were lower ($P = 0.714$) than in inflammatory masses. An optimal cut off ADC of 1.186 for abscess diagnosis, yielded a Sn of 100 % [95 % CI: 77 %-100 %], a Sp of 90 % [95 % CI: 66 %-100 %], positive and negative predictive values of 93 % [95 % CI: 82.8 %-100 %], and 90 % [95 % CI: 78 %-100 %] respectively. 31 out of 34 fistulas (91.2 %) were detected with T2-WI TSE while 34/34 fistulas (100%) were depicted on DWI. The study concluded that DWI was very accurate at differentiating between inflammatory mass and a perianal abscess. The fistula in-ano was more visible with DWI than with T2 TSE weighted imaging¹⁸⁸.

The value of DWI in diagnosis of perianal fistula compared with T2-WI alone was reported in a prospective study which included 92 patients with pCD. The authors found that the confidence scores and diagnostic accuracy for combined DWI and T2-WIs were higher than those of T2-WIs alone for both radiological reviewers (reviewer 1, $p = 0.032$ and reviewer 2, $p < 0.001$)¹⁹⁰. Also, they found that for both observers the Sn and Sp of the

combination of T2-weighted and diffusion images were greater than those of T2-WIs alone¹⁹⁰.

Application of DCE in pCD

Spencer *et al.* were the first to suggest the use of DCE techniques to evaluate the presence of active inflammation in perianal fistula. The authors reported that lower signal intensity was observed in healed tracts compared with vivid enhancement in active tracts¹⁹¹.

Since the first report, studies have proven the diagnostic accuracy of DCE sequences for determining the degree of disease activity in patients with pCD^{4, 192}. Horsthuis *et al.*¹⁹² used semi-quantitative DCE for the evaluation of disease activity in 33 patients with pCD. TICs, SoE and ME were calculated in a ROI drawn around the fistula. The results of DCE parameters were compared with reference standards (MRI-based score, CRP and PDAI values). No significant differences were observed in dynamic contrast-enhanced MR imaging parameters between patients with clinically active disease versus patients with clinically inactive disease. Significant, but weak-to-moderate correlations were shown between PDAI, and the region of interest volume; mean ME $r=-0.093$ ($P=0.646$), mean SoE $r=-0.169$ ($P=0.401$), as well as between PDAI absolute numbers of certain TIC curves. The mean ME was significantly higher in patients with active disease than in non-active disease patients ($p=0.001$). The ratio of rapid enhancement against slow enhancement pixels correlated with higher MRI scores as did the ROI volume. No significant differences were found

between CRP levels or in any of the TIC type numbers. A significant weak correlation was found between CRP levels with mean ME. Moreover, a larger number of rapidly enhancing pixels was seen in patients who required further escalation of therapy. Also, the authors observed a weak correlation between MRI-based score of disease activity developed by Van Assche *et al.*, and a perianal disease activity index.

Ziech *et al.* performed a prospective study of 16 patients with pCD, performing a quantitative and semiquantitative analysis of DCE parameters (extravascular space fractional volume (Ve), K^{trans} , ME, and the initial slope of increase), to evaluate disease activity and response to anti-TNF therapy of pCD⁴. The reference standards were MRI-based score, CRP and PDAI values⁴. Moderate correlations were shown between PDAI, and volume of enhancing pixels ($r = 0.79$, $p < 0.001$), initial slope of increase ($r = 0.58$, $p = 0.018$) and ME ($r = 0.67$, $p = 0.005$), but not with K^{trans} or Ve. This was probably due to increased perfusion caused by the high vascularisation in inflamed tissues. This was not demonstrated in Horsthuis *et al.*'s study, but the researchers did not use the same protocol DCE-MRI (differing in field strength, whether 2D or 3D were used, imaging parameters, and whether the entire lesion volume was analysed in place of a single slice). Moreover, volume of enhancing pixels correlated with MRI-based score ($r = 0.79$, $p < 0.001$) and CRP levels ($r = 0.52$, $p = 0.041$). The values of K^{trans} had significantly decreased 6 weeks after the start of treatment indicating that it might be a predictive parameter of the response to treatment. As such, the conclusion was that semi-quantitative parameters' initial slope of increase,

and maximum enhancement might be used to assess disease activity in patients with pCD, and a quantitative parameter volume transfer constant could be used as an indicator of the effect of treatment⁴.

In a retrospective study¹⁹³ which evaluated both DCE and IVIM sequences for quantitative characterization of anal fistula activity in 43 patients, the authors used surgical assessment or clinical outcome as the reference standard. They used different DCE parameters to quantify semi-quantitative maps of perfusion, such as, the area under the curve, brevity of enhancement (the time difference between the wash-in and wash-out), time to peak, time of arrival, maximum relative enhancement, wash-in and out rates. The authors found that only two DCE parameters (time-to-peak and brevity of enhancement) were significantly longer in active fistulas compared with inactive fistulas whereas the other parameters were not significantly different. Also, for the IVIM parameters, none achieved statistical significance for differentiating between active and inactive fistulas.

In summarising these studies, signal on T2WI, the degree of contrast enhancement and wall thickness were useful features to evaluate CD in MRI. However, monitoring response to treatment, and the accurate estimation of the extent of disease, remain as diagnostic challenges in both CD and pCD. For these reasons, evolving advanced quantitative imaging sequences, such as MT, DCE, DWI and T2 relaxometry in the abdomen can be very important in terms of quantifying and monitoring the responses to treatment. These quantitative techniques provide measurements that are

less subjective than observer scoring systems making them very suitable for monitoring purposes. DWI and DCE have high sensitivity to distinguish between active and inactive inflammation in bowel segments, and for assessing disease activity as well as possibly helping to predict responses to treatment. Also, MT has proved to be a very useful sequence in differentiating between fibrosis and inflammation. The conventional T2WI is a useful sequence in many MRI scores in evaluating of CD and its complications. The potential improvement of T2 relaxometry over the more subjective T2-based imaging can help in the treatment progression.

4.4 Hypothesis and Aims of this project

MRI is particularly well suited to evaluate disease activity and its complications in patients diagnosed with CD as an imaging biomarker of therapeutic efficacy because of its lack of ionizing radiation, which makes it an appealing modality for repeated assessment before and during treatment.

Based on background knowledge, and literature summarised above, little is known about using newer MRI techniques, such as T2 relaxometry, MT, IVIM-DWI and DCE individually or combined as an imaging biomarker for therapeutic efficacy in CD (luminal and pCD) and its complications.

The overall hypothesis of this thesis is that these newer MRI techniques are well suited to evaluate disease activity and its complications in patients diagnosed with CD, and to measuring the inflammatory disease

in luminal and pCD. This will be put to the test by assessing whether these imaging techniques are better at predicting the response to biological therapy compared to conventional scores.

The aims of the project are, therefore, to carry out three studies:

Study 1 aims (MAP):

1. To measure disease activity within pCD using MRI sequences before and after biological therapy.
2. To investigate changes in clinical indicators and MRI parameters after biological therapy.
3. To investigate the inter-relationship between these sequences, and to compare the utility of these sequences on both 1.5T and 3T MRI platforms.

Study 2 aims (REPEAT):

1. To evaluate the repeatability and inter-observer variability of quantitative MRI parameters (MT, T1, T2 and IVIM-DWI) in the luminal bowel wall in a cohort of healthy volunteers.

Study 3 aims (Motility plus):

1. To determine the changes over time in T2, MTR and IVIM-DWI parameters, disease activity and clinical indicators applied in a cohort of patients with luminal CD before and after biological therapy.
2. To assess the correlation between these quantitative measurements.

3. To assess the correlation of these parameters with clinical and MRI-based measurements of CD activity

This work will investigate whether it is possible to more accurately quantify the inflammatory burden within luminal and pCD by using quantitative MRI and allow for better patient follow-up and more effective clinical decision-making.

Chapter five: A new Mr-based Perianal Crohn's disease activity score: the MAP study

Abstract

Background: Perianal Crohn's disease (pCD) is a potential complication of CD. Absence of reliable disease measurements makes disease monitoring unreliable. Clinical measurements, such as, the Perianal Disease Activity Index (PDAI) and Fistula Drainage Assessment are inaccurate with high inter-observer-variability. MRI has been increasingly used for diagnosing and monitoring pCD patients and has shown potential for assessing therapeutic response and inflammation. Quantitative MRI sequences, such as, diffusion-weighted image (DWI), dynamic contrast enhancement (DCE), and magnetisation transfer (MT), in addition to T2 relaxometry, offer opportunities to improve diagnostic capability.

Objectives: To measure the disease activity within a pCD patient cohort using quantitative MRI sequences (DWI, DCE, MT, and T2) before and 12 weeks after biological therapy.

Methods: Twenty-five participants with pCD were scanned using the above sequences at 1.5T and 3T, once before starting biological therapy, and once after 12 weeks of biological therapy.

Results: No significant differences in T2, DWI, MTR and DCE parameters were observed after treatment. No significant correlation

between clinical indicators and MRI parameters was observed. A significant negative correlation was found between MTR and T2, MTR and DCE parameter k^{trans} and MTR with an apparent diffusion coefficient, reflecting competing disease effects of inflammation, and fibrosis.

Conclusion: These quantitative sequences have not changed after treatment, and were also not different in responder and non-responder groups. However, negative correlations between the MTR, and T2, DCE, and K^{trans} were observed.

5.1 Introduction

CD is a chronic inflammatory bowel disease, often associated with perianal complications, such as fistulas. pCD is a potential complication in a third of CD patients¹⁹⁴. pCD causes various debilitating symptoms such as perianal discharge, pain and bleeding that impose various functional limitations that negatively affect a patients' quality of life. The disease's natural history is characterized by periods of active disease followed by periods of clinical remission. As a result, it requires multiple medical and surgical interventions and represents a major therapeutic challenge.

To date, it is very difficult to quantify inflammation and optimise therapy in patients with pCD due to the absence of reliable disease activity measurements. Clinical measurements such as PDAI⁹⁰ and FDA¹⁹⁵ are crude, and prone to inter-operator-variability making those indices less sensitive to assess responsiveness after medical therapy. Previous studies have shown that complete resolution of the internal fistula tracks does not occur in response to short-term treatment, despite the healing of the external orifices^{101, 155}. Those indices are, therefore, questionable when monitoring disease activity after medical therapy. Consequently, to provide patients with a correct treatment plan, and to avoid recurrences it is important to define a method for measuring perianal fistula activity.

MRI is a highly accurate non-invasive approach that allows for morphological evaluation, and perianal fistula classification¹⁹⁶. Pelvis MRI has been shown to be useful, not only in defining the anatomy of the pelvic

region in pCD, but also in measuring the inflammatory activity of the fistula^{156, 197}. MRI is the preferred test for assessing pCD, and its response to biological therapy^{4, 154, 198, 199}. However, radiological scoring of features in a conventional MRI sequence, is also prone to inter-observer variability. The Van Assche score is the most frequently used MRI index for pCD. This score provides combined information concerning anatomical fistula description, and features reflecting inflammatory activity. Evaluation of this score is difficult because no gold standard exists in determining fistula healing. This index has shown a weak correlation with clinical response to therapy according to several small studies^{156, 157, 192}; but, this method has not been completely validated^{102, 157}. A Van Assche score is insensitive to clinical response^{102, 200}, with no significant difference shown in clinical responders to anti-TNF therapy before and 6 weeks after treatment¹⁹⁷, nor was there a difference in the score between clinical responders and non-responders¹⁰². Recent modifications of this score have been put forwarded but have not yet been externally validated^{201, 202}.

Quantitative MRI sequences, such as DWI, DCE, and MT, along with T2 relaxometry, offer opportunities to improve diagnostic capability. DCE and DWI have been shown to have potential in the assessment of activity in pCD^{186, 198} while MT may be a feasible tool to distinguish active from inactive fistulas¹⁸⁵. These quantitative MRI measures have never been investigated together prospectively in an active pCD cohort. Moreover, it is not yet clear if these measures are feasible on a 1.5T MRI platform or solely on a 3T MR platform.

The aims of this study were (1) to measure disease activity within a pCD patient cohort using quantitative MRI sequences (DWI, DCE, MT, and T2) before and after biological therapy, (2) to investigate changes in clinical indicators, and MRI parameters after biological therapy, (3) to investigate the inter-relationship between these sequences, and (4) to compare the utility of these sequences on both 1.5T and 3T MRI platforms.

5.2 Materials and Methods

Ethical permission was obtained for the study from the National Research Ethics Committee (REC), and the National Health Service (NHS) Research and Development (R&D) department (ref 16/EM/0433) on November 2016, and registered in ClinicalTrials.gov (Identifier: NCT03325582) on April 2017. All subjects in this study provided written informed consent.

5.2.1 Patients

MAP was a multicentre prospective cohort study conducted at three sites in Nottingham (Nottingham University Hospitals), University of Manchester (Salford Royal NHS Foundation Trust), and London (Northwick Park NHS Foundation Trust), with 3T scanning in London supported through University College London Hospitals. Recruitment and scanning were undertaken at these sites. Recruitment was also supported through the Pennines Acute Hospital NHS Trust, and Sheffield Teaching Hospitals NHS Foundation Trust.

All participants received biological treatment in the form of either Infliximab intravenous (IV) 5 mg/kg at weeks 0, 2, 6, and 8, weekly thereafter or with Adalimumab subcutaneously (s.c.) at week 0 (160 mg), 2 (80mg), 40 mg every 2 weeks thereafter or with Ustekinumab 6mg/kg IV at week 0, and a weight-based s.c. every 12 weeks thereafter.

The inclusion criteria involved active pCD as defined by clinical assessment, and a PDAI score of >4, a clinical decision to start biological therapy with ages of 16-75 years. Exclusion criteria included an absence of a diagnosis of CD, perianal fistulising disease not secondary to CD, rectovaginal fistulas, malignant disease, significant cardiovascular or respiratory disease and hepatic disease or renal failure, pregnancy or breastfeeding, inability to consent, history of proctectomy or unwillingness to undergo biological therapy or contraindication with respect to MRI.

To complete the study participants had to attend three research visits. Up to four weeks before the start of biological therapy, participants had their visit, one which included screening to ensure participants met the inclusion criteria, and possessed none of the exclusion criteria. Visit two involved the first MRI visit (before starting biological therapy), and visit three was the second MRI visit (12 weeks after the biological treatment onset). On the same day of the MRI scan, each participant underwent a clinical assessment of their fistulas according to the FDA, PDAI and CRP tests were calculated (Figure 21).

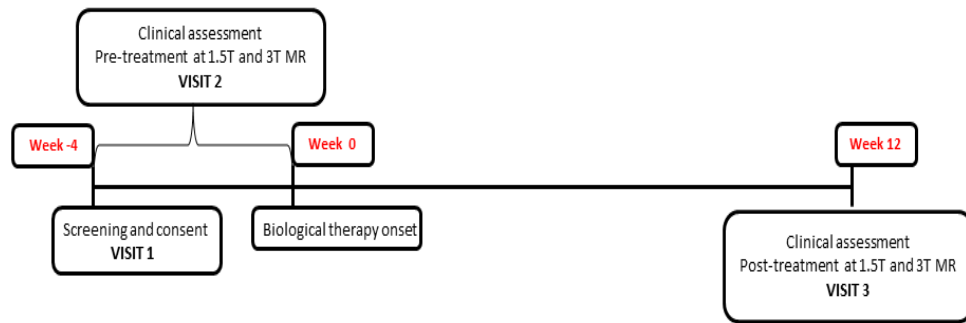


Figure 21: Patient journey during the study

5.2.2 MRI image acquisition

Participants were scanned on 1.5 Tesla scanners (one GE Signa Healthcare and two Philips, Achieva and Intera platforms), and 3T (all Philips, 2 Achieva and Ingenia) using either pelvic or torso phased array coils across all centres at the baseline, and 12 weeks after biological therapy.

All participants were scanned in a supine position (feet first) on both 1.5T and 3T MRI scanners. No special preparation was needed prior to scanning. The details of the sequence parameters used at 1.5T and 3.0T MRI scanners are displayed in Tables 9 & 10.

DWI and MT (on and off) sequences were added to the standard clinical protocol undertaken on the 1.5T MRI scanner, which was sagittal, coronal and axial T2-weighted images FSE sequences, and coronal and axial oblique STIR sequences.

At 3T, DWI, DCE, T1, T2, and MT sequences were added to the standard clinical protocol of coronal, axial, and sagittal T2-weighted turbo spin echo, oblique axial and oblique coronal fat-suppressed T2-weighted, and pre/post contrast-enhanced -T1 weighted scans.

Axial DWI was performed using a diffusion-weighted single-shot spin-echo EPI sequence with the following acquisition parameters: flip angle = 90 degrees, in GE (1.5T) two values were acquired 0 and 600 s/mm² and in Philips 4 b-values acquired for 3 orthogonal directions (b = 0, 100, 300, 600 s/mm²) mean scan time was 5 min 26s.

T2 was measured from fast spin-echo sequences in the coronal-oblique plane, acquired at 2 different echo times: T2 weighted FSE with fat saturation with a long TE (80 ms) and short TE (7.30ms), whilst keeping all other scanning parameters the same.

MT coronal oblique scanning was performed using a coronal 3-dimensional (3D) gradient-echo sequence without fat suppression. Two dynamics were acquired, one with the pre-saturation pulse for MT and one without. This sequence and the T2 sequences were planned parallel to the anal canal to include as much of the fistula region as possible.

The DCE protocol involved 2 stages: first, a variable flip angle (2°,10°,20°,30°) T1 3D gradient-echo sequence without any fat suppression was used to generate a pre-contrast T1 relaxation time map of the tissue;

secondly, dynamic imaging was carried out using a 3D T1-WI spoiled gradient echo (SPGR) sequence, with fat suppression, with the following acquisition parameters: coronal oblique images, voxel size 0.875 x 0.875 x 4 mm, flip angle = 15 degrees. To generate a pre-contrast T1 map according to the steady-state SPGR equations (8 and 9, Chapter 2). Imaging was performed for a total of 12 dynamic slices with a temporal resolution of 5ms. Gadoteridol (Bracco International B.V, Strawinskylaan, Amsterdam) was intravenously injected (0.2mmol/kg) at a rate of 3.0 mL/s using an automated injection pump (Medrad ® Spectris Solaris® EP) after 6 pre contrast acquisitions to acquire an adequate baseline data before contrast entered the tissue. This was followed by a 20 mL saline flush administered at the same rate. There was a delayed reconstruction of this data to allow immediate acquisition of the T1-post contrast data.

Table 9: MRI parameters for all sequences on the 1.5T scanner

Parameters	Imaging Plane	TR/TE (ms)	FOV (mm)	Slice Thickness(gap)mm	Matrix
T2W FSE	Sagittal	4500/92.9	260×260	4(0.4)	244×288
T2W FSE	Coronal	7000/114	320×320	4(0.4)	320×224
T2W FSE	Axial	9740/114	320×320	4(0.4)	320×224
STIR FSE	Oblique coronal	4100/57.3	270×270	3(0.3)	256×224
STIR FSE	Oblique axial	3900/57.5	270×270	3(0.3)	256×224
MTR	Oblique coronal	33/3.8	380×380	4(0.4)	288×244
DWI	Axial	2000/70.8	350×350	4(0.4)	144×144
TE: echo time; TR: repetition time; FOV: field of view; GAP: Spacing between Slices					

Table 10: MRI parameters for all sequences on the 3T scanner.

Sequences/ Parameters	Imaging plane	TR/TE (ms)	Manchester TR/TE (ms)	FOV (mm)	Slice Thickness (gap)mm	Matrix
T2W FSE *	sagittal	5417/100	8978/100	260×260	4(0.4)	228×220
T2W FSE *	coronal	6500/100	10774/100	320×320	4(0.4)	280×252
T2W FSE *	axial	10472/100	17359/100	320×320	4(0.4)	280×220
T2W FSE SPAIR long TE *	coronal oblique	4526/80	10631/80	270×270	3(0.3)	272×237
T2W FSE SPAIR short TE *	coronal oblique	4526/7.3	10631/7.3	270×270	3(0.3)	272×237
T2W FSE FAT SAT long TE *	axial oblique	4385/80	4627/80	270×270	3(0.3)	244×244
DWI EPI	axial	6154/88	4547/77	320×296	4(0.4)	160×146
3D Dyn MTR*	coronal oblique	72/4.5	144/4.5	298×298	3(0)	248×228
3D T1 with VFA 2,10,20,30		9.2/1.79	9.2/1.79	280×280	4(0)	216×216
T1W GRE FAT SAT Pre contrast*	coronal oblique	3.2/1.52	3/1.4	260×260	1.6(0)	164×184
T1W GRE FAT SAT Pre contrast*	axial oblique	2.1/1.47	3/1.4	260×260	1.6(0)	164×162
4D THRIVE DCE	coronal oblique	3.3/1.6	3.3/1.6	280×280	4(0)	218×216
T1W GRE FAT SAT Post contrast*	coronal oblique	3.2/1.52	3/1.4	260×260	1.6(0)	164×184
T1W GRE FAT SAT Post contrast*	axial oblique	2.1/1.47	3/1.4	260×260	1.6(0)	164×162
*At UoM, these sequences had a longer TR/TE values as the scanner did not have the multi-transmit capability which reduces the power deposited into the patient. THRIVE is T1-weighted High-Resolution Isotropic Volume Examination.						

The quantitative MRI parameters calculated from the images at 1.5T were MTR and ADC, whereas at 3T MTR, ADC, T1, T2 and DCE features including K^{trans} , V_e and V_p were calculated.

Scan card set-up for the multi-centre study

5.3 Optimisation of scanning parameters

Exam cards were established over several optimisation scanning sessions at the University of Nottingham 1.5T GE and 1.5 T Philips and 3T Phillips MRI scanners with the new sequences to incorporate DCE, DWI, T2, and MT. These sequences were shared with other scanning sites. There were three parts to testing the exam cards. First, to generate T1 from a variable flip angle technique (VFA). To determine whether this approach would be suitable, VFA data of a quadrant phantom with different T1 values were acquired using a 3T MRI scanner. The T1 of the different quadrants was measured using both the VFA method and an inversion-recovery EPI method as the gold standard. Data were obtained from 7 flip angles (2, 5, 10,15,20,25 and 30°).

For DCE modelling, the T1 of the tissue was needed. The highest signal intensities are normally found at lower flip angles as seen in Figure 22. As the flip angle is increased the SAR increases, therefore, for a fixed TR the SAR at 30° is significantly higher than at 10°. Increasing the TR of the VFA sequence reduces the SAR of the sequence. To determine whether there was any effect on the measured T1s of the phantoms at different TR values, three different values were used which corresponded to low,

medium and high SAR. The accuracy, of the T1 values along the 12 slices of the phantom, was also investigated. This is because the FA at the edges of a 3D volume is lower than that in the central region, thus reflecting the RF slice profile, which will affect the accuracy of the measurement due to the flip angle involvement in the calculation of T1.

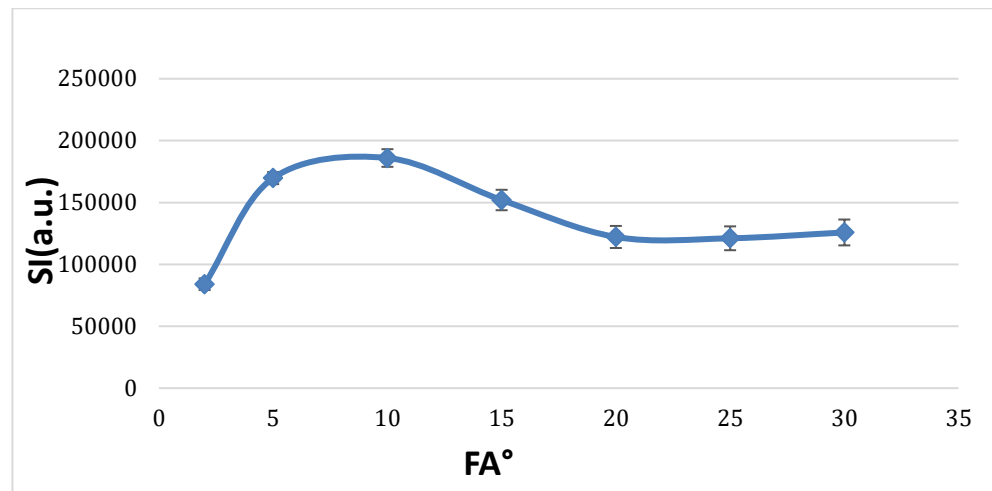


Figure 22: Line graph showing that smaller flip angles have a higher signal intensity than higher flip angles.

Secondly, we ensured that the exam cards were loaded correctly onto the other site scanners, and ensured that there were no conflicts, which could occur if other sites did not have the same access to different applications software, and made alterations to the sequences if needed. Finally, we ran the exam cards on two healthy subjects to ensure that the image contrast was consistent between the different sites.

5.4 MRI Evaluation

All MR images were assessed by three consultant GI MRI radiologists (UB, KL, CC) all with > 5 years of MRI experience who were blinded to the

clinical results. For each patient, radiologists calculated the Van Assche score¹⁰¹ only using 1.5T images before the 3T images were viewed. This score ranged from 0 to 22, the higher scores indicating more severe disease.

The radiologists then defined diseased tissue regions of interest (ROIs) on MRI scans on all slices where the fistula, including all branches, were visible using the coronal FSE IR at 1.5T (Figure 23, a), and on the T1 contrast coronal images at 3T (Figure 24, a).

Once the regions were identified on the coronal FSE IR and T1 post-contrast coronal sequences, the reader (the author, myself) co-located the same regions on the DWI axial, MT coronal (for both scanner data), DCE coronal and T2 coronal sequences on the raw images, (Figures 23, 24). These could then be directly copied on to the calculated maps (see details below and Figures 24 e&f) using Analyze® software (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN, USA). The distribution of the data across the ROI was non-gaussian in many of the datasets, therefore, the median, of the data from the ROI, was used as the data value (Figure 25).

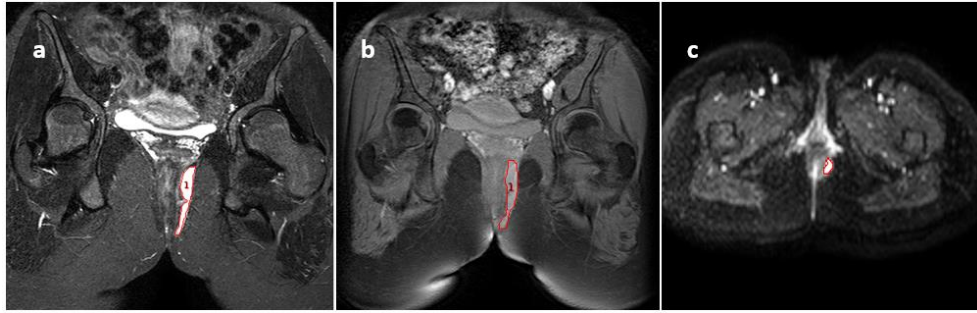


Figure 23: Patient with clearly visible fistula. Coronal FSE IR (a) where a radiologist drew the ROI and (b) MT, (c) DWI shows where the ROI was copied at 1.5T

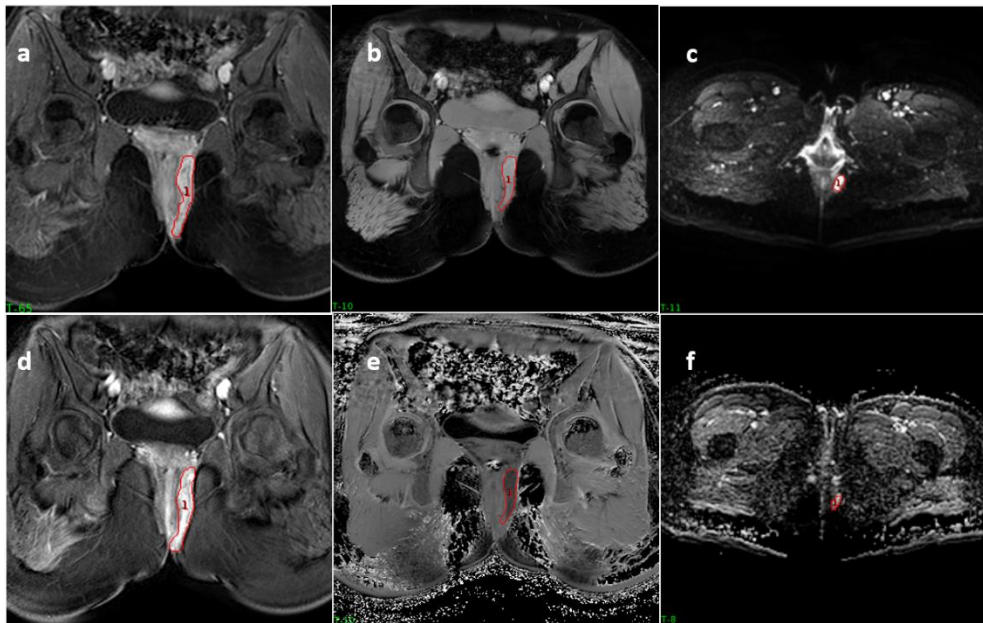


Figure 24: Images for the same patient in figure 23. Coronal T1 post contrast(a) where a radiologist drew the ROI and (b) MT, (c) DWI (d) DCE shows where the ROI was drawn. MAP of MT (e) and (f) DWI where the results were calculated.

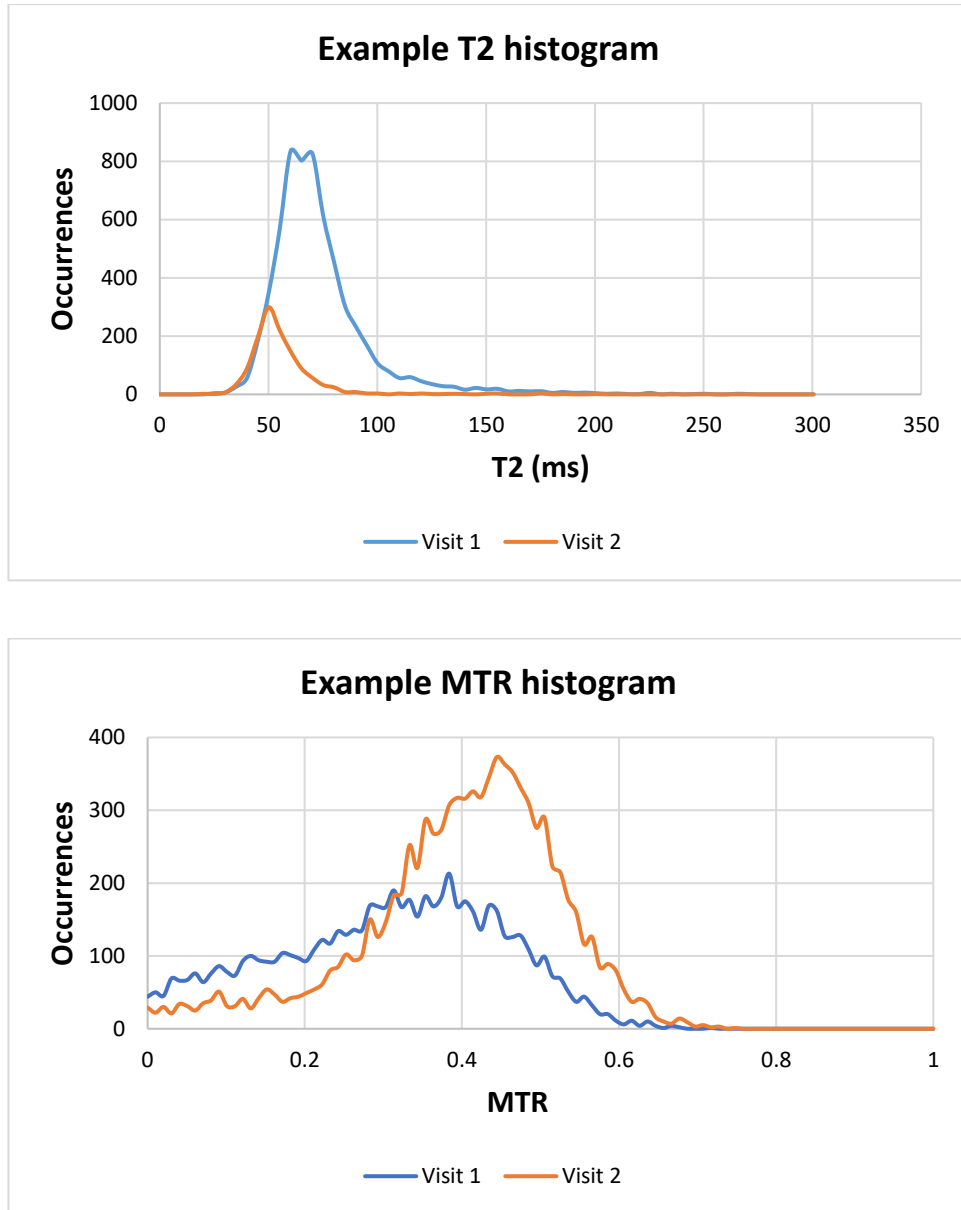


Figure 25: An example of the histograms depicting the ROI data which shows that the data from the diseased area did not have a gaussian distribution. T2 top and MTR bottom; data drawn from two different participants.

Maps of the ADC were generated using a mono-exponential decay (all 4 b-values, b-0 and 600 only, and b0 and 100 only) using equation 6 in chapter 2. MTR was generated from the MT-on and MT-off data using equation 5 (as described in chapter 2). The Extended Tofts Model (ETM)²⁰³ was used to generate the DCE parameters (vascular transfer constant

(K^{trans}), the volume fraction of the extravascular, extracellular space (V_e), and the volume fraction of the plasma space (V_p)) from the tissue uptake curves. A T1 map was generated from the pre-contrast variable flip angle gradient echo sequence (using equations 8 and 9 as described in chapter 2). T2 was calculated from FSE sequences acquired at 2 different echo times using a mono-exponential decay.

Internal Validation of MRI data sets

As the data were acquired at two different time points it was important to understand how the size of the ROIs drawn influenced the accuracy of the quantitative data, as small ROI sizes are likely to have relatively larger amounts of noise associated with them. To be confident that differences found in the diseased tissue would not just be noise from the measurement, the influence of the volume size of the ROI on the data measured was investigated.

To determine the effect of different ROI sizes, 3T MTR muscle data were investigated for the scans both time points. Muscle tissue was used as 'control' data as the quantitative signal derived from it should not alter over the time period between the two scans (unlike the diseased tissue regions). The MTR sequence was chosen as this provided excellent structural information of the muscle tissue, allowing for similar anatomical regions to be located for the measurements at the two different time points.

Sixteen ROIs from two subjects were drawn on the MTR images from both visits (Figure 26) with the size of each different sized ROI identical for the 2 time points. These ROIs were located in a similar anatomical region and were reduced in size from a maximum volume of approximately 10 mL to 0.1mL. The MTR visit1 – MTR visit 2 data was then plotted against volume size. As the ROI sizes were reduced, the difference between measurements became noisier and less stable. To determine a minimum voxel size the standard deviation (SD) of the MTR subtracted data was measured for each of the subjects and plotted against ROI volume; with the standard deviation calculated from the MTR data between the volume measured and the largest volume . (i.e. SD data plotted at 5mL were calculated from the MTR data from 5-10mL; SD data plotted at 0.5mL were calculated from the MTR data from 0.5-10mL). A minimum volume was then selected, which was above the point in each graph where a sharp rise in SD occurred as the volume size decreased.

Data sets from MR sequence parameters were only used in the statistical analyses of diseased areas above the pre-specified cut-off volume.

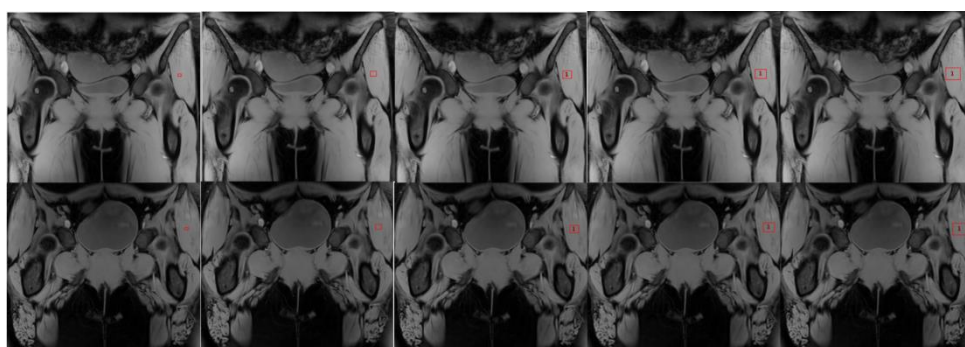


Figure 26: An example of five regions of muscle starting from the smallest to the largest ROIs. Visit 1 - top and visit 2 - bottom.

In addition to understanding the role of the ROI size in the accuracy of the data, the reproducibility of the data generated from the quantitative sequences was also investigated. Muscle tissue was again selected as a ‘control’ tissue as it should not alter its MR characteristics between the two visits. To check the reproducibility of the MR parameters (MTR and ADC both 1.5 and 3T and T2 -3T only) between the two visits at the same field strength, identical sized regions located in the same muscle region at each visit were drawn on the data (Figure 27). The median from the muscle data were calculated

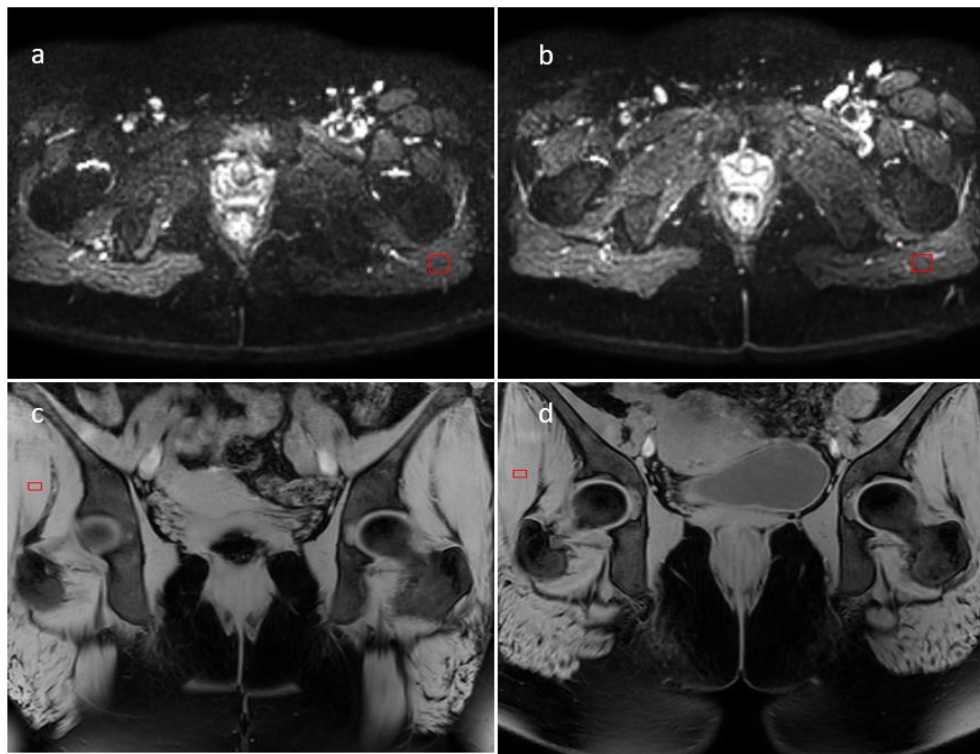


Figure 27: An example of muscle area ROIs on DWI (a&b) and MT(c&d) with visit 1 (a&c) and visit 2 (b&d) at 3T.

5.5 Clinical Evaluation

Clinical assessment was performed by experienced gastroenterologists with >10 years' experience in IBD (GM, KA, JW). The PDAI was scored by interviewing the patient, and undertaking a visual assessment of the perianal region on the same day as the MRI scan. The PDAI included five items: degree of induration, type of perianal disease, restriction of sexual activity, restriction or pain of activities in daily living, and the presence or absence of discharge (Table 11). Scores ranged from 0 to 20, with higher scores indicating more severe disease⁹⁰.

Table 11: Perianal Crohn's Disease Activity Index.

Descriptor	Description	score
Discharge	No Discharge	0
	Minimal mucous discharge	1
	Moderate mucous or purulent discharge	2
	Substantial discharge	3
	Gross fecal soiling	4
Pain/restriction of activities	No activity restriction	0
	Mild discomfort, no restriction	1
	Moderate discomfort, some limitation activities	2
	Severe pain, severe limitation	3
Restriction of sexual activity	No restriction sexual activity	0
	Slight restriction of sexual activity	1
	Moderate limitation of sexual activity	2
	Marked limitation of sexual activity	3
	Unable to engage in sexual activity	4
Type of perianal disease	No perianal disease/skin tags	0
	Anal fissure or mucosal tear	1
	<3 perianal fistula	2
	>3 perianal fistula	3
	Anal sphincter ulceration or fistula with significant undermining of skin	4
Degree of Induration	No induration	0
	Minimal	1
	Moderate	2
	Substantial	3
	Gross fluctuance/abscess	4

CRP (mg/L) determined as a biological marker of disease activity was performed on the same day as MRI at the biochemistry department of Nottingham University Hospitals NHS Trust.

5.6 Statistical analysis

All analyses were carried out using GraphPad Prism (version. 8.1.2; GraphPad Software, Inc.). MRI and clinical (CRP, PDAI) datasets were tested with the Kolmogorov–Smirnov test to assess normality of distribution before analyses. Normally distributed data were expressed as mean and standard deviations (SDs), whereas non-normally distributed data were expressed as median and interquartile ranges (IQRs). At the follow-up MRI, treatment response was defined as a drop in PDAI score by 50% or more of the baseline SD¹⁰³. Participants were divided into clinical responders and non-responders to treatment. For non-normally distributed matched datasets, the Wilcoxon signed-rank test was used to compare pre- and post-treatment results for both clinical indicators, and MRI parameters, and a paired t-test was used for normally distributed data. The strength of the relationship between the MRI parameters MTR, T2, T1, k^{trans} , V_e , V_p , and ADC, and clinical parameters were assessed using Pearson's correlation coefficients for normally distributed data, and Spearman's correlation coefficient for non-normally distributed data. Correlation between the different MRI parameters was also assessed with the data corrected for multiple comparisons by using the Bonferroni method. Unmatched comparisons between clinical responders and non-responders were undertaken with a Mann–Whitney U test. The responsiveness was evaluated by quantifying the ability of the indices to detect a meaningful

change, defined as an improvement, if there were a drop in the PDAI score by half or more in SD. P values of less than 0.05 were deemed significant.

5.7 Results

5.7.1 Optimisation results

Table 12 shows the data from the quadrant phantom VFA experiment compared with the gold standard EPI data. In the middle of the 3D block (slice 6), the T1 values are most accurate as the RF slice profile is flattest there, whereas at the edges of the 3D block the RF is lower, and, therefore, incorrectly represented in the T1 calculation. Figure 28 shows how the accuracy of T1 using the 4 FA sequence varied in relation to the slice number.

Table 12: VFA T1 data (in seconds) from the four quadrants shown for three different slices (1, 6, and 11) along with the phantom.

	quadrant	LOW SAR	MOD SAR	HIGH SAR	EPI
Slice 1	1	0.80	0.84	0.85	1.20
	2	0.58	0.60	0.59	0.84
	3	0.56	0.58	0.56	0.80
	4	0.28	0.29	0.28	0.44
Slice 6	1	1.22	1.16	1.07	1.20
	2	0.92	0.89	0.84	0.85
	3	0.93	0.91	0.89	0.80
	4	0.43	0.43	0.44	0.43
Slice 11	1	0.98	0.90	0.84	1.19
	2	0.79	0.76	0.71	0.85
	3	0.72	0.70	0.69	0.81
	4	0.32	0.32	0.32	0.44

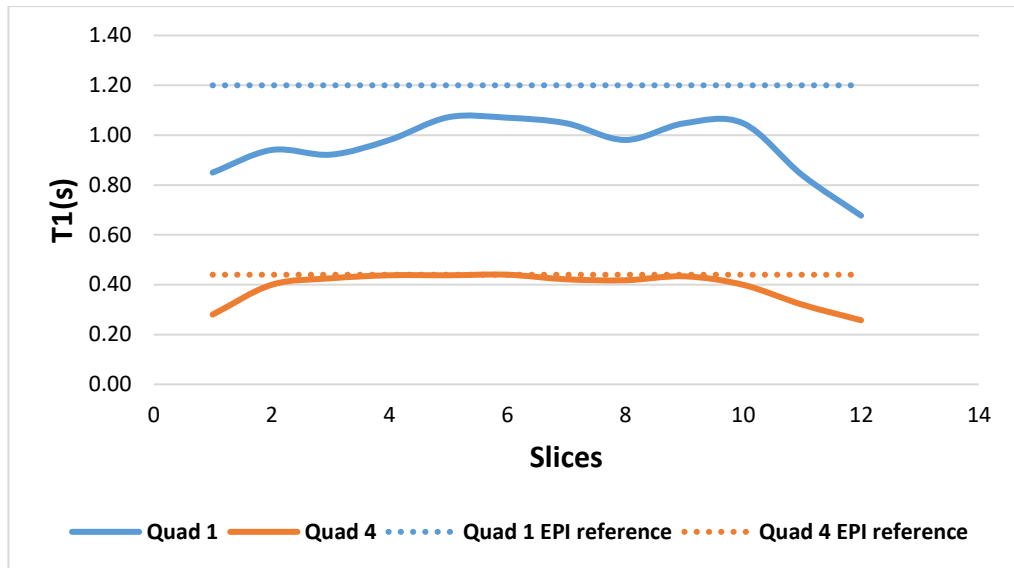


Figure 28: Graph showing the T1 calculated from two quadrants along with the 12 slices. The central slices produced more accurate T1 values when compared with the edge slices. The reference EPI data are shown as a dotted line.

To reduce the time needed to acquire the T1 map we used only 4 flip angles (5, 10, 20 and 30°) for the in vivo study²⁰⁴. As a final test of the exam cards, we scanned two healthy volunteers using the full exam cards (but without using any contrast agent) to make sure they were executed correctly, and that the images provided the appropriate image contrast, before recruiting the first patient.

5.8 Study results

5.8.1 Patient characteristics

Twenty-five participants were recruited. 6 subjects were withdrawn from the per protocol analyses (3 subjects stopped treatment before 12 weeks, 1 subject was later diagnosed with rectal cancer, 1 started treatment

before MRI and 1 subject had poor data quality due to motion artefacts in the MRI data).

Internal validation: effect of volume on the signal-to-noise ratio

There was a marked inconsistency in the MTR datasets acquired from the ROI with volumes less than 2 mL (Figure 29,a). Effect of Volume change on accumulated SD of 3T data (larger volumes to smaller volumes) (Figure 19,b) .In contrast, there was a significant improvement in the repeatability of the MTR sequence datasets acquired from the ROI with a cut-off volume of 2 mL or more. Any subjects with a fistula volume of less than 2 mL were excluded from the analysed group.

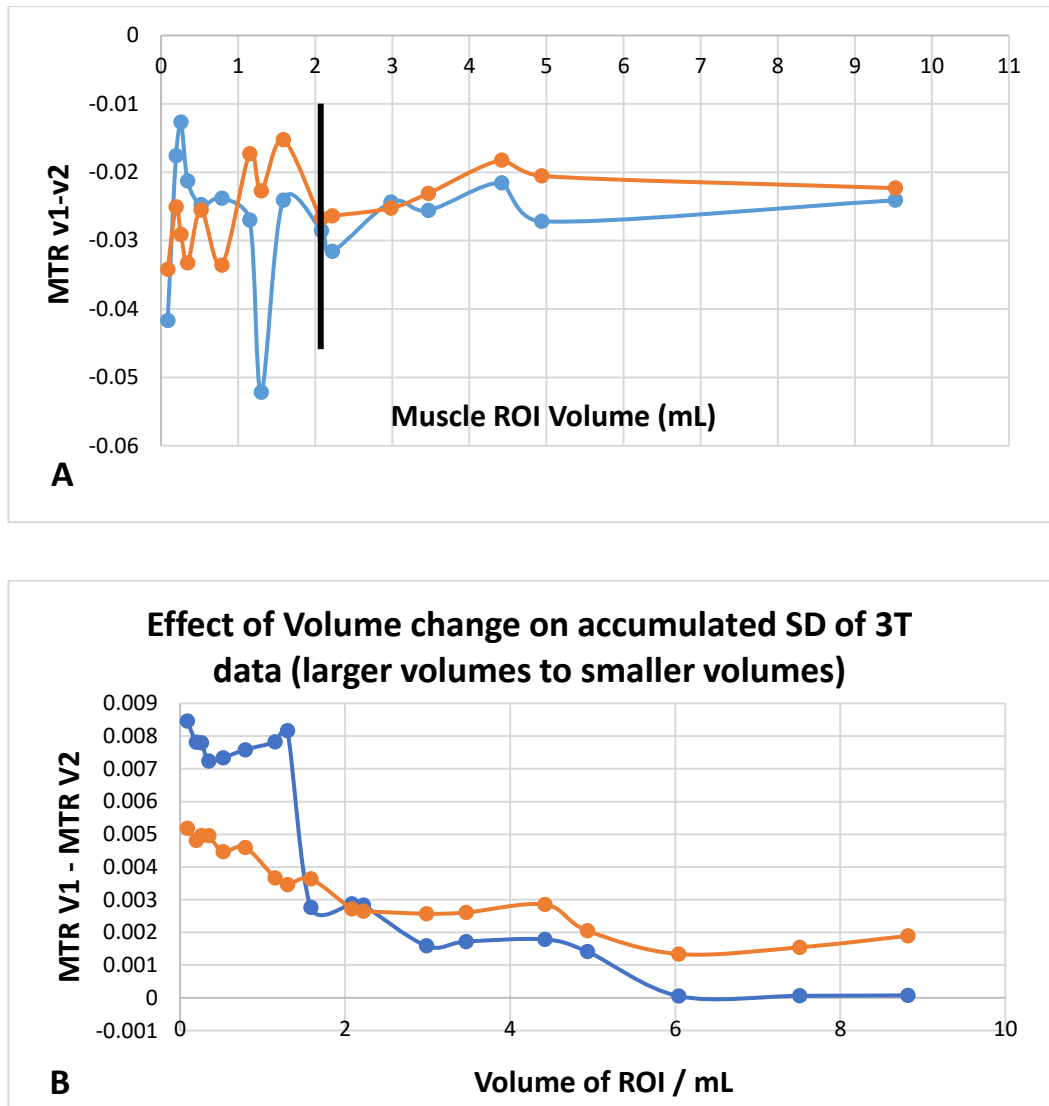


Figure 29: Data investigating the accuracy of the MTR measurement with size of ROI volume drawn. Muscle data from two subjects. The scatter plots showed more random data from just below 2 mL compared with values above 2 mL (a). An example of the effect of volume change on accumulated SD of MTR 3T data from same participants (b). The vertical line (black) at 2 mL depicted the cut off value used to exclude small volumes of fistulas.

A further 6 subjects were excluded due to low fistula volumes leaving a total of 13 participants (median age 38 ± 13 years) for final analysis. Where specific sequence data were missing, in some participants, these were excluded in individual analyses. An example of fistula volumes is presented in Figure 30. The flow chart for the subjects is included for the study in Figure 31. Initial clinical characteristics of participants are presented in Table 13.

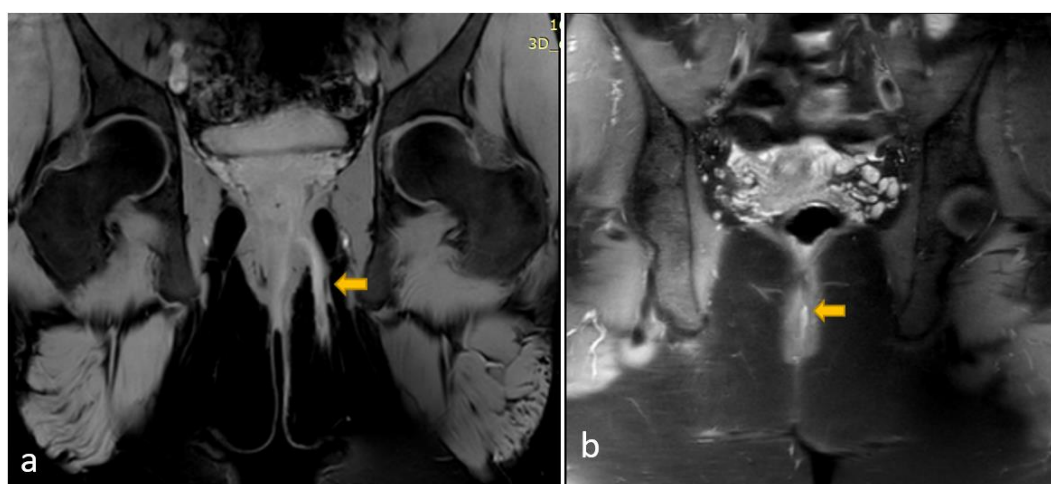


Figure 30: Example of fistula indicated by yellow arrow on MT images with (a) big volume (> 2 mL) and (b) small volume (< 2 mL) slice slice shown, however large fistula covers multiple slices .

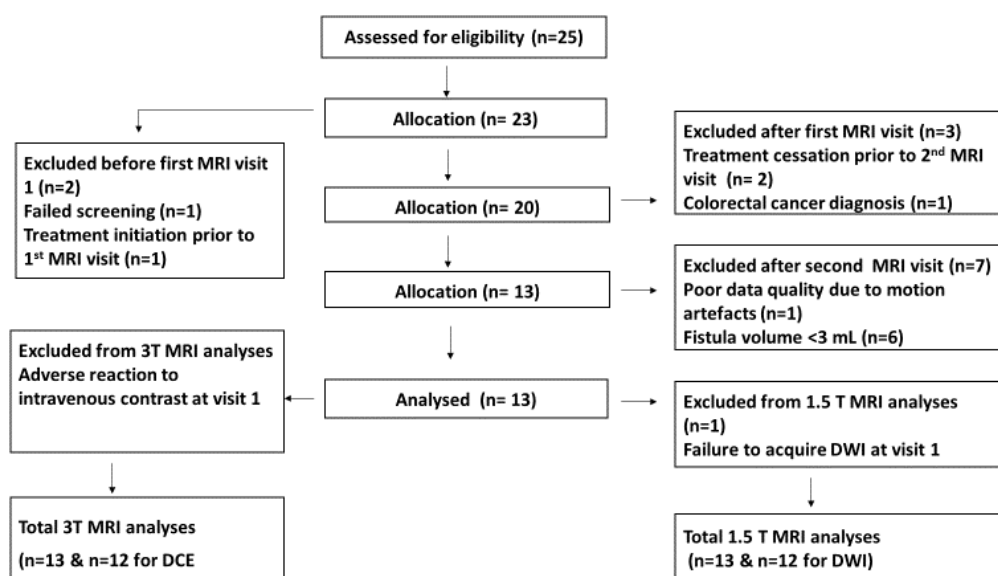
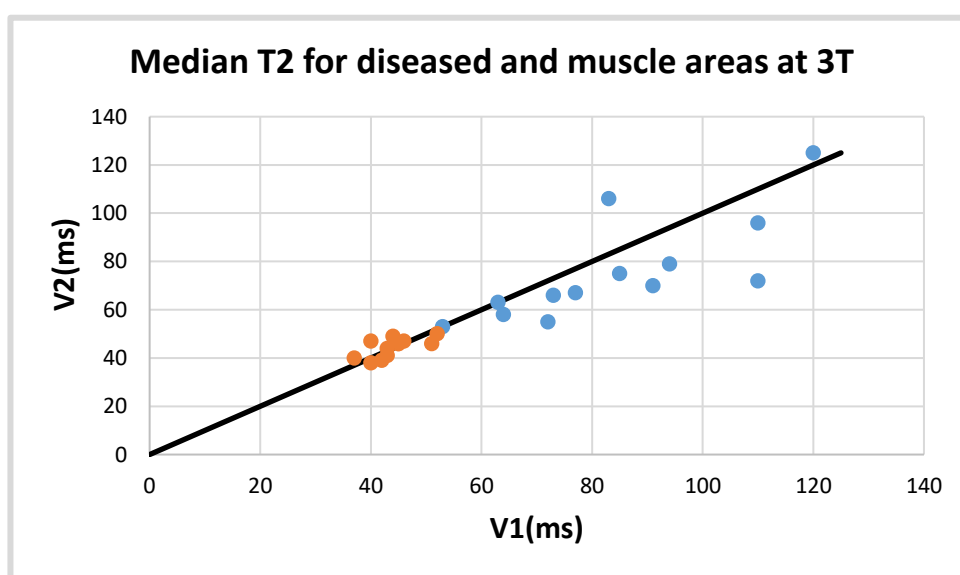


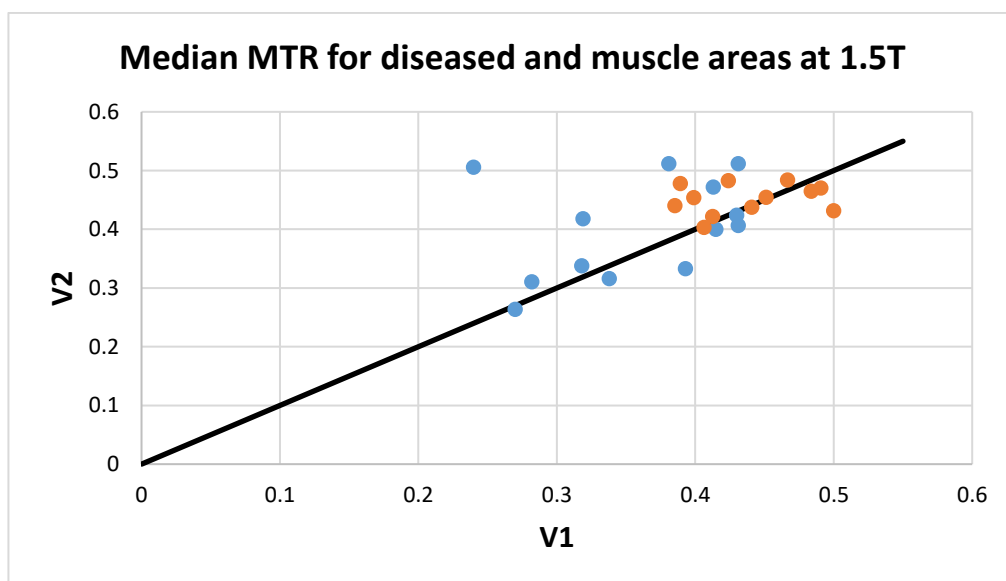
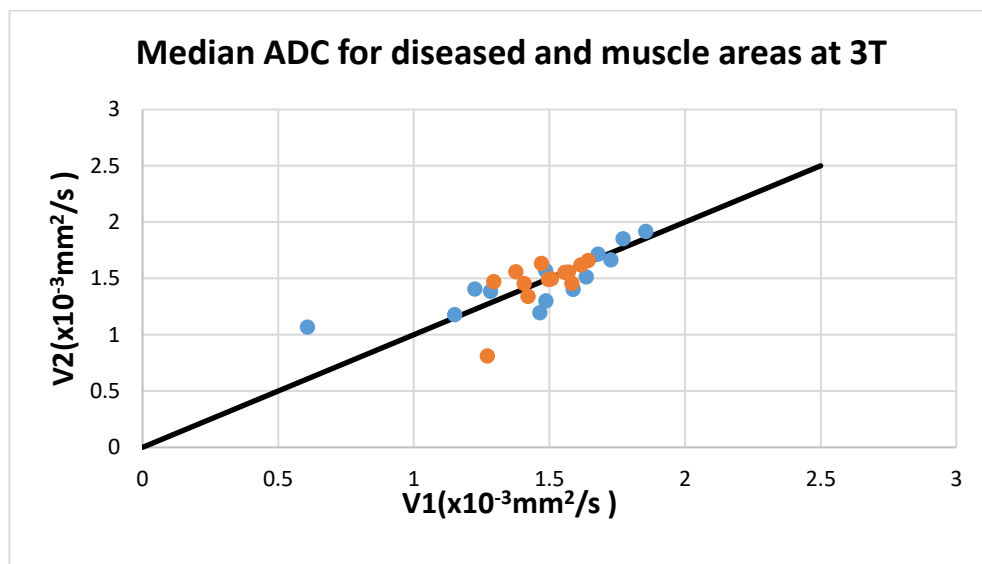
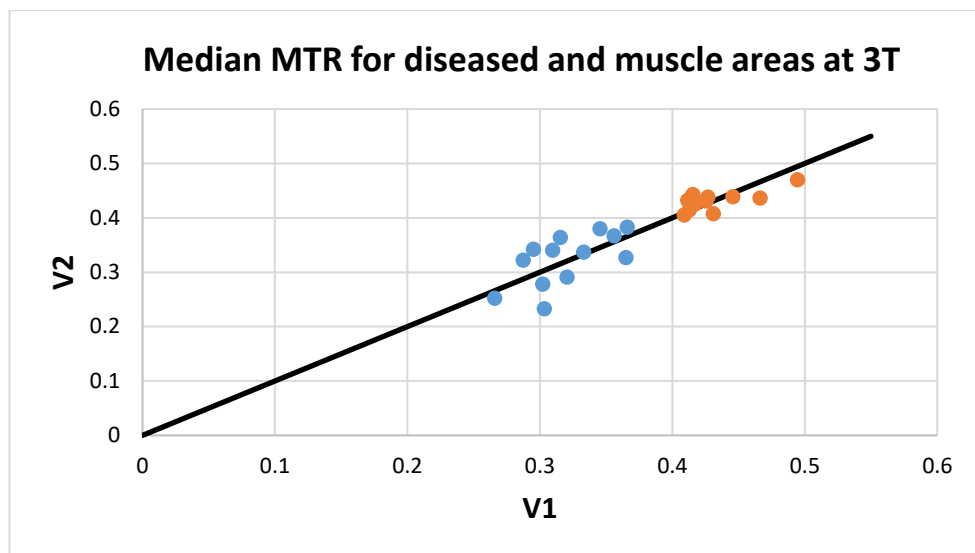
Figure 31: Flow chart.

Table 13: Participants' demographic data, and PDAI/CRP/Van Assche score results. The results in red indicate visit two while black represented in visit one.

Patient No.	Age years	M/F	PDAI	CRP mg/l	Van Assche Score	Responder Yes/No
1	27	M	5 4	10 <5	9 11	No
2	24	M	8 0	47 <5	22 7	Yes
5	37	F	7 4	<5 <5	7 9	Yes
6	45	F	8 2	12 <5	22 11	Yes
8	23	M	6 5	27 <5	20 14	No
9	27	M	11 8	<5 <5	15 7	Yes
11	52	F	11 1	74 <5	14 7	Yes
12	64	F	14 10	22 <5	16 13	Yes
13	49	M	11 4	20 <5	13 12	Yes
14	53	F	14 9	15 8	22 14	Yes
15	33	F	8 4	8 <5	20 11	Yes
19	39	F	9 10	20 26	22 22	No
20	29	F	8 9	1 1	17 19	No

Figure 32 depicts the comparison between visit 1 and visit 2 data for muscle, and the diseased tissue for the MRI parameters T2 (3T only), MT and DWI (both scanners), to determine the repeatability of these sequences. Visit 1/visit 2 muscle data showed a small clustering of the muscle data compared with the diseased tissue data (Figure 32), hence implying a stable repeatable and homogenous signal, at 3T. At 1.5T the DWI data showed a wider spread in the muscle data in comparison with other sequences. This spread was similar to that of the diseased tissue. Due to the larger spread in the muscle data at 1.5T compared with 3T, this data were not analysed further in the study.





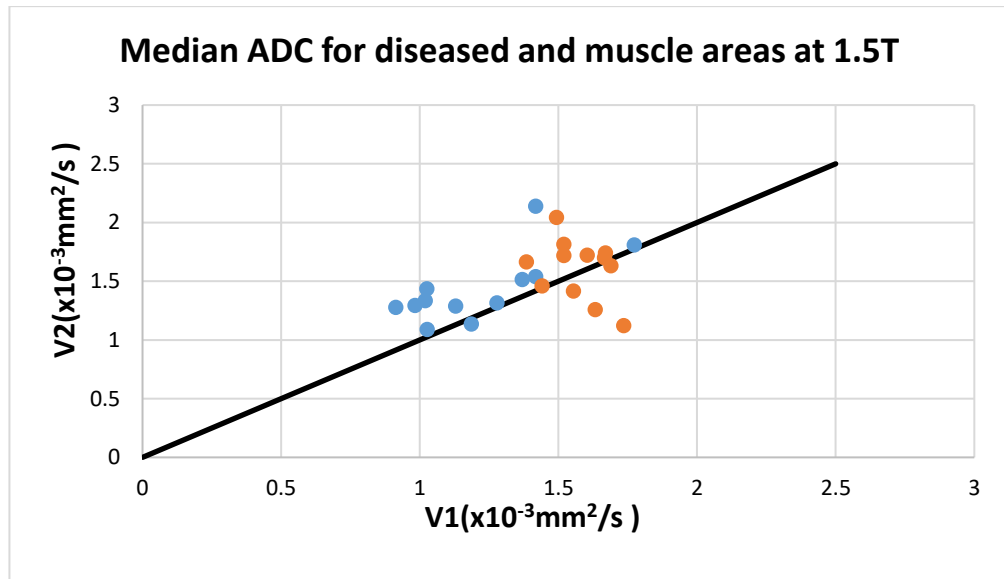


Figure 32: The muscle data (orange dots) showed a tighter cluster of results around the line of identity compared with the diseased tissue data (blue dots) for the 3T sequences, but a larger spread for the 1.5 T.

5.8.2 Comparison of findings before and after therapy in both clinical and MRI parameters

5.8.2.1 Comparison between changes in the MRI sequences after treatment

The mean and SD of MRI sequences were not significantly different before and after treatment (Table 14): T2 84 ± 20 ms vs 75 ± 20 ms ($P=0.056$), MTR 0.32 ± 0.03 vs 0.23 ± 0.04 , ($P=0.7$), DWI $1.45 \pm 0.33 \times 10^{-3} \text{mm}^2/\text{s}$ vs $1.47 \pm 0.26 \times 10^{-3} \text{mm}^2/\text{s}$ ($P=0.74$), for DCE parameters : ETM $k^{\text{trans}} 0.099 \pm 0.033 \text{ min}^{-1}$ vs $0.10 \pm 0.044 \text{ min}^{-1}$ ($P=0.45$), ETM $V_e 0.28 \pm 0.06$ vs 0.34 ± 0.12 ($P=0.06$) and ETM $V_p 0.0041 \pm 0.005$ vs 0.0038 ± 0.005 ($P=0.84$). However, there was a difference in T1 parameter before 2.36 ± 0.70 s, and 1.99 ± 0.73 s ($P=0.01$) after treatment.

The Van Assche score was significantly differenced after therapy, with the score decreasing from 16 ± 5 at baseline to 12 ± 4 ($P=0.009$) after treatment.

5.8.2.2 Comparison of changes in the clinical data after treatment

For the clinical indicators, there was a significant difference before and after treatment as shown in Table 14. After 12 weeks of biological therapy, PDAI and CRP values decreased significantly when compared to baseline ($P=0.0014$; $P=0.009$). CRP decreased from 15 (6-24) mg/l at baseline to 5 (5-5) mg/l after therapy.

Table 14: Comparisons of MRI parameters (3T MRI platforms), and clinical indicators before and after treatment.

	Pre-treatment	Post-treatment	P value
MRI parameters on 3T			
T2 (ms)	84 ± 20	75 ± 21	0.056
ADC ($\times 10^{-3} \text{mm}^2/\text{s}$)	1.45 ± 0.33	1.47 ± 0.26	0.74
MTR	0.32 ± 0.03	0.23 ± 0.04	0.7
ETM K^{trans} (min^{-1})	0.099 ± 0.033	0.10 ± 0.044	0.45
ETM V_e	0.28 ± 0.06	0.34 ± 0.12	0.06
ETM V_p	0.0041 ± 0.005	0.0038 ± 0.005	0.84
T1(s)	2.36 ± 0.70	1.99 ± 0.73	0.014
Van Assche score	16 ± 5	12 ± 4	0.009
Clinical indicators			
CRP (mg/l)	15 (6-24)	5 (5-5)	0.009
PDAI	9 ± 2	5 ± 3	0.0014

5.8.3 Disease Response

Clinical responses to treatment were determined by a drop in the PDAI score by 50% or more of the baseline SD¹⁰³. After the initiation of biological treatment, nine of the 13 participants were considered responders, and 4

participants did not exhibit a response on the therapy (Table 15). MRI parameters were not significantly different between responders and non-responders with no change in sequence data seen in the responder and non-responder groups before and after biological therapy.

There was a significant change in the Van Assche score for the responder group 16 (13-22) at baseline vs 11 (7-12) at 12 weeks ($P=0.01$) also for CRP and PDAI scores as well.

Table 15: MRI parameters and Clinical indicators [median (IQR)] at baseline, and following treatment with biological therapy for pCD using a 3T MRI scanning platform.

Scanner	MRI sequence	Non-Responders (n=4)			Responders (n=9)		
		Baseline	Follow-up	P-value	Baseline	Follow-up	P-value
3T	T2(ms)	100 (70-117)	83 (61-117)	0.25	77(67-89)	67 (59-77)	0.2
	ADC x10 ⁻³ mm ² /s	1.70 (1.51-1.82)	1.69 (1.31-1.86)	0.87	1.4 (1.18-1.61)	1.40 (1.24-1.54)	0.82
	MTR	0.29 (0.27-0.31)	0.30(0.25-0.35)	0.62	0.33 (0.30-0.36)	0.34 (0.30-0.37)	0.82
	K ^{trans} (min ⁻¹)	0.10(0.09-0.10)	0.12(0.12-0.16)	0.25	0.079 (0.06-0.12)	0.09 (0.06-0.12)	0.95
	Ve	0.31 (0.233-0.33)	0.37(0.29-0.45)	0.25	0.27 (0.23-0.32)	0.30 (0.21-0.41)	0.2
	Vp	0.009(0.0005-0.013)	0.007(0-0.009)	0.5	0 (0-0.0006)	0.0004 (0-0.0006)	0.81
	T1(s)	3.15(1.72-3.40)	2.06(1.22-3.14)	0.25	2.36 (1.6 -2.71)	1.94 (1.37-2.68)	0.09
	Van Assche score	18 (11-21)	15(11-20)	>0.99	16 (13-22)	11 (7-12)	0.011
Clinical Indices	CRP (mg/l)	15 (3-25)	5 (2-20)	0.75	15 (6-34)	5 (5-5)	0.015
	PDAI	7 (5-20)	7 (4-9)	>0.99	11(8-12)	4 (1-8)	0.009

5.8.4 Correlation of clinical indicators with MRI parameters.

No significant correlations between MRI parameters and clinical indices were observed as shown in Table 16. The Van Assche score was positively correlated with both PDAI and CRP ($p=0.002$). $P=0.006$, respectively.

Table 16: Correlation of MRI parameters with clinical indicators after treatment. The Pearson correlation used for the correlated PDAI score and the Spearman correlation used for the correlated CRP with MRI parameters.

			Correlation from Pearson / Spearman	
MRI parameter	Clinical Indicator	N	R	P value
T2	PDAI	26	0.27	0.16
	CRP	26	0.22	0.26
MTR	PDAI	26	0.08	0.67
	CRP	26	-0.03	0.86
ADC	PDAI	26	-0.0007	0.91
	CRP	26	0.05	0.79
k^{trans}	PDAI	24	-0.06	0.75
	CRP	24	-0.08	0.7
ETM Ve	PDAI	24	0.15	0.47
	CRP	24	-0.38	0.06
ETM Vp	PDAI	24	-0.2	0.32
	CRP	24	-0.19	0.36
T1	PDAI	24	0.15	0.47
	CRP	24	0.07	0.71
Van Assche score	PDAI	26	0.57	0.002*
	CRP	26	0.51	0.006
* Those are significant >0.005				

5.8.5 Correlation between MRI Parameters

The correlation between MRI parameters is presented in Table 17 with the MTR parameter negatively correlating with several other parameters, such as, T2, K^{trans} and ADC (Pearson correlation coefficient: $r=-0.73$, P

<0.0001, $r = -0.56$, $P = 0.0005$; $r = -0.61$, $P = 0.0009$, respectively). No correlation was found between MTR with T1 ($r = -0.26$, $P = 0.21$), V_e ($r = -0.02$, $P = 0.9$) and V_p ($r = -0.44$, $P = 0.02$). T2 correlated with T1 ($r = 0.64$, $P = 0.0006$). No correlation was found between T2 with K^{trans} ($P = 0.19$), V_e ($P = 0.22$), V_p ($P = 0.46$), and ADC ($P = 0.039$). There was no correlation between ADC with T1 and DCE parameters. The Van Assche score was positively correlated with T2 ($r = 0.55$, $P = 0.0003$). Figure 33 shows scatter plots for some of the key correlations between the different MRI parameters measured.

Table 17: Correlation between MRI parameters.

				Spearman /Pearson Test	
Scanner	Variable 1	Variable 1	N	R	P value
3T*	T2	MTR	26	-0.73	<0.0001*
		ADC	26	0.406	0.039
		T1	24	0.64	0.0006*
		K ^{trans}	24	0.27	0.19
		Ve	24	-0.25	0.22
		Vp	24	0.15	0.46
		Van Assche score	26	0.55	0.003*
	MTR	ADC	26	-0.61	0.0009*
		T1	24	-0.26	0.21
		K ^{trans}	24	-0.65	0.0005*
		Ve	24	-0.025	0.9
		Vp	24	-0.44	0.02
		Van Assche score	26	-0.37	0.056
	ADC	T1	24	0.44	0.02
		K ^{trans}	24	0.32	0.12
		Ve	24	-0.11	0.57
		Vp	24	0.14	0.49
		Van Assche score	26	0.18	0.35
	k ^{trans}	T1	24	-0.24	0.24
		Ve	24	0.5	0.01
		Vp	24	0.35	0.09
		Van Assche score	24	0.14	0.5
	ETM Ve	T1	24	-0.71	<0.0001*
		Vp	24	0.34	0.1
		Van Assche score	24	0.19	0.36
	ETM Vp	T1	24	-0.15	0.47
		Van Assche score	24	0.19	0.35
	T1	Van Assche score	24	0.41	0.04

*Bonferroni correction adjusted significant p = 0.0071 for 3T

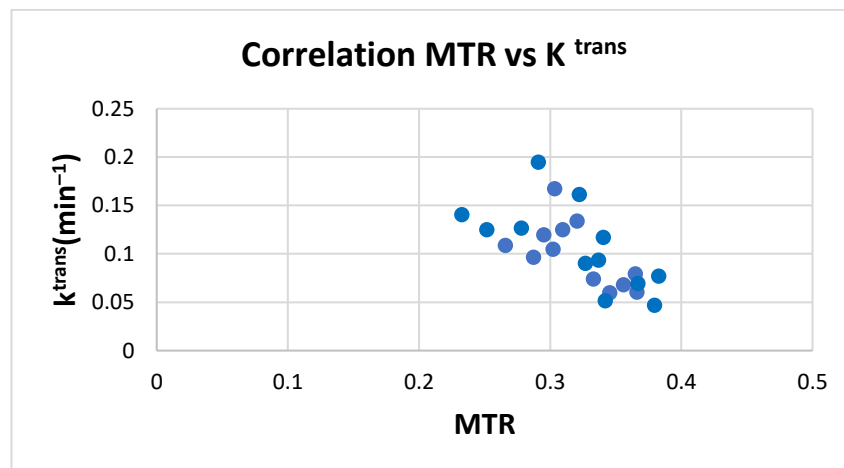
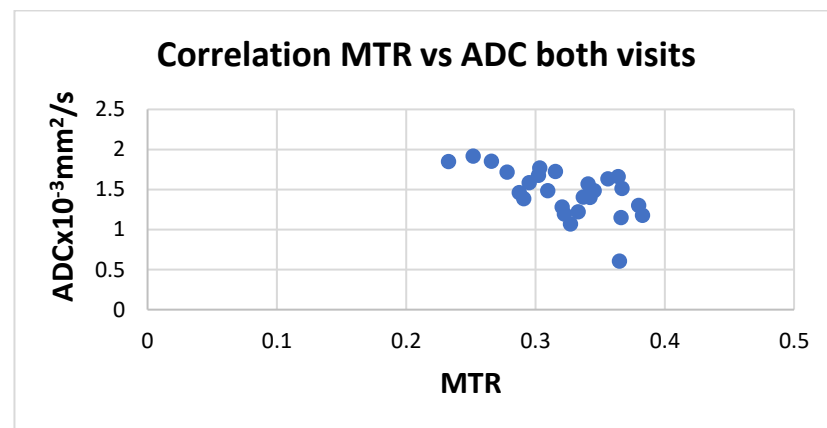
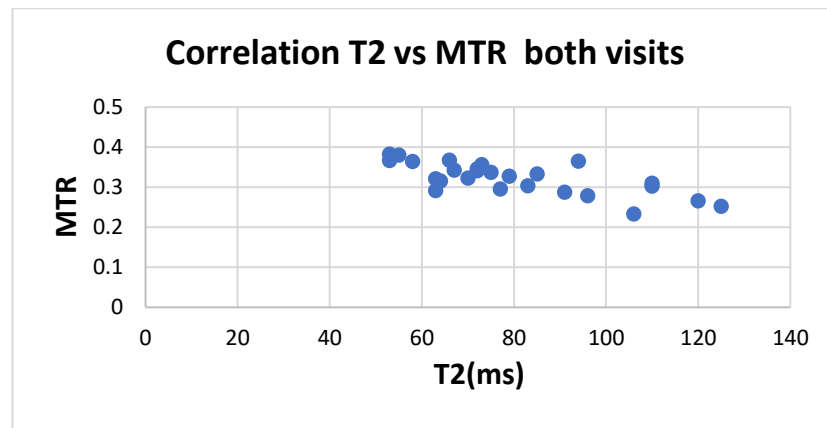


Figure 33: Correlation between median values of some of the MRI quantitative parameters.

5.9 Discussion

In this study, we investigated the feasibility of quantitative MRI sequences (T2, DWI, MT, and DCE) for the assessment of perianal fistulas in CD, its utility for the assessment of disease activity, and variations that reflected the treatment effects.

Our major finding is that in the MRI assessment of perianal CD, the repeatability, of acquired data sets, is relatively poor on 1.5T MRI platforms and for fistula volumes less than 2mls (albeit indicated from MTR data) irrespective of which MRI platform was used. This finding may be of some clinical significance as at present most worldwide health care systems use 1.5T MRI systems to monitor the response of biological treatment in pCD rather than 3T systems. However, for quantitative measurements our data suggested that 3T systems would be more accurate instead of 1.5T. Having said this, the non-quantitative measure of activity (Van Assche Score) which was measured from the 1.5 T data, did show good correlation with the clinical indicators (CRP and PDAI). We did not however directly compare the standard Van Assche scoring from the T2 weighted imaging at the different field strengths.

Also, we found that in patients undergoing biological therapy, for the majority of MRI parameters, no significant differences after treatment were observed. This may have been because of the heterogeneous response to treatment, small sample size, and early assessment at 12 weeks. Looking at 12 weeks may have been too early to determine treatment response,

however the techniques described may be useful to check after a longer time period and may better indicate deep remission and healing and therefore aid the clinical decision to stop therapy. Furthermore, these quantitative sequences did not correlate with CRP, or PDAI. A significant difference was observed in the Van Assche score after treatment that also strongly correlated with both PDAI and CRP.

MTR²⁰⁵ and T1 signals are expected to increase with fibrosis, as the tissue has a predominantly macromolecular composition, whereas the presence of fibrosis is associated with a low T2 signal. The amount of fibroblasts and collagen is associated with a decreased T2 signal in the bowel wall¹⁶². T2 and k^{trans} are expected to increase with inflammation as they directly express the amount of fluids within the pathological wall (increased tissue perfusion and vascular permeability). ADC can be affected by changes in perfusion, and cell size from inflammation and fibrosis.

We have observed a negative correlation between DCE (K^{trans}), T2 and ADC with MTR dataset. This may suggest that as tissue inflammation recedes due to response to therapy, the residual tissue may have a higher MTR response due to the residual fibrosis, or that the MTR signal may also be affected by inflammation²⁰. In the absence of histological tissue it is not possible to offer any further conclusions.

Dynamic contrast-enhancement is a sensitive non-invasive measure of tissue inflammation, and offers more physiological information. This

sequence has the ability to quantitatively reflect tissue characteristics due to its dynamic evaluation of contrast agent inflow. DCE has been shown to have great potential as a non-invasive measure pCD activity^{4, 102, 191}.

Experimental work in vascular biology has highlighted the important relationship between CD activity and neo-angiogenesis. Angiogenesis is a fundamental component of complex biological processes including chronic inflammation and cancer, which refers to the creation of new capillaries in existing blood vessels. Acute inflammation is characterised by an increase in vascular perfusion, while advanced chronic disease progression is associated to fibrotic tissue deposition as a consequence of a reduction in vascularisation that results in a reduction in tissue perfusion³⁴. Angiogenesis and inflammation are intertwined in CD patients. Inflammatory cells produce diverse angiogenic factors that promote the growth of micro-vessels. This increased permeability is highlighted by high K^{trans} value. Vascular remodelling and neo-angiogenesis are often present in CD patients²⁰⁶, and biological treatment decreases this inflammation-induced angiogenesis in CD patients²⁰⁷. K^{trans} measurement may help in the prediction of outcome. A decrease in K^{trans} value after biological therapy indicates changes in vessel permeability, and a reduction of angiogenesis, and a possible initial anti-inflammatory effect of therapy on those patients.

Earlier research has been focused on qualitative assessment¹⁹¹, whereas more recent studies have investigated the correlation between fistula activity with quantitative⁴ (complex pharmacokinetic modelling) or

semi-quantitative¹⁰² (analysis of the parameters of the time-intensity curves) analysis. We have demonstrated a weak correlation between a perianal disease activity index, and the MR-based Van Assche score. Nevertheless, we observed a difference in the Van Assche score before and after treatment. Moreover, when the groups were dichotomised in responder and non-responder cohorts a difference was observed in the responder group. Ziech *et al.*⁴ performed the first study using a quantitative DCE model to evaluate disease activity in patients with pCD. They observed a correlation between the quantitative parameters, and the perianal disease activity index. They found that the quantitative parameter (K^{trans}) in the responder group had decreased considerably 6 weeks after the start of treatment with anti-TNF therapy ($p = 0.027$), indicating a predictive parameter of the therapeutic response. Unlike our study, we have not found a reduction in quantitative parameters in the responder group. The reason for the contradiction between our results and Ziech *et al.* may be related to sample size, where, in their study, their results were obtained from a small cohort (only six patients underwent follow-up MRI), whereas in our study, 19 participants treated with biological therapy.

Also, similar to our findings, the quantitative DCE parameters K^{trans} , V_p and V_e , did not correlate with the Van Asche score, CRP and PDAI. These observations might be explained by the poor performance of the clinical scoring system, and by the early and possibly premature assessment at 12 weeks. Moreover, the small sample cohort and the heterogeneity of the perianal disease burden might have weakened a possible correlation.

Diffusion imaging is another MRI method we have investigated in the MAP study. This sequence is based on restrictions of the free movement of tissue water molecules due to ischaemia, the presence of macromolecules or increased cellularity. These restrictions can be calculated through ADC. DWI offers an excellent contrast between lesions, such as, inflammatory foci or cancer, and the surrounding tissues. DWI sequences have recently been studied for the evaluation of fistulas^{186-189, 208}. In a retrospective study involving 24 patients with 41 lesions (23 active lesions and 18 inactive perianal fistulae), the mean value for ADC in the active perianal fistula group ($0.908 \pm 0.171 \times 10^{-3} \text{mm}^2/\text{s}$) was significantly lower than for the inactive group ($1.124 \pm 0.244 \times 10^{-3} \text{mm}^2/\text{s}$). A significant decrease in ADC in active pCD was replicated in other studies¹⁸⁹. However, no significant difference in mean ADC between active and inactive lesions was found by other researchers^{188, 208}.

In MAP, we observed no difference in ADC before and after treatment. Moreover, when the groups were dichotomised in responder and non-responder cohorts no difference in ADC was observed. The difference in results between these studies and ours could be attributed to the fact that our study used two b values 0 and 600 s/mm² whereas Dohan *et al.*¹⁸⁸ used DWI with b-values of 0, 600 and 1000 s/mm² and Hori *et al.*¹⁸⁷ used DWI with b-values of 0 and 800 s/mm² also the main aims of their studies were completely different from our study.

The magnetisation transfer sequence has been the most recent and promising technique for assessing tissue fibrosis. To the best of our knowledge, there has only been a single study assessing the feasibility of MT in pCD ¹⁸⁵. MTR will be high in tissues that are abundant in macromolecules (which is typically the case in fibrosis due to collagen deposition) ¹⁸⁵. A group of 29 patients with pCD underwent MT sequences (one with an off-resonance saturation pulse of 800 and one with 1200 Hz), T2/T1-weighted gadolinium-enhanced and DWI sequences. They characterized active fistula if the fistulas presented with bright contrast enhancement, diffusion hyperintensity and T2 hyperintensity. In non-active fistulas, MTR was significantly lower at 800 Hz than in inactive fistulas while no significant difference was observed between both groups in MTR at 1200 Hz. MTR at 800 Hz was significantly correlated with the Van Assche average score. In our study treatment did not induce a difference in MTR, no difference in MTR was observed between responders and non-responders. The reason for the contradiction between our results and Pinson *et al.* might be related to the way the 'active' fistulas were defined, in their study, they used fistulas presenting with bright T2 hyperintensity, diffusion hyperintensity (at b1000), and contrast enhancement were characterised as active, whereas in our study, we divided the clinical findings (PDAI>4), and all should have presented as 'active' disease to correspond with the previous study as they were going to receive treatment for it.

To best of our knowledge, quantitative T2 relaxometry has not been used in pCD. STIR or fat-saturated T2W images are most sensitive for

detecting fluid and inflammation including fistula tracts. In this study, we observed no difference in T2 before and after treatment. Moreover, when the groups were dichotomised into responder and non-responder cohorts no difference in T2 was observed either. This might be explained by the fact that we defined the fistula in follow-up visit to include only the visibly 'inflamed' regions of the fistula, which could automatically lead to similar T2 measures albeit covering a possibly smaller region. Future work might need to incorporate the T2 signal intensity and fistula volume into a composite measure.

The van Assche score was calculated from T2 weighted images, and used for evaluation to treatment response in pCD. The evaluation of an MRI based score is difficult because there is no gold standard for determining whether a fistula has healed or responded. Longer-term studies replicated those findings too, and also showed a dis-concordance of at least 12 months between clinical and radiological remission¹⁹⁹. The score was insensitive to clinical response, with no significant difference shown in clinical responders to anti-TNF therapy before and 6 weeks after treatment, nor was there any difference in the score, between clinical responders, and non-responders, that did not change significantly before or after treatment¹⁹². No correlation was found between these MR-based measures of fistula activity, and other known markers of disease activity, such as, CRP and the PDAI¹⁹². Previous studies assessing the application of the original van Assche index used various definitions and methods for the determination of fistula response, healing and remission^{101, 156, 159, 160, 197}.

This score was assessed in short and long terms. In both short-term and mid-terms, the score decreased significantly but not in the long-term. Van Assche *et al.* found that there was no significant difference in responder baseline activity as opposed to the non-responder group, but a greater decrease in activity was observed in the responding group after six to ten weeks of treatment¹⁰¹.

In a study which assessed 59 infliximab-treated patients, the authors found a significant decrease in the Van Assche score in the short-term ($p < 0.002$) and med-term ($p < 0.0001$), but not over the long-term (1 year) ($p = 0.1$)¹⁵⁶. However, Ng *et al.*¹⁹⁷ assessed 34 patients who received anti-TNF drugs in another long-term follow-up study, did not find a significant variation in the Van Assche score between responders and non-responders, also, they found no significant difference in a responder group at 6, 12, and 18 months. Similarly, when more than 20 patients were evaluated at baseline, and after one year of anti-TNF therapy, the researchers found that the Van Assche score did not change in responders, non-responders and in remission patients¹⁵⁴. In another study, involving 16 patients, who were treated with anti-TNF, no significant changes in the score have been observed after treatment, despite clinical improvement or a reduction in biological markers, such as, CRP¹⁰².

Our findings are similar to almost all of those studies, which found that the Van Assche score was not significantly different between responder and non-responder groups.

In the present study, we found sensible correlations in terms of MRI parameters, but the majority of these parameters did not correlate with clinical indexes. Further studies are required with larger sample sizes, and follow up scans at more than 6 months, which may result in a better correlation between these quantitative measurements with clinical indicators, and the Van Assche score.

Our study had a number of limitations. First, the sample size was relatively small, which limited the results, which could be produced. Second, for each case, only 2 MRI scans were evaluated (in baseline and in the follow up), and the time window ranged considerably from 1 to follow-up MRI scans (at around 12 weeks). This time window proved not long enough to identify if the patient was responding or not responding to biological treatment. The twelve-week period was chosen due to time constraints from the grant funder (MRC – Confidence in Concept). Therefore, future studies should include serial MRI scans for patients using prolonged follow-ups in order to gather evidence on the best timing for MRI scans. Third, we did not carry out intra- and inter-rater reliability for these MRI results, in terms of fistula definition from the radiologists. The 1.5T quantitative data revealed poor repeatability in the muscle regions but in newer 1.5 T systems with more advanced hardware could provide more reliable data if used in future studies. This study also had many outcome parameters and used multiple comparisons to investigate the relationship between them which is another limitation of the study. Moreover, pCD studies have been hampered by a lack of an externally validated gold-standard measure. Nevertheless,

previous studies have suggested that baseline symptoms as measured by PDAI, CRP and a baseline T2 signal had a moderate ability to predict response as early as 12 weeks. Present MRI sequences should be revalidated in a larger sample cohort with larger fistulas, with a longer disease follow-up using 3T MRI platforms. These MRI measures together with a clinical core-outcome set should form the basis of objective assessments of future therapies in pCD

In conclusion, this study compared parameters of quantitative T2, MT, DWI and DCE-MRI in pCD patients, and found negative correlations between the 3T MTR-MRI data with T2, ADC and K^{trans} . These correlations will be used to help design a larger further study. There was no difference before and after treatment, and no difference between responders and non-responders (defined clinically) was observed. These advanced techniques might play an important role in the treatment of pCD patients in the future. However, as a relatively small number of patients were included in the study, a larger sample should be investigated in future studies.

Chapter six: Using non-contrast sequences to characterise and quantify the small bowel wall in healthy volunteers (REPEAT study)

Abstract

Background: Quantitative sequences including: Intravoxel Incoherent Motion (IVIM) diffusion-weighted imaging (DWI), relaxometry (T1 and T2) and Magnetisation Transfer (MT) sequence have been investigated as objective imaging biomarkers of fibrosis and inflammation in Crohn's disease (CD). To ensure a complete technical validation of these sequences, a repeatability measure is needed to ensure the stability of the measured parameter. Objectives: to measure the repeatability of the measurement from the sequences in the normal small bowel.

Methods: Ten healthy volunteers were scanned at 3T on 2 separate occasions (minimum 2-week gap) using the above quantitative sequences to determine the repeatability of this methodology. Participants ingested 1 litre of oral contrast agent 45 minutes prior to scanning, and two doses (20mg each) of buscopan (hyoscine-butylbromide) were administered intravenously to reduce peristaltic motion during the scans. To assess the inter-observer variability in the measurements, two observers independently delineated multiple regions of interest in the small bowel for the different imaging sequences. Test-retest repeatability and inter-observer agreement were assessed using the coefficient of variation (CoV), and the intra-class correlation coefficient (ICC).

Results: Test-retest repeatability of these sequences in the bowel wall was excellent for ADC, MTR, T1, and D (mean CoV 5%(N=10), CoV= 7%(N=10), 8% (N=9), and 10%(N=10), respectively, and good for PF (20%,N=10), and acceptable for T2 (21%,N=8), while poor for the D*(CoV 31%, N=10).

Inter-observer variability was almost perfect for the T2 and D sequences (ICC =0.89, N=16), (ICC=0.86, N=20), good for ADC (ICC= 0.76, N=20) and moderate for T1 and PF(ICC=0.55, N=16) , (ICC =0.41, ,N =20), respectively, but poor for MTR, and D* (ICC=0.08, N=20: ICC=0.14,N=20), respectively. The dynamic range of MT data in the healthy volunteers was low, thereby, affecting the measured ICC.

Conclusion: Excellent repeatability for T1, MTR ADC and D was observed, and, in general, acceptable for PF, and T2, but this approach showed poor repeatability of D*. Our study indicated that good to excellent inter-observer variability could be obtained during the measurements of T2, T1 relaxations, D, ADC and PF of the small bowel which could serve as a potential imaging marker in the diagnosis, and, especially, in the follow-up of CD. T2 and T1 of the small bowel were repeatable measurements, which might prove to be accurate imaging biomarkers in IBD, and MT, and might also be useful for fibrosis. In general, these parameters produced reliable and repeatable results in the healthy bowel wall.

6.1 Introduction

Quantitative analysis of MRI sequences such as T1 and T2, DWI and MT has been increasingly used for the evaluation of IBD^{20, 168, 179}. The success of accurate interpretation of bowel pathologies requires an optimal preparation and acquisition. Fold visualization, and luminal distension are the determining factors in the imaging of the gastrointestinal tract. An oral contrast agent is necessary, which should cause uniform intra-luminal signal attenuation, high contrast between the bowel wall, and luminal content, minimal mucosal absorption leading to maximum distension, absence of artefact formation with no significant adverse effects.

IVIM is a technique based on DWI with multiple b values, and is used to quantitatively assess pure molecular diffusion and blood perfusion -with the parameters of perfusion-related diffusion coefficient (D^*), perfusion fraction (PF), and pure molecular diffusion coefficient (D). In this technique, multiple b-values, such as, 0, 20 and 800 s/mm², are typically used. A b-value of 20 s/mm² has a minimal weighting in diffusion that makes fluid more conspicuous by loss of signal in the blood vessels. A high b-value of 800–1000 s/mm² negates the signal intensity of fluid, and normal bowel mucosa, and is best used to identify inflammation²⁰⁹. IVIM has been used to characterise tumours, and to assess tissue response to treatment in a number of organs, such as, in the kidney, liver and prostate²¹⁰⁻²¹⁴. This model has recently been used to measure inflammatory activity in CD and to predict histological fibrosis¹⁷⁹⁻¹⁸².

Another quantitative MRI approach, MT reflects the exchange of magnetisation between protons in free water and protons bound to semisolid macromolecules through chemical exchange, and/or dipole-dipole interactions. The simplest imaging method to obtain an estimate of the MT effect is the MTR calculated from the signal intensity with and without the off-resonance pulse. MT-MRI is a promising sequence for the evaluation of CD fibrosis^{20, 168}.

T2- and T1-weighted sequences are used as disease activity biomarkers in inflammatory CD²¹⁵, with T2-weighted images showing inflammation as a hyperintense signal. Relaxometry is a quantitative technique that allows the absolute quantification of MRI parameters, such as, T1 and T2 relaxation times. T2 and T1 can be measured in the abdomen by using fast image sequences, such as, spin-echo-prepared ²¹⁶ or inversion recovery-prepared sequences ²¹⁷. This assessment is assumed to be more reproducible, and sensitive than the visual assessment of T1- and T2- weighted images for identifying minor contrast changes, such as, tissue oedema. Therefore, it is used for quantifying well-defined pathologies, such as, cartilage degeneration, cardiac iron overload, and cerebrovascular accidents ²¹⁸⁻²²⁰. Abdominal T1 and T2 mapping have proven more difficult because of the movement of intra-abdominal viscera with diaphragmatic movement during breathing. However, this method has already been successfully applied in pancreatic ^{221, 222} and hepatic imaging²²³ but has not been widely used in intestinal imaging.

To ensure accurate technical validation of these potential imaging biomarkers, a thorough assessment of precision and bias needs to be undertaken. A repeatability assessment should be undertaken, especially, when investigating a disease with certain tissue parameters or discriminating between healthy and pathological tissue. The knowledge of measuring repeatability is important in any study because it enables differentiation of true change caused by tissue biology, from a change in signal related to technical inaccuracies. There is limited literature investigating the repeatability of these quantitative sequences in the small bowel. Repeatability studies have been published for IVIM-DWI in the orbit²²⁴, and liver²²⁵. However, the repeatability of MT and relaxometry sequences have not been widely evaluated outside the central nervous system²²⁶. Moreover, the oral preparation used prior to MRE may have an effect on the signal intensity due to differential small bowel distension. However, evaluating the repeatability of these types of sequence in the bowel is important before embarking on disease-specific imaging biomarker validation studies. Therefore, this study aimed to measure the precision and bias in T2, T1, IVIM-DWI and MT small bowel wall measurements through an assessment of repeatability following bowel preparation, and administration of an anti-spasmodic agent in healthy volunteers on a single MRI platform, and an assessment of inter-observer variability.

6.2 Materials and Methods

6.2.1 Participants

This was a single-centre study potentially recruiting healthy volunteers. The exclusion criteria included any history of gastrointestinal disease, pregnancy, contra-indications to the anti-spasmodic agent, and use of medication known to affect gastrointestinal transit. The study was approved by the University of Nottingham Medical School Ethics Committee (J/3/2007/17). All participants gave written informed consent, and were screened for contraindications to MRI before participating.

Participants were scanned twice using an identical protocol, with a minimum of a two week interval between sessions 1 and 2. Participants were asked to fast from 22:00 hours the previous evening, and to avoid ingesting caffeine and alcohol, and taking part in strenuous exercise the day before the study start time. Forty minutes before scanning, volunteers were given 1000 mL of bowel preparation (2.5% mannitol with 0.2 % locust bean gum), to ingest slowly before scanning, to distend the small bowel. After initial planning scans, and before the quantitative acquisitions, participants were given two separate doses of an anti-spasmodic agent (hyoscine butylbromide 20 mg) intravenously to immobilise the small bowel. One dose was administered before the T2 and DWI measurements, and the second dose before the T1 and MT sequence acquisition (Figure 34).

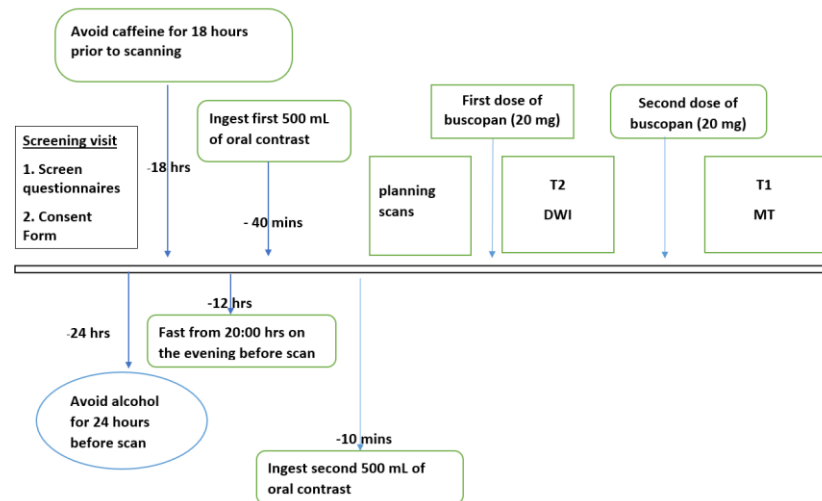


Figure 34: Protocol schematic for the repeatability study in healthy volunteers.

6.2.2 MRI protocol

Imaging was performed using a 3T Ingenia scanner (Philips, Best, The Netherlands) with participants positioned in a feet-first prone position. A respiratory belt was placed between the subject's back and the MR coil using a velcro attachment to ensure a stable position, and a good respiratory trace for triggered scanning. Sagittal, axial, and coronal localisers were obtained to plan the position of the other sequences. The quantitative sequence information is given below and is also presented in Table 18.

- T2 data was acquired from a single coronal slice spin-echo prepared bTFE sequence²¹⁶, using six echo times (TE) of 20, 50, 80, 120, 180, 300 ms. FOV 340mm x 352mm, slice thickness 5 mm, and TR/TE of 3.4 /1.68 ms were used. A separate breath-hold for each TE was used with a minimum wait time of 15 seconds between different TEs for full relaxation before the next acquisition. The position of the slice

was towards the anterior of the body to maximise coverage of many small bowel loops.

- Coronal IVIM-DWI respiratory triggered data were acquired using a single-shot spin-echo echo-planar sequence (SE-EPI), with 9 b values: 0, 50, 100, 200, 300, 400, 600, 800, 1000 s/mm². Other parameters for this sequence included TE-71 ms, TR-1278 ms, FOV 400mm x 400mm, slice thickness of 5 mm and matrix 132 x 133. A total of 12 slices were acquired for the DWI. They were positioned coronally to include as much of the small bowel as possible starting from the most anterior region of the visible small bowel.
- T1 data was acquired from a single coronal slice using an inversion recovery prepared TFE sequence positioned in the same location as the T2 data. Nine inversion times (TI) were acquired with TI 500, 650, 800, 950, 1000, 1150, 1300, 2500, and 5000 ms. An additional image with no inversion was also acquired, to obtain the M₀ part of the equation for fitting the data. Data were collected with a FOV 375mm x 351mm, slice thickness 5 mm, matrix 252 x 176, and TR/TE of 10 / 2.3 ms. Separate breath-holds with 15s of free breathing between acquisitions were used for each of the different TI data sets to provide a complete MRI signal recovery between images.
- MT data were acquired from a single coronal slice TFE scan positioned at the same location as the T2 data. Parameters: FOV

375mm x 351mm, slice thickness 5 mm, matrix 252 x 176, and TR/TE of 20 /2.3 ms were used. This sequence was a breath-hold scan. There were two different dynamics during the breath-hold (MT-on and MT-off), with a 10-sec gap between the two dynamics of each scan. The MT on – pulse used an off-resonance frequency pulse of 1000Hz.

Table 18: Parameters of MRI scans.

MRI parameters	T2 scan	T1 scan	DWI scan	MT scan
Sequence	single coronal slice spin-echo prepared bTFE	single slice T1 weighted inversion recovery spoiled TFE	coronal single-shot spin echo-planar	single coronal slice TFE
Slice thickness(mm)	5	5	5	5
TR/TE (ms/ms)	3.4 /1.68	10 /2.3	1278/71	20 /2.3
field of view (mm ²)	340 x 352	375 x 351	400 x 400	375 x 351
Number of slices	1	1	12	1
Breath-holding (BH)	1 BH per TE value with a 15-sec gap between TE acquisitions	1 BH per TI value with The 15-sec gap between TI acquisitions	respiratory triggered	10s BH to cover both 'on' and 'off' resonance image acquisition
Parameter variation	TE-prep values: 20, 50, 80, 120, 180, 300 ms.	TI values:500, 650, 800,950 ,1000,1150 ,1300,2500,5000 ms.	b-values:0,50,100,200,300, 400,600,800,1000 s/mm ² .	Presaturation MT on /off pulse

6.3 MRI Data Analysis

Parameter fitting

T2 measurements: Small bowel wall T2 was measured from motion-corrected images to remove distortions due to respiration and peristalsis. Multiple regions of interest (ROIs) were defined using custom-written software in MATLAB (R2017a, Mathworks) to identify the bowel wall on each data set (written by Dr Hannah Williams). This software used a combination of edge-detection and thresholding to identify the walls semi-automatically²²⁷ (not published yet). The bowel was isolated so that further analysis was only run on the bowel rather than the whole image. Following that, a mask of the bowel wall was made using edge detection and thresholding, with any misidentifications, manually removed (Figure 35) (such as the wall of the bladder, stomach or colon). To obtain information about the T2 of the bowel content an ROI was drawn inside the bowel, to include only the bowel content. This step was repeated three times to obtain three different content ROIs. The purpose of this step was to determine whether significant through-plane motion had occurred during the acquisition, and so use its content to determine whether any echo times needed to be deleted. This step was also used to measure the content signal, so that it could be taken into account during the fitting. The data from the ROIs were then fitted to a model of the signal evolution to calculate the T2 of the ROI, taking into account the T2 of the content, and partial volume effects. The programme fitted the data for T2, T1 and M_0 .

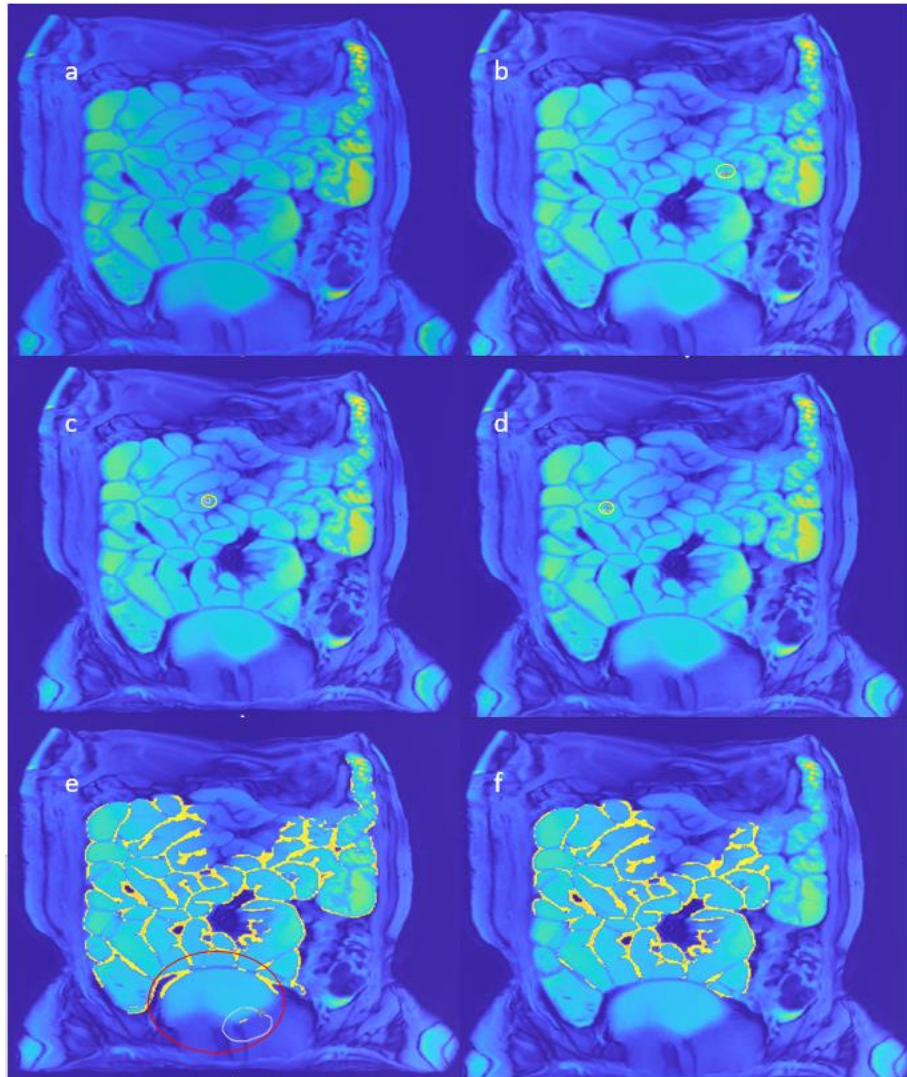


Figure 35: An example image of the highlighted bowel wall that is automatically selected using a combination of thresholding and edge detection to discover where the walls are. (A) shows small bowel after motion-corrected, (b-d) where 3 ROIs were drawn of the bowel contents; yellow ring, (e) mask image for small bowel wall, which helps to delete mesentery and unwanted regions, such as, large bowel and bladder wall (red ring) on the map, (f) an example of the wall after deleted mesentery or unwanted regions.

T1 data

Custom written software in Matlab was used for this analysis (written by Dr Caroline Hoad). An individual ROI was identified across the nine inversion recovery images, and this ROI moved manually if the wall moved position

across the images to keep the data consistent (Figure 36). The mean signal from these ROIs, was fitted to equation 10 by using the least-squares non-linear curve-fitting algorithm in Matlab.

$$S = S_0 \left[1 - (1 - \cos(\alpha)) \exp\left(-\frac{t}{T_{1\text{wall}}}\right) \right] \quad (10)$$

where S_0 represents the equilibrium magnetisation, and $S(TI)$ is the magnetisation at the measured TI.

To provide an estimate of alpha, at least three regions in the small bowel content were fitted for T1 prior to the wall measurements. An average of these values (T1 content and alpha) were used in the T1 fit for the wall, again to allow for partial volume effects in the ROI. This process was repeated for several bowel wall ROIs to obtain a better estimate of the T1.

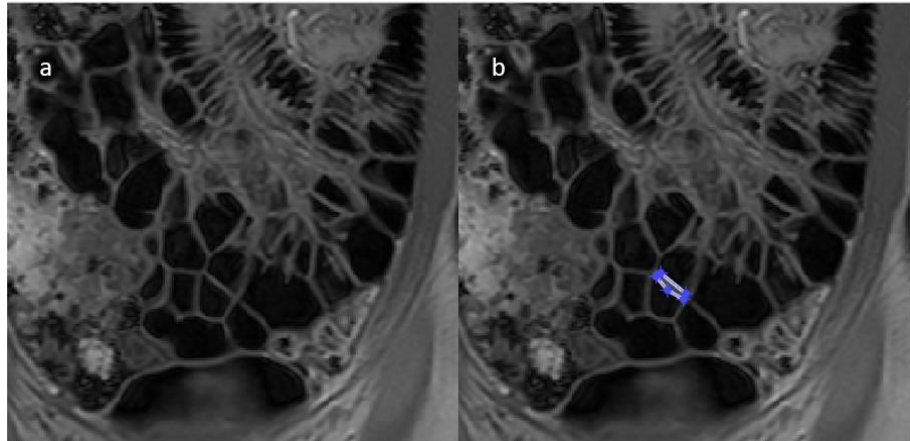


Figure 36: T1 representative example of the ROI. a before drawing the ROI, and b after drawing the ROI (white line and blue dots).

IVIM-DWI data

Custom written software in Matlab (R2017a, MathWorks) was used for this analysis (written by Dr Caroline Hoad). Many steps were included in the IVIM analysis. Initially, motion correction was run on a small section of the image to see if this improved the alignment of the data, and, if not, the raw data images were used to define the ROIs. The ROI was then drawn on the bowel wall, and the position inspected across the different b-value images. The ROI could be moved between different b-value images if necessary. The mean signal intensity values were then calculated from the ROI for each b-value image, and then analysed quantitatively according to both bi-exponential IVIM and mono-exponential models. This process was repeated multiple times for different bowel wall ROIs to obtain a better estimation of parameters for the bowel wall.

Mono exponential ADC maps were computed using all the b-value data except b1000, thus reflecting b-values that were typically used for DWI in clinical MRE protocols²²⁸. The IVIM signal was evaluated using equations 6 and 7, as described in chapter 2.

A two-step analysis was used to derive the IVIM parameters, similar to the procedure described previously²²⁹. First, an estimate of D and PF were calculated by a mono-exponential function $SI/SI_0 = (1-f) \exp(-bD)$ using b-values larger than 300 s/mm². These values were used as the first estimate in a non-linear model of an IVIM equation using all b-values to calculate the final best fit of model parameters²⁶.

MTR data:

MTR is a quantitative measure of the interaction between high and low-mobility proton pools of a tissue. MTR was calculated voxel by voxel after motion correction using equation 5 (as described in chapter 2).

MTR values were determined from the mean signal intensity MT values from ROIs. To generate each ROI steps undertaken have been listed below using custom-written software in Matlab (written by Dr Caroline Hoad).

- Selected small region
- Motion corrected in the region
- Checked motion correction to see if this improved the alignment of the data, and if not, the raw data were used to define the ROIs. Also, checked the MTR map was inspected for sharp lines, which would suggest a good correction and looked between the on and off data for obvious movement of the wall between the 2 images.
- Selected MTR image
- Drew ROI

This process was repeated multiple times to acquire multiple regions. An MT representative example of the ROI is shown in Figure 37.

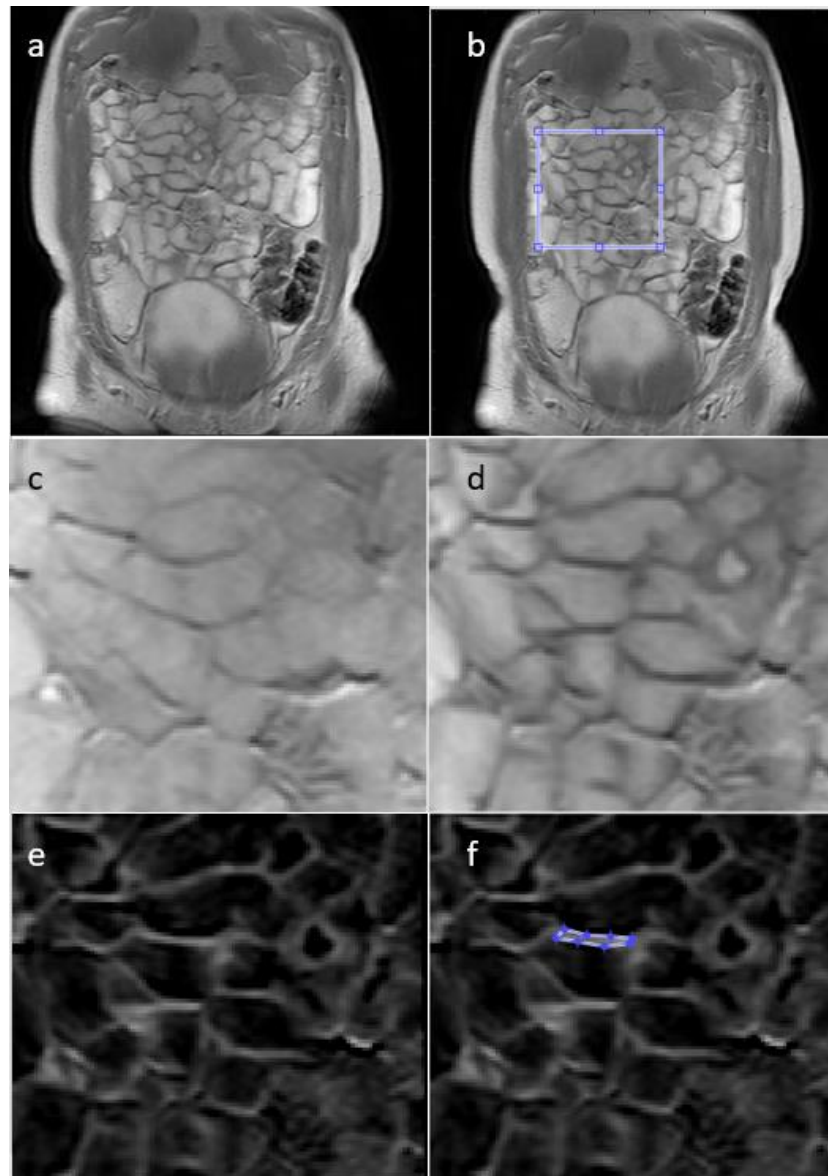


Figure 37: MT representative example of defining the ROI. The ROI was drawn on the small bowel wall after motion correction of the images. A shows the raw MT image, b cropped small area (blue square) that was used to run motion correction, c cropped area before motion correction, d cropped area after motion correction, bowel wall from MTR image (e) with an ROI along the bowel wall demonstrated as a white line with a blue dot (f).

Repeatability and inter-observer variability assessment

Two observers examined the quantitative measurements independently (the author (observer1) and Dr Hannah Williams (observer 2) for MT, T2 and T1 and the author (observer 1), and Dr Caroline Hoad for IVIM-DWI data. Observer 1 measured the data for test-retest repeatability. The measurement results between observers 1 and 2 were used for evaluating inter-observer variability. The participants were scanned twice in two weeks to assess the repeatability of the measurements. The observers independently drew nonspecific ROIs on each imaging dataset. Table 19 presents the number of regions, each of which was identified on these sequences by observers.

Table 19: Number of regions, which have been identified by observers for each parameter.

		No. of ROIs for T1		No. of ROIs for MT		No. of ROIs for T2		No. of ROIs for D, D*, PF and ADC	
Sub. No.	Visit	Obs.1	Obs.2	Obs.1	Obs.2	Obs.1	Obs.2	Obs.1	Obs.2
1	1	12	10	15	19	84	74	5	14
	2	10	10	12	18	53	44	4	4
2	1	10		27	15	95	143	8	12
	2	12	12	17	30	75	100	7	11
3	1			27	33	37	51	7	16
	2	12	12	18	28	175	232	11	28
4	1	6	3	12	10	43	38	4	5
	2	3		10	16	27	54	2	5
5	1	12	11	13	31	213	189	4	18
	2	10	10	17	30	70	109	5	15
6	1	9	5	13	26	88	88	3	15
	2	6	6	13	29	42	72	4	9
7	1	16	10	19	35	246	244	3	13
	2	10	10	28	34	149	176	5	20
8	1	13	10	27	24			6	17
	2	11	10	35	32			4	13
9	1	12	10	15	28	162	149	7	15
	2	13	10	27	35	185	180	9	13
10	1	14	11	21	32			8	14
	2	9		22	30			2	9

Optimisation work for IVIM data analysis.

Motion correction may be needed for DWI data acquisition as this was acquired using respiratory triggering, and the slice position between b-values might change. However, as the contrast changed considerably between the different b-value data we wanted to investigate what effect changing the 'fixed' image had on the motion correction. The fixed image was the anchor or reference image, and all the other images were motion corrected too. Data from 6 participants were motion corrected using a custom-written programme in MATLAB. This motion correction programme ran 3 times for each dataset to generate different results by changing the image used as the fixed image (b0, b100 and b300). Following that, each ROI was drawn on a single image from each dataset of these motion-corrected images and propagated across all nine b values using custom-written software written in IDL (version 6.4). We defined 4-6 different regions of the small bowel wall across several slices as well as 2-3 regions in the abdominal muscles and kidney for each of the six participants. Finally, a Matlab programme was run twice with and without b1000 data to generate, D, D*, PF and ADC from the mean ROI data. These results were compared between the different fixed images used in the motion correction to determine the performance in terms of a number of datasets, which produced fitted parameters of the correct order of magnitude expected from the data.

The following inclusion criteria were used to define poor fitting data: any data with negative values, PF above 70% (even highly perfused tissues

are unlikely to have values higher than this), and $D^* > 1$. This relatively pragmatically set was decided upon to allow the inclusion of a reasonable amount of data in the absence of published literature.

6.4 Statistical analysis

All statistical analysis was carried out using SPSS software (IBM SPSS Statistics for Macintosh, vers. 25.0. IBM Corp).

For all data sets, normality was assessed by using the Kolmogorov-Smirnov normality test. Where normality could not be confirmed, non-parametric tests were used, and data expressed as median, and in the Interquartile range (IQR), while data have been expressed as mean, (standard deviation) for normally distributed data.

To investigate the test-retest repeatability of each sequence for one observer, we used the coefficient of variation (CoV%). As the data were non-normally distributed, the median and IQR measurements from all the ROIs have been calculated by both observers. The CoV %, was computed as the percentage of the standard deviation (SD) of the mean ($SD \times 100\% / \text{mean}$) calculated from visit 1 and visit 2 data for each participant individually, and then averaged across all subjects. The repeatability of each parameter was defined as poor when CoV was $> 30\%$, acceptable when CoV was between 20–30%, good when CoV was between 10–20%, and excellent when $CoV \leq 10\%$ ²³⁰. To assess the spread in the data across all the participants (biological variation), a CoV was measured across visit 1

(observer 1) data from all participants (sd across all Visit 1 participants' data*100%/mean across all Visit 1 participant data). This across-subject data was compared with the within-subject data to determine whether the test-retest variability was lower than the variation seen across the different participants.

The inter-observer variability was assessed by calculating the ICC with a two-way random model of absolute agreement and was interpreted as follows: 0.81–1: almost perfect correlation; 0.61–0.8: good correlation; 0.4–0.6: moderate correlation; 0.21–0.4: fair correlation; 0.0–0.2: poor correlation ²³¹. The ICC was calculated from both observers in both visits together.

6.5 Results

6.5.1 Optimisation results

All participants (six females) mean age (26± 6 yrs) completed the scan. The best performing algorithm across the different regions of the small bowel was the b300 data as this produced the least errors across the different categories. The results of this optimisation have been presented in Table 20.

Table 20: Results from the motion correction data using 3 different b-value images as the reference image. Data is presented for small bowel, kidney and muscle regions. Poor p-value data (D and PF) had model parameter estimates of $p > 0.05$. Poor perfusion estimates from the mono-exponential fit of high b-value data were negative values or $PF > 0.7$. Non-fitted data for D and PF were defined as error in the programme or not get a value.

ROI Anatomical Region and fixed b-value used for MC	No. of ROIs	poor p-value (PF)	poor p-value (D)	PFestimates for p_est	number of non-fitted data D	number of non-fitted data PF
Small bowel b0	25	3	3	9	4	4
Small bowel b100	25	5	6	7	4	3
Small bowel b300	25	3	4	5	3	3
Kidney b0	9	3	3	0	0	0
Kidney b100	9	1	2	2	0	0
Kidney b300	9	2	2	1	0	0
Muscle b0	16	0	1	2	0	0
Muscle b100	16	1	1	2	0	0
Muscle b300	16	0	1	1	0	0

6.6 Study Results

6.6.1 Participants' characteristics

Ten participants were recruited. All participants (9 female, 1 male: mean age 30 ± 8 yrs) completed both scans. Two participants were excluded from T2 data analysis from both observers due to motion artefact, one from T1 owing to the same reason from one observer, and four from the second observer. As the data were non-normally distributed, the median and IQR measurements from all the ROIs have been calculated by both observers. The flow chart for the subjects included in the study is shown in Figure 38. Figure 39 and 40 show example images for T1 and T2 data. Tables 21 and

22 show the T2, T1, MTR and IVIM_DWI parameters median (IQR) data for each subject at both visits.

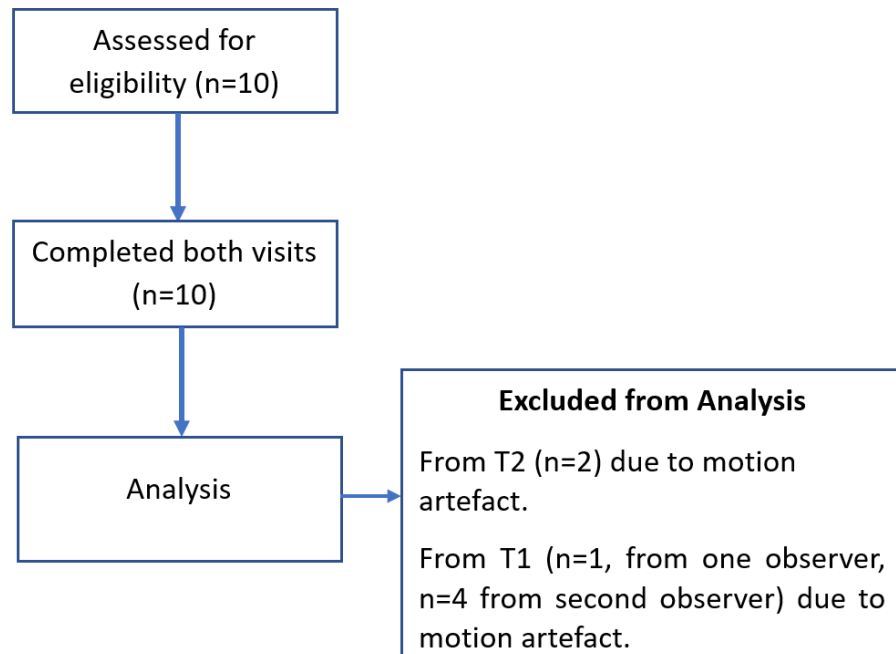


Figure 38: Flow chart.

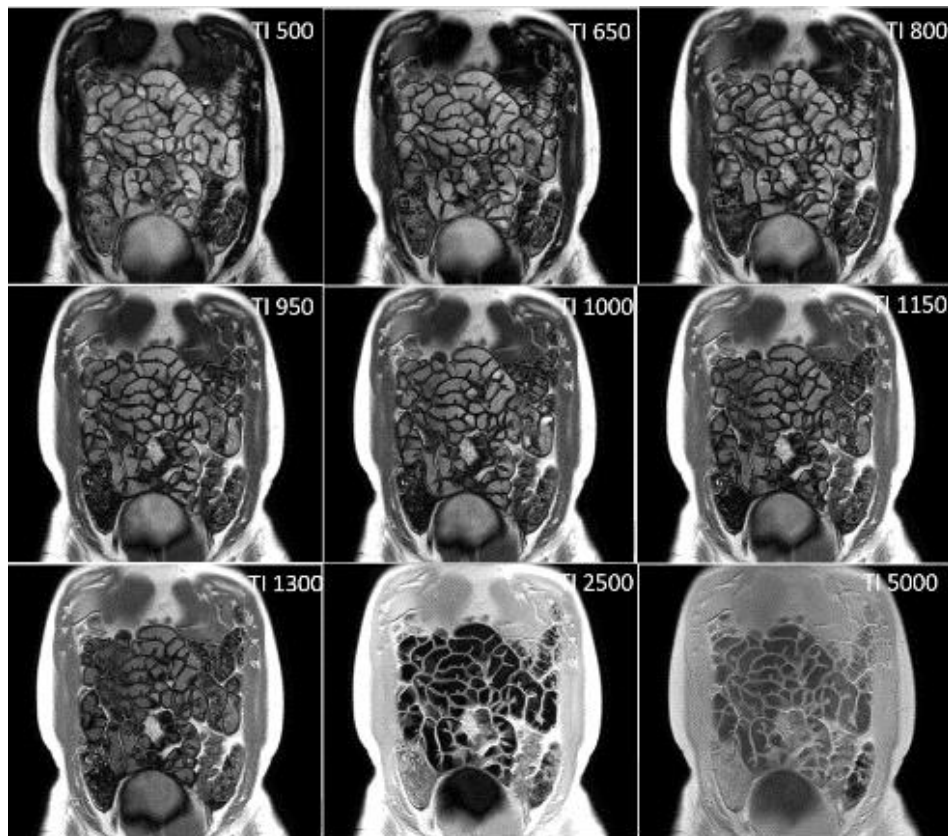


Figure 39: Coronal images through the abdomen showing the T1 recovery of the bowel wall with different TIs in ms.

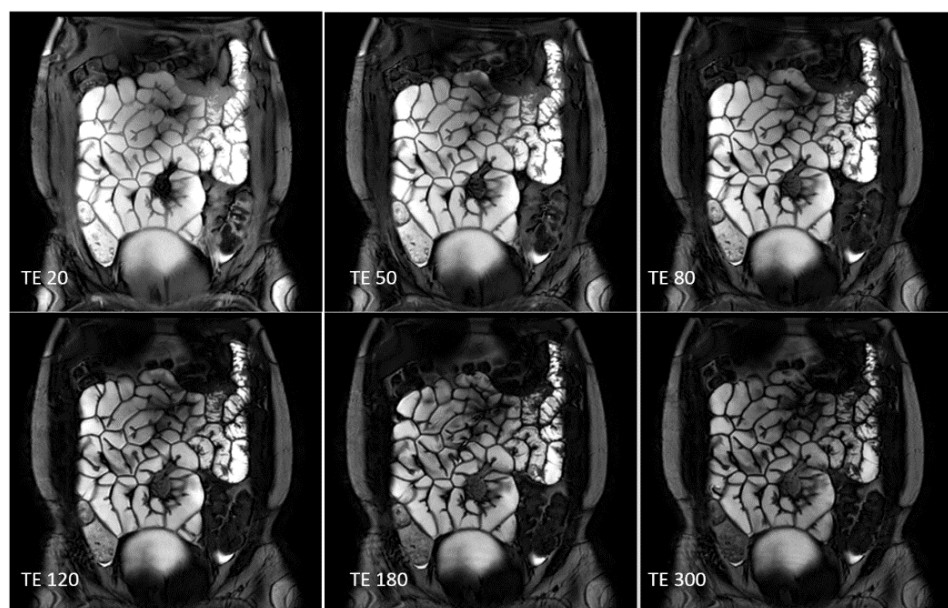


Figure 40: Coronal images through the abdomen showing T2 decay of the bowel wall with different TEs in ms.

Table 21: The median and interquartile range of T2, MTR, T1 measurements form all the different ROIs for two observers. Missing data in the table due to motion artefact.

		T1				MT				T2			
		Obs.1		Obs.2		Obs.1		Obs.2		Obs.1		Obs.2	
		Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Subject 1	Visit 1	0.881	0.481	0.902	0.351	0.314	0.113	0.265	0.152	68.6	52.7	66.3	48.8
	visit 2	0.913	0.316	0.848	0.290	0.241	0.072	0.301	0.229	82.6	109	87.2	33.8
Subject 2	Visit 1	0.951	0.358			0.276	0.102	0.364	0.261	88.2	115	90.4	122
	visit 2	0.984	0.391	0.973	0.599	0.279	0.054	0.321	0.267	36.7	57.1	43.0	56.7
Subject 3	Visit 1					0.240	0.082	0.260	0.177	64.3	77.0	56.3	64.9
	visit 2	1.118	0.503	1.137	0.514	0.272	0.089	0.321	0.233	63.4	62.7	61.6	45.9
Subject 4	Visit 1	1.068	0.451	0.797	0.617	0.239	0.090	0.376	0.376	90.8	59.7	96.4	37.1
	visit 2	1.138	0.551			0.220	0.114	0.280	0.280	55.2	80.6	68.2	61.6
Subject 5	Visit 1	0.701	0.191	0.674	0.208	0.317	0.098	0.342	0.245	49.8	48.2	52.2	40.7
	visit 2	0.699	0.182	0.891	0.364	0.306	0.079	0.346	0.267	47.6	46.0	54.0	30.9
Subject 6	Visit 1	1.009	0.520	0.912	0.776	0.311	0.110	0.300	0.190	71.0	63.8	70.8	66.1
	visit 2	0.841	0.473	0.707	0.330	0.288	0.114	0.315	0.200	58.0	46.8	52.0	46.3
Subject 7	Visit 1	0.925	0.375	0.909	0.377	0.267	0.064	0.297	0.233	66.9	64.0	61.5	423
	visit 2	0.719	0.279	0.734	0.229	0.297	0.080	0.308	0.227	87.7	90.7	68.5	217
Subject 8	Visit 1	1.175	0.506	1.150	0.996	0.279	0.076	0.323	0.247				
	visit 2	0.874	0.337	0.631	0.443	0.274	0.070	0.304	0.234				
Subject 9	Visit 1	1.021	0.193	1.039	0.338	0.275	0.128	0.369	0.240	51.3	33.8	50.5	259.4
	visit 2	0.902	0.323	0.879	0.272	0.284	0.119	0.334	0.215	73.4	73.8	69.4	359.4
Subject 10	Visit 1	1.011	0.293	0.626	0.375	0.319	0.085	0.332	0.247				
	visit 2	0.951	0.361			0.254	0.086	0.321	0.235				
*missing values due to motion artefact.													

Table 22: The median and interquartile range for IVIM-DWI parameters for form all the different ROIs for two observers.

		D($\times 10^{-3}$ mm ² /s)				PF				D*($\times 10^{-3}$ mm ² /s)				ADC($\times 10^{-3}$ mm ² /s)			
		Obs.1		Obs.2		Obs.1		Obs.2		Obs.1		Obs.2		Obs.1		Obs.2	
Subject	Visit	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
1	V 1	2.54	0.55	2.57	1.16	0.337	0.246	0.464	0.218	27.18	22.40	15.82	21.62	2.95	0.41	3.27	0.94
	V 2	2.78	0.74	3.33	0.83	0.259	0.065	0.162	0.19	20.28	13.67	80.71	487.50	3.16	0.52	3.93	0.19
2	V 1	2.79	0.95	3.03	1.04	0.452	0.198	0.357	0.082	12.38	18.81	21.04	9.81	3.20	0.29	3.57	0.91
	V 2	2.84	0.41	2.77	0.69	0.315	0.059	0.31	0.123	22.12	7.71	20.21	19.24	3.28	0.37	3.18	0.63
3	V 1	3.73	0.63	3.22	0.48	0.381	0.306	0.341	0.175	15.75	21.03	17.94	20.06	4.10	0.33	3.88	0.67
	V 2	3.10	0.97	3.48	1.21	0.495	0.128	0.393	0.206	21.51	20.87	22.65	18.05	3.56	0.49	3.84	1.04
4	V 1	1.53	1.16	1.77	0.79	0.458	0.505	0.21	0.202	5.99	5.81	21.91	20.27	2.32	0.28	2.06	0.88
	V 2	2.25	1.46	2.67	1.09	0.401	0.376	0.327	0.104	36.26	61.13	50.76	53.32	2.90	0.73	3.61	1.75
5	V 1	1.89	1.24	1.78	0.30	0.222	0.392	0.284	0.244	14.15	26.15	11.07	22.75	2.42	1.11	2.09	0.67
	V 2	1.86	0.55	1.95	0.50	0.499	0.245	0.364	0.208	9.67	2.83	9.88	6.17	2.47	0.52	2.41	0.71
6	V 1	2.41	1.03	2.50	0.96	0.307	0.076	0.305	0.309	36.58	39.58	18.60	33.84	2.45	0.89	3.09	1.00
	V 2	1.69	0.65	2.04	0.85	0.407	0.176	0.367	0.385	24.37	37.59	13.92	14.16	2.35	0.59	2.59	0.52
7	V 1	2.07	0.89	1.75	0.90	0.412	0.092	0.347	0.244	35.71	65.63	8.98	11.59	2.61	0.28	2.62	1.17
	V 2	2.12	0.82	1.99	0.48	0.511	0.377	0.269	0.177	23.65	15.36	13.63	11.03	2.75	0.50	2.34	0.43
8	V 1	2.70	1.52	2.45	0.42	0.367	0.333	0.31	0.19	31.73	38.19	27.16	31.96	3.19	1.10	2.75	0.43
	V 2	2.65	1.08	2.77	0.66	0.466	0.320	0.478	0.189	37.78	30.62	26.26	18.87	3.25	0.31	3.43	0.35
9	V 1	2.42	0.35	2.73	0.94	0.365	0.383	0.359	0.288	11.87	8.11	16.34	16.32	3.31	0.71	3.59	0.68
	V 2	3.09	0.97	3.24	1.03	0.278	0.362	0.381	0.327	17.48	42.48	25.71	24.31	3.50	0.37	3.60	0.79
10	V 1	2.60	0.70	2.61	1.17	0.567	0.173	0.497	0.213	24.81	12.81	15.08	27.28	3.37	0.62	3.54	1.00
	V 2	2.40	0.05	2.75	0.92	0.612	0.069	0.455	0.214	28.50	17.52	31.23	28.15	3.03	0.01	3.52	0.96

6.6.2 Test-retest repeatability

For the first observer, test-retest repeatability in the small bowel walls was excellent for ADC, MTR, T1 and D (mean CoV 5%, N=10: CoV 7%, N=10: 8%, N=9 and 10%, N=10) : acceptable for T2 (CoV 21%, N=8), good for PF (mean 20%, N=10), and poor for D* (mean CoV 31, N=10) as seen in Table 23.

The test-retest data (within-subject) showed lower variability compared with the variability seen across the different subjects at visit 1 (biological variation).

Table 23: Test-retest repeatability mean coefficients of variation (CoVs) of T1, MTR, T2, D, PF, D* and ADC within-participant data and across-subject visits, and biological variations across all subjects of same observer data V1 only.

Test retest (within participant)			Biological Variations (across participants)	
Parameter	N	CoV %	N	CoV %
T1	9	8	9	13
MTR	10	7	10	10
T2	8	21	8	21
D	10	10	10	24
PF	10	20	10	24
D*	10	31	10	50
ADC	10	5	10	18

6.6.3 Inter-observer variability

Table 24 shows the ICC for the inter-observer variability. Figure 41 shows an example of inter-observer data. Inter-observer variability was almost perfect (ICC =0.89, N=16) for T2. A moderate ICC was found for T1 (ICC=0.55, N=16), while it was poor for MTR (ICC=0.08, N=20).

For IVIM-DWI parameters, inter-observer agreement was excellent in the bowel walls for D (ICC=0.86, N=20), good for ADC (ICC= 0.76, N=20) and moderate (ICC =0.41, N =20) for PF. However, a poor correlation was found for both D* and ADC (ICC= 0.14, N=20).

Table 24: Inter-observer agreement: intra-class correlation coefficients (ICCs) of T1, T2, MT and IVIM-DWI parameters made between two observers for the small bowel wall regions.

Parameter	N	ICC	[95% CI]
T1	16	0.55	[0.13-0.81]
MTR	20	0.08	[-0.13-0.37]
T2	16	0.89	[0.71-0.96]
D	20	0.86	[0.68-0.94]
PF	20	0.41	[0.007-0.708]
D*	20	0.14	[-0.33-0.54]
ADC	20	0.76	[0.49-0.9]

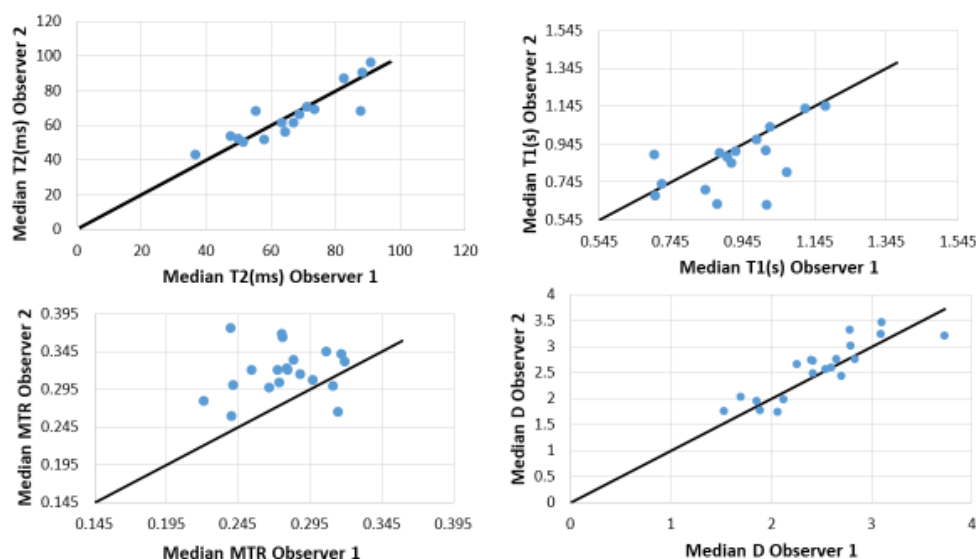


Figure 41: Correlation between the two observers across both visits. Line of identity is shown in black.

6.7 Discussion

The main goal of this study was to evaluate the repeatability of MRI-based quantitative sequences of the small bowel wall using IVIM-DWI, MTR, T1 and T2 relaxometry sequences with 3T MRI. We kept as many variables as constant as possible, using the same scanner, the same operator, and the same procedure. Our results showed that in terms of test-retest repeatability of these sequences in the bowel wall were excellent for MTR, T1 and D, and good for ADC and D, and acceptable for T2, but poor for D*. Regarding the Inter-observer variability, agreement was almost perfect for T2, D, good for ADC moderate for T1 and PF, while poor for D*, ADC and MTR in the bowel wall.

In clinical practice, the use of imaging biomarkers requires a well-structured procedure step by step, which must first follow selected criteria. These criteria include providing precise, accurate and feasible measurements, associated with a clinical end point, and performance in its proposed use in a specific context. These criteria have been classified into three distinct but interrelated fields: the analytical validation, qualification, and utilisation. Analytical validation plays the most important role in determining the technical performance of biomarker while qualification and utilisation are major parts for assessing the clinical impact of it²³²

For the imaging of biomarkers, both the technical and the clinical performances need to be rigorously evaluated for the future evolution of

precision medicine. The technical performance evaluates how a test performs under controlled conditions in reference subjects or objects.

In this study, technical validation was assessed by measured repeatability and variability of IVIM-DWI, MTR, T1 and T2 relaxometry sequences in a cohort of healthy participants in order to address whether these biomarkers were measurable accurately and precisely. Knowledge concerning repeatability is essential to understand the change of quantitative MRI derived parameters, and to assess their potential impact on their use as an imaging biomarker in disease characterisation or treatment response. A lack of reproducibility or repeatability may severely affect the potential utility of an imaging biomarker in measuring a biological process. Reproducibility refers to measurements performed using different types of equipment, at different times and sites or using different operators or software either in the same or with different participants while repeatability, in contrast, refers to measurements performed several times in the same participant using an unchangeable procedure, such as, the same operators and software, equipment, over a short timeframe. In this study, the test-retest repeatability of most of these parameters varied from acceptable to excellent, except in respect of D^* , as well as, variability, which showed a moderate to almost perfect result in T2, D, T1 and PF, and poor for the rest of measurements (MTR, D^* and ADC).

In clinical decision making, the use of quantitative biomarkers, such as IVIM-DWI, is not just linked to the Sn and Sp of the underlying

microstructure and physiology, but also with respect to the stability of these measurements themselves. The ability of the pseudo-diffusion components and the diffusion components of the IVIM signals to be measured or detected meaningfully does not depend only on the properties of the system, but also on the MR scanner hardware performance, and acquisition schemes being used.

Abdominal DWI commonly has some technical challenges that have an impact on the quality of these quantitative biomarkers, such as, hardware limitation on setting and selecting b-values and TEs, triggering vs breath hold vs free breathing for averaging to gain increased SNR, however this may lead to more possibilities of motion artefacts from both peristalsis and respiration. Field inhomogeneities and the presence of susceptibility artefacts from gas may also create variability in the data.

The repeatability of T1, T2 relaxometry, MTR and IVIM-DWI have been explored in several studies in different tissues in the body, but not in the bowel walls. This is the first study in small bowel segments to the best of our knowledge. The T2 measurements were deemed to be almost perfect in terms of inter-observer variability, but acceptable in test-retest repeatability. The overall CoV value for test-retest repeatability of the T2 measurement indicated acceptable repeatability in the measurement (CoV 21%). A previous study on inter-vertebral discs reported high levels of reproducibility for T2 measurement, with similar results as compared with those obtained in our study ²³³. Previously, some studies documented T2-

mapping repeatability in other organs²³⁴⁻²³⁶. Fei-Yun et.al.²³⁴ reported that the T2 value showed satisfactory short-/long terms scan–rescan (ICC=0.75-0.86 vs ICC=0.720-0.81), and observer–observer agreements (ICC>0.7), and repeatability found in extra-ocular muscles at 3.0-T MRI. In another lumbar inter-vertebral disc study using T2 mapping with 3T, they found that the reproducibility of the T2 measurements was found to be fair to good in terms of both test-retest reproducibility and Inter-observer variability. Also, the repeatability of the T2 mapping sequence was reported to be good in a myocardial study²³⁵ in test-retest repeatability, and excellent in variability measurements, which is in line with our study. Our results showed that the inter-observer agreements were almost perfect. This was, perhaps, because the process was far more automatic compared with the other methodologies as they used freehand ROI.

The acceptable test-retest repeatability, and almost perfect inter-observer variability found in our study indicated that T2 mapping was a steady and reliable technique, which could serve as a potential imaging marker in a diagnosis, and, especially, in the follow-up for CD. Also, if large differences in the T2 of diseased tissues are found this would make it easy to differentiate from healthy tissue. The range of T2 values across participants was not very small (20%) and this would have had a positive effect on the ICC measured in our study.

For T1, we found that this sequence had excellent test-retest repeatability and moderate inter-observer variability. In healthy volunteers'

liver, at 1.5T Hoad *et al.* assessed the repeatability of T1 measures between two imaging sessions, and found that T1 was repeatable with a very small CoV of $1.8 \pm 1.8 \%$ ²³⁷. The wider dynamic range of T2 rather than T1 could be a possible explanation for higher inter-observer variability of T2 measurements as compared with that of T1. Another plausible explanation was that we identified the ROI automatically for T2 data, while using freehand for T1 data. Moreover, the other explanation of this result might be owing to the fact that as the ROIs were not identified in the same areas by observers, thereby leading to regional differences in the bowel being detected (that is, there is a variable distribution of vascularity and lymphoid tissue throughout the bowel).

In our study, the precision as measured through CoV and ICC of MTR was excellent in test-retest repeatability, and poor in inter-observer variability. A previous study on myelin imaging in the human spinal cord reported low variability for MTR as well²³⁸. Relatively low ICC values of MTR measurements were found in our study, which might have been because of several reasons. The MTR data could not take into account any partial volume effects of the small bowel contents in the ROI, which might have introduced additional errors, and these were accounted for in the T1 and T2 measurements. This would also mean that the choice of ROI would be particularly important, and might significantly affect the measured results. Also, the dynamic range of the MT data was very small (10% variation across all subjects), which would have an impact on the ICC measured. In addition to this, the MT image acquisition was undertaken after other

sequences, which could have led to more motion artefacts due to the relatively short half-life of buscopan. This might have influenced the repeatability of the MTR value.

Repeatability of DWI and ADC have already been evaluated in the head and neck^{239, 240}, liver, and kidney^{241, 242}, but only a few studies have evaluated the IVIM repeatability in these domains^{225, 243, 244}, and none in the small bowel. In the abdomen, especially, in the liver, the majority of studies previously published, evaluated reproducibility or repeatability and accuracy of IVIM. The repeatability of the D and ADC parameters was higher than the perfusion-related parameters D* and PF. The true diffusion coefficient D appeared to be the most reproducible parameter in the kidney and the liver^{242, 245, 246}, whereas D* appeared to be the least reproducible parameter among IVIM metrics, with the CoV ranging from 24.8% to 193.8%^{225, 242, 244, 247}. In orbit mass, test-retest repeatability of IVIM parameters was good for D and ADC (mean CoV 14% and 12%), poor D* and PF (mean CoV 110% and 43%)²²⁴. Our findings agreed with these studies with excellent test-retest repeatability of ADC and D (mean CoV 5% and CoV 10%, respectively), and good for PF (mean CoV 20%), and poor test-retest repeatability for D* (CoV 31%).

In terms of inter-observer variability, we found that in D, ADC and PF there were more repeatable parameters compared with D* and ADC. Other studies contrasted with our findings showing, poor inter-observer variability for PF and D* in liver parenchyma and hepatocellular carcinoma²²⁵. In

orbital mass, the inter-observer repeatability was almost perfect in the orbital mass for all the IVIM parameters²²⁴.

This low test-retest repeatability and inter-observer variability of IVIM parameters might have been the result of the following: first, we used respiratory-triggered DWI, which might have introduced blurring, from poor triggering, this would affect the images produced using signal averaging across different gradient directions, bowel peristalsis introduced a further error. Secondly, the measurement of DWI in the normal bowel wall is often difficult as it is a very thin region, given the typical thickness of the image slice (normal bowel wall 1 – 2 mm, typical DWI slice 5–8 mm), we used small freehand ROIs encompassing the wall area, and this might have had an effect on both test-retest repeatability, and the inter-observer variability, and specially perfusion-related parameters, which are more susceptible to noise in the measurement. Moreover, there is no direct IVIM parameter measurement, but there are different methods for calculating them from exponential decay data, such as, the Bayesian probability theory or the non-linear least squares. Bayesian analysis performs better than nonlinear least square analysis, especially, in the condition of low SNR ²⁴⁸, so using this approach may give a better result. Finally, the dynamic range for the measurement of perfusion-related parameter D^* was poor, and the quality of parameter estimates depended upon a number of factors involving the number of b values obtained, especially of low b values, and the signal-to-noise ratio, which has to be high enough.

In our study, changes in parameters related to perfusion specifically D^* require caution for future studies when considering their poor repeatability. In contrast, D and ADC showed good repeatability suggesting that these parameters might be more reproducible with reliable quantitative biomarkers to characterise bowel disease, to quantify the severity of the disease, and to predict its course, but also to evaluate therapeutic responses under treatment.

Our study had some limitations. The sample size of this study was quite small. Further studies with more participants could provide more evidence to our conclusions. We did not do any intra-observer variability work. Also, we did not evaluate the repeatability of IVIM-derived parameters in other abdominal organs, such as, the liver or kidney to check the repeatability and variability in these organs, to determine whether the data were noisier in the bowel. Moreover, most of the data analysis involved manual drawing of the ROIs, which added noise to the measurements (and probably provided much of the differences between the 2 observers), and much of the data were single slice, so it might have been that the same loops of bowel were not investigated on both occasions, which again might have introduced further variability. Expanding the measurements to multiple slices would be one way to overcome this particular limitation.

In conclusion: our study showed the robustness and feasibility of these quantitative parameters in the assessment of small bowel walls. In bowel wall, test-retest repeatability was excellent for ADC , D , MTR , $T1$, and good

for ADC and PF, and acceptable for T2, but was poor for D*, whereas the inter-observer agreement repeatability was almost perfect for T2 and D values, moderate for T1, ADC and PF, and poor for the rest of the parameters. In general, T1 and D parameters showed a good measurement in terms of test-retest repeatability and inter-observer variability.

Chapter seven: Using non-contrast sequences to characterise and quantify the small bowel as a predictor of long-term response in patients with Crohn's disease commencing biological therapy (Motility plus study).

Abstract

The use of quantitative MRI such as magnetisation transfer, intravoxel incoherent motion (IVIM), diffusion-weighted imaging (DWI), and T2 relaxometry, in patients with Crohn's disease (CD) has increased in recent years. There are, however, few data available on how MRI sequences of active disease alter during biological therapy, and whether these changes correspond to clinical biomarkers and symptoms. This study aimed to evaluate the changes over time in T2, MTR and IVIM-DWI (D, PF, D* and ADC) parameters during treatment with biological therapies in CD patients, to determine whether there was any correlation between these MRI parameters with clinical indices, and to assess the difference between normal and abnormal bowel segments.

Methods: We performed a prospective single centre study involving 25 patients with active CD. All participants underwent MRI and clinical assessments at baseline, and at 20-28 weeks after biological therapy. The differences of clinical indicators (Harvey–Bradshaw index [HBI], C-reactive protein [CRP] level and faecal calprotectin [FCP]), Crohn's Disease MRI Index (CDMI) and T2, MTR and IVIM-DWI parameters between pre- and

post-treatment were calculated by using a paired t-test. The correlations between variables were analysed with Pearson's correlation coefficients.

Results: After treatment, the values of clinical indicators did not change (CRP 5(5-7) vs 5(5-5) mg/L, $P=0.34$; HBI 4(3-8) vs 3(1-6), $P=0.08$; FCP 514(192-810) vs 98(43-713), $P=0.12$). Also, no difference before and after treatment were observed in MRI parameters: T2 (69 (53-79) vs 65(52-75)) ms , $P=0.95$), MTR (0.28 ± 0.04 vs 0.26 ± 0.09) $P=0.46$), D (1.28 ± 0.27 vs $1.42\pm0.52 \times 10^{-3}$ mm²/s, $P=0.42$), PF (0.26 ± 0.075 vs 0.32 ± 0.149 , $P=0.19$), D* (14.9(9.58-21) vs 21.3(11-36.3) $\times 10^{-3}$ mm²/s, $P=0.19$), ADC(1.57(1.47-1.69) vs 1.63(1.38-2.25) $\times 10^{-3}$ mm²/s, $P=0.28$) and CDMI (6.4(5.9-7.6) vs 6.4(5-6.7), $P=0.3$). No significant correlations were observed between clinical and MRI parameters, and in-between MRI parameters.

Conclusion: These sequences have not shown any changes before and after a biological treatment. Moreover, no significant correlation was observed among MRI parameters, and with clinical indices. The exact role of these parameters for CD management should be assessed in a larger cohort with histopathological validation.

7.1 Introduction

A paradigm shift in IBD management has begun to show that a treat-to-target approach might be superior to a simple symptom resolution²⁴⁹⁻²⁵¹. This approach has been advocated for IBD in an attempt to reduce the need for surgery, and to improve long-term patient outcomes that involve close monitoring of specific and objective measures of inflammation. The implementation of this approach requires continuous close monitoring of patients by a multidisciplinary team of advanced practitioners, medical technicians, physicians, nurses and, other health care professionals. The ultimate therapeutic goal in CD is to control disease and stop progression. Biological therapies are effective in the maintenance of the induction and maintenance of remission in CD²⁵².

The objective assessment of disease activity, the detection of changes and complications in disease behaviour, and the response to treatment continue to be challenging. In terms of diagnosis and follow-up, several approaches have been proposed to assess CD characteristics, including disease activity and complications, but a consensus regarding the best exam has yet to be reached²⁵³. Endoscopy is the current reference standard for assessment of disease activity, and to quantify the inflammatory burden^{209, 254, 255}. Endoscopy, however, is poorly accepted by the patients, is invasive, only permits the examination of the intestinal mucosa, and only provides a limited assessment of the small bowel visualizing only the terminal ileum⁷⁹.

Clinical scores such as HBI and CDAI are commonly used to assess the severity of disease activity²⁵⁶, especially, in clinical studies rather than in a real-world healthcare setting. However, the indicators are based on subjective criteria, and do not correlate well to more objective outcomes of mucosal healing²⁵⁷.

CRP and FCP are non-invasive, effective biomarkers as decision-making tools, but should not be used as treatment endpoints²⁵⁸. CRP is a useful serum marker of inflammation, being cost-effective and non-invasive.

Multiple studies have indicated that the TNF antagonist therapy is inversely associated with baseline CRP, which can predict a better treatment response by a high baseline CRP^{68, 259}. Reinisch *et al.* identified the possibility of clinical remission with infliximab therapy more than twice as effective for CD patients with baseline CRP concentrations of >7 mg / L than for those with low values. However, there is no optimal cut-off point for ROC baseline CRP as a response predictor after 14 weeks, and it is, therefore, difficult to make firm clinical suggestions on the role of CRP in therapeutic alterations at 14 weeks⁶⁸.

FCP is a calcium-combined protein faecal biomarker, mainly derived from neutrophil, and has been shown to have high Sn and Sp for intestinal inflammation. FCP reflects mucosal inflammation, and may be an effective tool for assessing patients' response to therapy²⁶⁰⁻²⁶⁴. It demonstrates a

positive linear correlation with both endoscopic and histological activity²⁶⁵,²⁶⁶, and is a useful marker in predicting relapses post intestinal surgery²⁶⁷.

Cross-sectional imaging may provide a sensitive and non-invasive, safe measure of disease presence, activity, and extent. MRE is now recommended by consensus guidelines as a first-line test for the diagnosis and monitoring of CD activity^{84, 92, 93, 143, 268}.

Before instituting medical management, it is important to evaluate the inflammatory component of the disease. Unlike inflammatory disease behaviour where medical treatment is the most cost-effective first-line therapy, fibro-stenotic disease is usually best treated by intestinal surgery¹³⁴. Moreover, intravenous gadolinium adds cost, carries a risk of nephrogenic systemic fibrosis, an allergic reaction and long-term brain deposition following repeated use (US FDA 19/12/2017). Present MRI-based disease activity scores either do not have the potential capability to predict fibrosis or are gadolinium-based. Novel Imaging sequences, such as, MT, T2 relaxometry and IVIM_DWI, especially, (ADC) have shown significant correlation to histological fibrosis^{20, 162, 168, 176, 179, 269-272} in single centre preliminary studies, but their responsiveness to medical therapy or predictive role to non-response in medical therapy has never been investigated.

We hypothesise that an objective baseline disease characterisation using MT, T2 relaxometry or DWI may have clinical utility in predicting response to biological therapies.

In this study, we aimed to attain prospective-initial data in a cohort of patients with active CD exposed to biological therapy. We aimed to: (1) determine the changes over time in T2, MTR and IVIM-DWI parameters, disease activity and clinical indicators during treatment with biological therapy, (2) assess the correlation between these quantitative measurements, (3) to assess the correlation of these parameters with clinical and MRI-based measurements of CD activity, and to quantify MRI parameters in normal and abnormal wall segments in those participants.

7.2 Materials and methods

7.2.1 Ethical Considerations

This single-centre, prospective study, was undertaken as a local sub-study of the MOTILITY clinical trial (trial registration No: ISRCTN14481560 & NREC No.: 17/WM/0106). Written informed consent was obtained from all subjects.

7.2.2 Recruitment Criteria

Inclusion criteria for this study were CD patients aged ≥ 16 years with active disease in the small bowel based on MRE and/or ileo-colonoscopy, patients scheduled to start biological treatment including Infliximab, adalimumab and ustekinumab. Patients were excluded if they had small

bowel stricture causing upstream dilatation on endoscopy or imaging, small bowel surgery within the previous 3 months before the study, the primary aim of therapy being to treat perianal or colonic CD, any contraindications to MRI (e.g. cardiac pacemaker) or refusal to adhere to the protocol.

7.2.3 Trial Design

All patients with CD underwent clinical assessments, including measurements of HBI, laboratory tests (including CRP and FCP) and MRE twice, with the first research visit before starting biological therapy, and the second one performed 20-28 weeks after the onset of biological therapy.

At the two time points, clinical disease activity was determined by FCP, HBI and CRP. Participants completed an HBI questionnaire on the same day of MRI scan, as shown in Table 25. Blood and stool samples were provided on the same day of the scan or within 3 months of the MRI examination. Biological therapies were instituted as per Summary of Product Characteristics and licensing by relevant regulatory bodies (TA187, TA187, TA456). The dose was given dependent on body weight. For example, ustekinumab is given as an intravenous infusion at induction, and as a subcutaneous injection at maintenance: one intravenous induction treatment (approximately 6 mg/kg/ body weight), and maintenance subcutaneous treatment at week 8 (90 mg), then on every 12 weeks.

Table 25: Harvey-Bradshaw Index (HBI) for Crohn's Disease.

Descriptor	Description	score
General well-being	Very Well	0
	Slightly below par	1
	Poor	2
	Very poor	3
	Terrible	4
Abdominal pain	None	0
	Mild	1
	Moderate	2
	Severe	3
Number of liquid/soft stools	1 per occurrence	-
Abdominal mass	None	0
	Dubious	1
	Definite	2
	Definite and tender	3
Complications	1 per item	-
	Arthralgia	
	Uveitis	
	Erythema nodosum	
	Aphthous ulcers	
	Pyoderma gangrenosum	
	Anal fissures	
	New fistula	
	Abscess	

7.3 MRI Acquisition

All MRI scans were performed at 3.0 T (Ingenia; Philips, Best, The Netherlands), using a 32-channel receiver coil at the Sir Peter Mansfield Imaging Centre, University Campus, University of Nottingham, UK. Participants were requested to fast 6 hours before the scan. Participants were given 1L of 2.5% mannitol with 0.2 % locust bean gum to drink 50 minutes before the scan to achieve adequate small bowel distension²⁷³. The participants were placed in a feet-first prone position. To reduce bowel motility, up to two doses (20mg each) of hyoscine-butylbromide (Buscopan, Sanofi-aventis Ltd.) were administered intravenously before the quantitative

sequences, followed by a minimum 10-minute interval between doses. To reduce respiratory movements, and to provide higher image quality, all imaging was performed under breath-held conditions except for the IVIM-DWI sequence that was triggered by respiration.

The standardised assessment of the bowel consisted of coronal T2 weighted imaging without fat saturation, coronal and axial T2-weighted turbo spin echo imaging with fat saturation. In addition, at visit 1 only, some participants had a spoiled the 3D T1-weighted THRIVE gradient-echo before contrast and coronal and axial T1-weighted THRIVE at 3–4 min after a contrast injection to provide a full set of clinical scans, if this had been requested by their gastroenterologist. The b-values ($10^{-3} \text{ mm}^2/\text{s}$): 0, 50, 100, 200, 300, 400, 600, 800, 1000 were selected because they were the most common combinations among the different DWI protocols used. The acquisition parameters of the MT, T2 relaxometry and IVIM-DWI sequences are shown in Table 26.

Table 26: Acquisition parameters of T2, IVIM-DWI, MT.

MRI parameters	T2 scan	IVIM -DWI scan	MT scan
Sequence type	single coronal slice spin-echo prepared bTFE	coronal single-shot spin-echo-planar	single coronal slice TFE
Slice thickness(mm)	5	5	5
TR/TE (ms/ms)	3.4 /1.68	1278/71	20 /2.3
field of view (mm ²)	340 x 352	400 x 400	375 x 351
Flip angle (°)	50	90	15
Matrix	228 x 229	132 x 133	252 x 176
No. of slices	1	12	1
Breathholding (BH)	1 BH per TE value with a 15-sec gap between TE acquisitions	respiratory triggered	10s BH to cover both 'on' and 'off' resonance image acquisition
Parameter variation	TE-prep values(s): 20, 50, 80, 120, 180, 300.	b-values (10 ⁻³ mm ² /s): 0,50,100,200,300, 400,600,800,1000	Presaturation MT on /off pulse

7.4 Clinical MRI analysis

Conventional MRI severity index

Radiological scoring was assessed by two abdominal radiologists one (U.B) with 9 yrs experience in GI MRI, and one (N.K) clinical radiology fellow with 5-years' experience in abdominal cross-sectional imaging. The radiologists were blinded to the results of the quantitative sequences and clinical data results. The mural signal intensity and mural thickness (mm) at the most thickened bowel region on the coronal T2 TSE fat-suppressed sequence were used to quantify disease activity using the mural thickness

and T2 signal intensity^{94, 96}. The CDMI score at both visits was calculated by the radiologists using the following equation:

$$\text{CDMI score} = 1.79 + 1.34 \cdot \text{Mural Thickness} + 0.94 \cdot \text{Mural T2 Score} \quad (11)$$

Abnormal segments from the small bowel were evaluated by the scoring of mural T2 signal and mural thickness features from predominantly single slice data showing the most diseased segments (Figure 42). The mural thickness was measured using electronic callipers on the T2 TSE images and graded from 0 to 3 as 0:1-3mm, 1: >3-5mm, 2: >5-7mm and 3: >7mm. The grading of the mural signal intensity was compared with adjacent normal small bowel on fat-saturated coronal TSE images. This was graded from 0 to 3 as 0 : 0: Equivalent to the normal bowel wall, 1: Minor increase in signal - bowel wall appears dark grey on fat saturated images, 2: Moderate increase in signal - bowel wall appears light grey on fat saturated images, and 3: Marked increase in the signal- bowel wall contains areas of white high signal approaching that of luminal content⁹⁴.

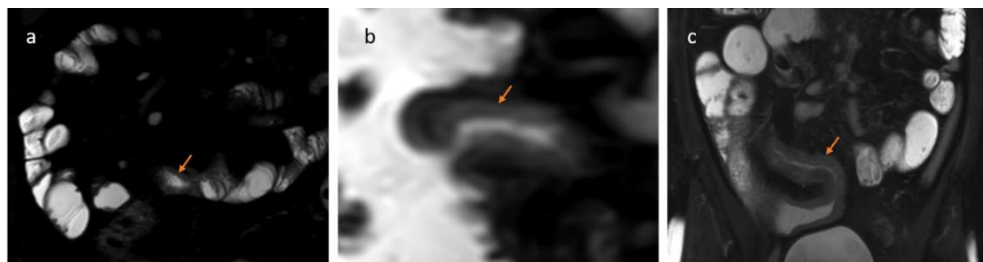


Figure 42: Qualitative examples of mural signal assessment using coronal T2 fat sat images. (a) the bowel wall (arrow) indicates a small increase in signal bowel wall looks dark grey(score 1).(b) the bowel wall indicates a moderate increase in signal it appears light grey (score 2).(c) the bowel wall (arrow) indicates a marked increase in signal it has areas of white high signal approaching that of luminal content.

Parameter fitting

T2 measurements

The Region of Interest (ROI) in the abnormal bowel segment, was defined during the clinical MRI analysis, and was drawn based on abnormal mural thickness. The regions on T2 TSE were drawn following input from radiologists with the ROI redrawn on the same regions on the bTFE images. Finally, all regions were then subsequently reviewed by the radiologists to validate the areas drawn. The ROI was drawn manually on the abnormal walls on T2 prep images across all TEs using Analyze9 (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN). The ROI could be moved between different TE images, if necessary, due to any misalignment due to bowel wall motions or respiration. A representative example of the ROI placement on the T2 prep images is shown in Figure 43.

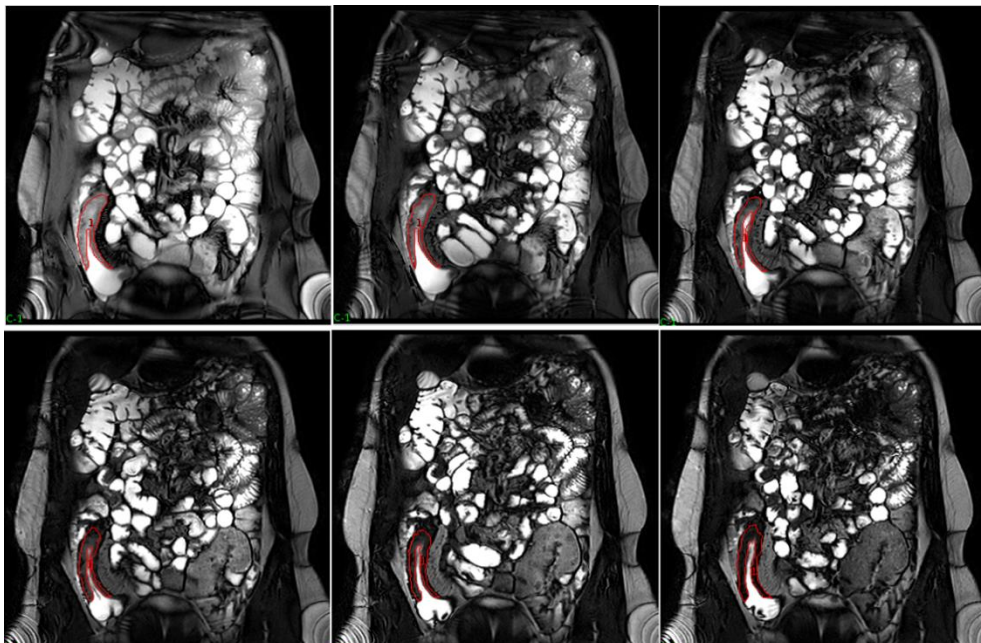


Figure 43: Illustrates the abnormal area of T2 decay of the bowel wall at TE 20,50 and 80(top) and TE 120, 180 and 300(bottom).

MT analysis

ROIs were drawn on abnormal areas included all affected bowel segments on the MT image (single slice) using an in-house Matlab script (written by Dr Caroline Haod) supported by one radiologist to validate the area. Normal bowel segments were identified as the bowel wall with a thickness of $< 3 \text{ mm}$ ¹²⁶. The (T2W) MR images were used as an anatomical reference for determining the abnormal areas. MT ratio (MTR) was calculated voxel by voxel after motion correction using equation 5 (as described in chapter 2)^{19, 20, 165}.

A representative example of the MT ROI is shown in Figure 44. The ROI was drawn on the abnormal small bowel wall after the motion-correction, and matched as closely as possible to the regions drawn on the T2 in the same visit. The in-house written software only allowed for a single ROI to be drawn at any one time, therefore, if more than one region was needed to define the diseased region, each was drawn separately onto the image, and combined afterwards using another programme to produce the mean MTR across the full region.

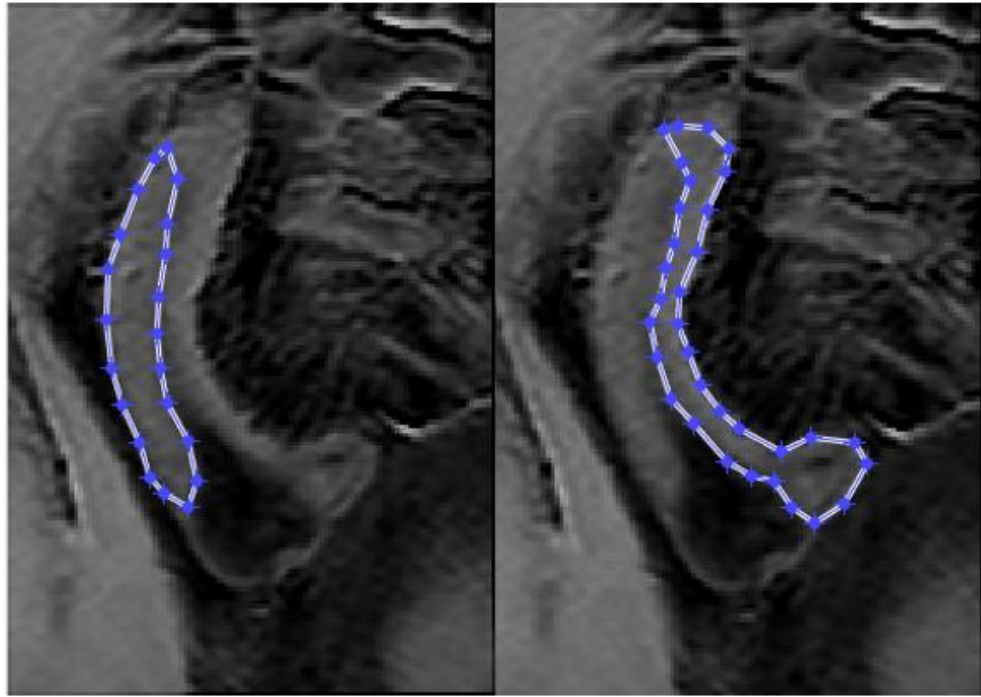


Figure 44: Illustrates the abnormal area of MT image.

IVIM-DWI data Analyses

IVIM-DWI data calculation and image analysis were all performed by using an in-house developed programme written in MATLAB (version 2019b, Mathworks, Natick, Massachusetts) (written by Dr Caroline Haod).

A similar process described for the REPEAT study was used in this workstream. Several steps were used for IVIM-DWI analysis. Initially, motion correction was run on abnormal wall segments of the image to see if this improved the alignment of the data, and, if not, the raw data images were used to define the ROIs. A freehand ROI was drawn on the motion-corrected data or DWI raw data across all b values in the abnormal segment wall with the maximum wall thickening and matched as closely as possible to the regions drawn on the T2. The ROI could be moved between different

b-value images if necessary. A representative example of ROI placement is shown in Figure 45.

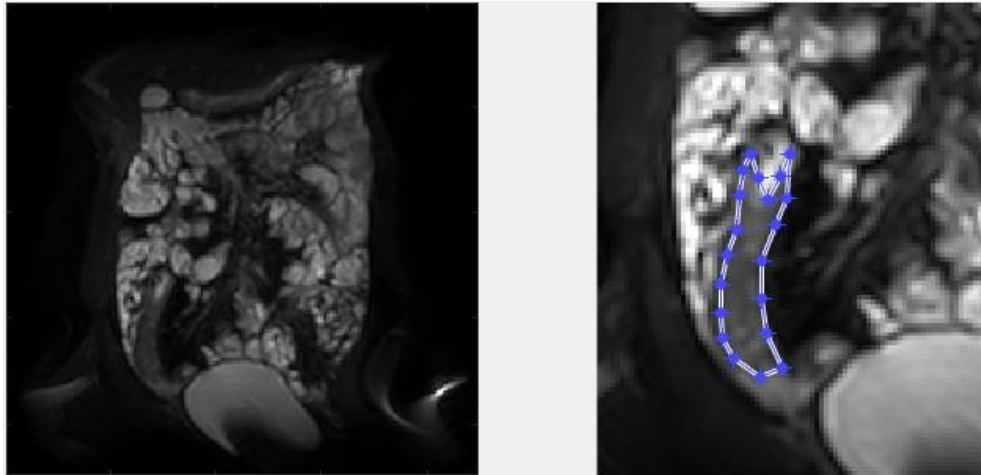


Figure 45: Illustrates the abnormal area of the DWI image.

In order to quantify the diffusion and the perfusion properties, the mean signal intensity values were then calculated from the ROI for each b-value image, and then analysed quantitatively according to both bi-exponential IVIM and mono-exponential models. This process was repeated multiple times if there were many abnormal bowel wall segments. Mono-exponential ADC maps were computed using all the b-value data, reflecting b-values that are typically used for DWI in clinical MRE protocols²²⁸. The IVIM signal was evaluated using equations 6 and 7 (as described in chapter 2).

A two-step analysis was used to derive the IVIM parameters, similar to that previously described in Chapter 6 (subsection 6.3)²²⁹.

The normal bowel for all these sequences was measured using the same methodology as in chapter 6 (subsection 6.3).

7.5 Statistical analysis

Reflecting the pilot nature of the study a per-protocol assessment has been undertaken. To assess the normality of distribution of the data, a Shapiro-Wilk test was undertaken for all data sets. Typically, normally distributed data were represented as mean and standard deviation (SD) while non-normally distributed data were expressed as median and interquartile range (IQR). A paired t-test was used for testing the statistical significance of changes before and after treatment for each parameter for normally distributed data. The correlations between the delta (visit 2 -visit 1)of the MRI parameters, and the delta of the FCP, HBI and CRP was used. Spearman's correlation coefficients were used to measure the strength of the correlation between clinical indicators and MRI parameters. Nonparametric tests such as the Wilcoxon test for pre- or post-treatment comparisons and Spearman analysis for relationship were used for data not normally distributed.

All the computations were performed using SPSS (version 125; IBM SPSS Corp.). Statistical significance was set at $P < .05$.

7.6 Results

Twenty-five participants were recruited to this study. Six participants subjects were later withdrawn from the study: due to protocol non-

compliance (n=4), requiring small bowel surgery before the start of treatment (n=1), and treatment cessation due to adverse events prior to visit 2 (n=1). Eighteen participants who received anti-TNF therapy, and one received ustekinumab (n=1) after the first MRI scan were analysed in this study. The 19 participants (M/F 9/10) had an average age of 43 ± 17 years. Of these patients, 5 were excluded from DWI-IVIM assessment (motion artefact 2 and 3 participants did not have a baseline DWI scan). Of the 19 participants, 5 were excluded from T2/MT analyses due to the abnormal area not included in the single slice, and 3 participants excluded from MT analyses due to motion artefact. Ten participants [53%] had a history of CD-related intestinal surgery. The flow chart for the subjects is included in the study in Figure 46. Initial clinical characteristics of participants are presented in Table 27.

FCP within a 3-month timeframe the MRI at visit one was available for 14 participants (average time between MRI and HBI measurements 20 ± 30 days, and at visit 2 FCPs were unavailable for three participants (two of them already out of three-month timeframe at visit1), leaving a total of 13 paired FCP datasets.

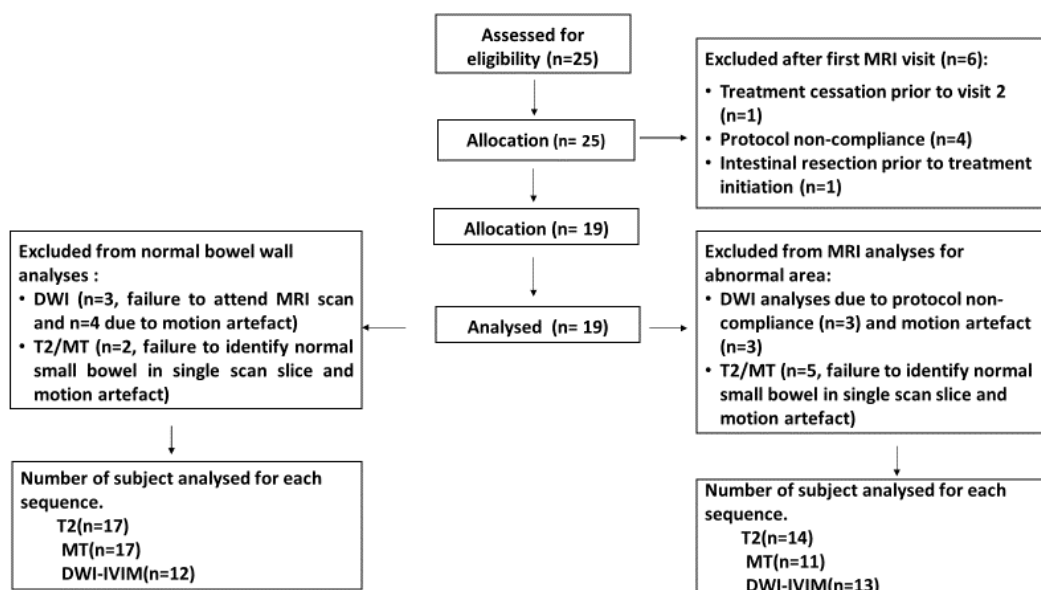


Figure 46: Flow chart.

Table 27: Participants demographic data and HBI/CRP/CDMI score results. The * indicates FCP acquired > 3 months from the visit, n/a the results unavailable, AD=adalimumab, INF=infliximab, UST= ustekinumab, 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.

Participants No.	Age	Gender	Montreal	medication	Smoking	Visit 1				Visit 2			
						FCP ug/g	CRP MG/L	HBI	CDMI	FCP ug/g	CRP MG/L	HBI	CDMI
1	21	F	A2L3B1	AD	No	514	8	4	5.01	940	5	1	5.01
2	40	M	A2L3B1	AD	Yes	1800*	5	2	6.35	n/a	5	2	6.35
3	33	M	A1L3B1	AD	No	567	8	9	6.35	87	5	5	1.79
4	21	M	A2L3B1	AD	No	1800	5	5	5.95	30	5	0	5.01
5	26	F	A2L3B5	AD	No	318	10	5	7.69	507	5	0	7.69
6	26	F	A2L1B1	AD	No	686*	5	3	8.63	30	5	4	4.07
7	46	M	A2L1B2	AD	No	834	5	4	8.63	1203	5	5	7.69
8	75	F	A3L1B2	AD	Yes	1800	30	4	5.01	1092	5	9	6.35
9	56	F	A2L1B2	AD	No	n/a	5	12	5.81	371	5	6	6.75
10	39	M	A2L3B2	AD	Yes	1162*	5	7	6.35	n/a	38	4	5.41
11	66	M	A3L3B1	AD	No	743	5	0	4.07	98	5	2	6.35
12	22	M	A2L1B1	AD	No	154	5	10	7.69	30	5	8	5.01
13	69	F	A3L1B2	AD	No	52*	6	0	6.35	30	5	1	4.07
15	58	M	L1B2	AD	No	787	5	1	6.35	522	7	1	7.69
20	57	F	A2L1B2	AD	Yes	230	5	6	6.35	134	5	9	5.41
21	51	F	A2L1B2	AD	Yes	32	5	8	6.35	60	5	2	6.35
22	46	F	A2L3B1	AD	No	1333	7	3	6.35	n/a	5	0	6.35
23	22	M	A3L1B2	AD	Yes	312	5	3	8.63	4.9	5	3	7.69
25	56	F	A2L1B2	UST	No	152	5	14	8.63	57	5	14	6.35

7.6.1 Comparison of data sets pre- and post-treatment

7.6.1.1 Comparison of changes in the MRI data sets

The median (IQR) of MRI sequences data and CDMI scores were not significantly different before and after treatment (Table 28).

7.6.1.2 Comparison of changes in the clinical data after treatment

After 20-28 weeks of biological therapy, CRP, HBI and FCP did not change significantly between study visits (Table 28).

Table 28: Comparisons of MRI parameters and clinical indicators before and after treatment for inflamed bowel segments.

	N	Pre-treatment	Post-treatment	P
MRI parameter				
T2 (ms)	14	69(53-79)	65(52-75)	0.9
MTR	11	0.28±0.04	0.26±0.09	0.46
D (x10 ⁻³ mm ² /s)	13	1.28±0.27	1.42±0.52	0.42
PF	13	0.26±0.075	0.32±0.149	0.19
D*(x10 ⁻³ mm ² /s)	13	14.9(9.58-21)	21.3(11-36.3)	0.19
ADC (x10 ⁻³ mm ² /s)	13	1.57(1.47-1.69)	1.63(1.38-2.25)	0.28
CDMI score	19	6.35(5.8-7.6)	6.35(5-6.7)	0.38
Clinical indicator				
CRP(mg/l)	19	5(5-7)	5(5-5)	0.34
HBI	19	4(3-8)	3(1-6)	0.08
FCP(μg/g)	13	514(192-810)	98(43-713)	0.12

7.6.2 Correlations between MRI Parameters

No significant correlations between MRI parameters were observed as shown in Table 29. Figure 47 shows scatter plots for some of the key correlations between the different MRI parameters measured.

Table 29: Correlation between MRI parameters in a group from both visits.

		Spearman Correlation		
Variable 1	Variable 2	N	R	P value
T2	MTR	22	0.18	0.43
	D	22	-0.28	0.21
	PF	22	0.23	0.31
	D*	22	-0.22	0.33
	ADC	22	0.20	0.37
	CDMI	28	0.12	0.56
MTR	D	20	0.22	0.36
	F	20	-0.01	0.95
	D*	20	-0.11	0.64
	ADC	20	0.07	0.77
	CDMI	22	-0.15	0.52
D	CDMI	26	-0.27	0.18
F	CDMI	26	-0.06	0.78
D*	CDMI	26	0.03	0.90
ADC	CDMI	26	-0.20	0.32

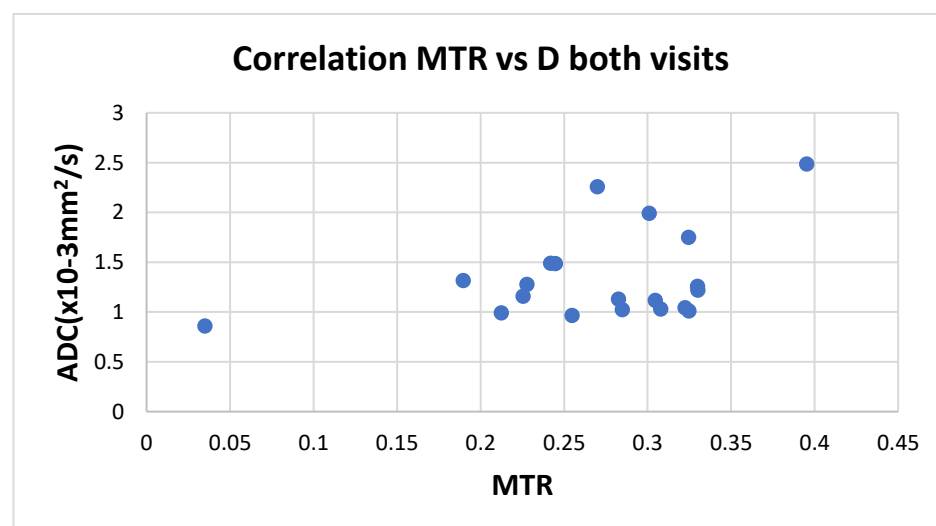
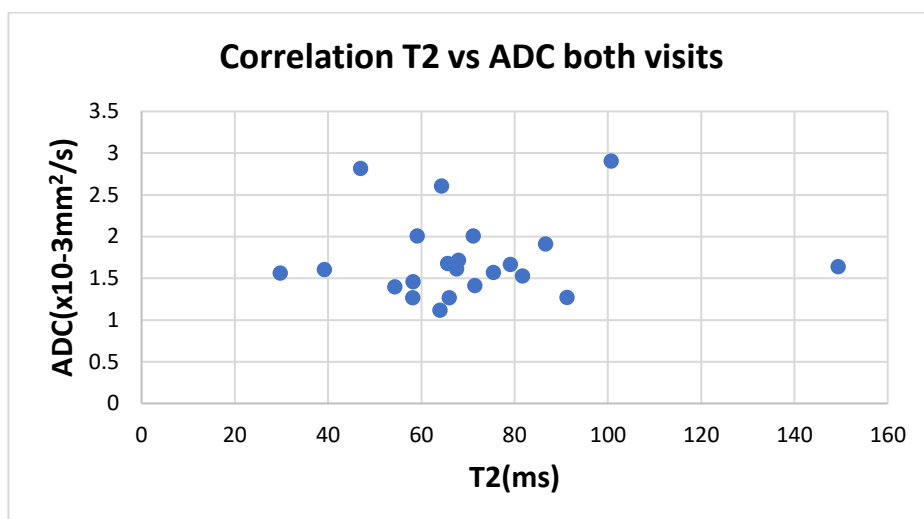
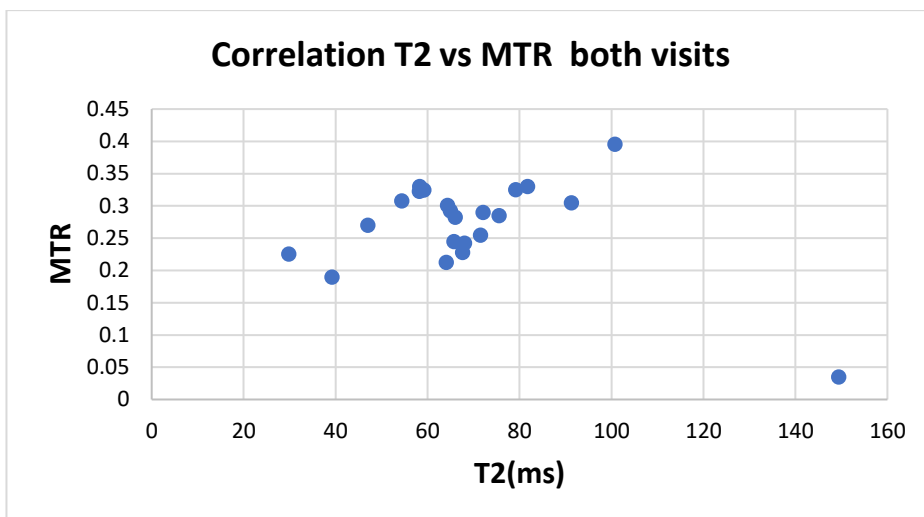


Figure 47: Correlation between median values of some of the MRI quantitative parameters.

7.6.3 Correlation of MRI parameters with clinical results

Table 30 shows the correlations of delta MRI sequences and delta MRI activity scores with delta clinical indices of the included participants. Both did not show any significant correlation with any of the assessed indices. None of the T2, MTR and IVIM-DWI showed a significant correlation with FCP, HBI and CRP values.

Table 30: Spearman correlations of delta T2, MTR and IVIM-DWI parameters and delta CDMI scores with delta clinical indicators.

			Correlation from Spearman	
Variable 1	Variable 2	N	R	P value
T2	CRP	14	-0.136	0.64
	HBI	14	-0.079	0.78
	FCP	9	0.450	0.22
MTR	CRP	11	0.867	0.06
	HBI	11	0.096	0.78
	FCP	7	-0.428	0.35
D	CRP	14	-0.077	0.79
	HBI	14	0.412	0.14
	FCP	8	0.500	0.21
PF	CRP	14	0.024	0.93
	HBI	14	-0.002	0.99
	FCP	8	0.306	0.46
D*	CRP	14	-0.077	0.79
	HBI	14	0.468	0.09
	FCP	8	0.309	0.46
ADC	CRP	14	0.063	0.83
	HBI	14	0.363	0.20
	FCP	14	0.142	0.75
CDMI	CRP	19	0.008	0.97
	HBI	19	-0.016	0.94
	FCP	13	-0.124	0.68
CRP	HBI	19	0.025	0.91
	FCP	13	-0.050	0.87
FCP	HBI	13	-0.215	0.47

7.6.4 Comparison of MRI sequences between normal and inflamed bowels

The results of the MRI sequences for the normal and inflamed bowel at baseline session are illustrated in Table 31. T2 and MTR values were not significantly different between normal and inflamed bowels at both visits.

For IVIM-DWI parameters, the ADC was significantly higher in normal than in inflamed bowels $3.22 \pm 0.72 \times 10^{-3} \text{ mm}^2/\text{s}$ vs $1.61 \pm 0.23 \times 10^{-3} \text{ mm}^2/\text{s}$ ($P < 0.0001$) at visit one and also a follow-up visit $3.20 \pm 0.71 \times 10^{-3} \text{ mm}^2/\text{s}$ vs $1.78 \pm 0.53 \times 10^{-3} \text{ mm}^2/\text{s}$ ($P < 0.0001$).

Significant statistical difference of pure diffusion coefficients D was also found between the inflamed and normal bowels at baseline visits ($P=0.0001$), and at follow up visit ($P=0.0007$), and also for PF at both visits ($P=0.001$ and $P=0.014$), but not for D^* ($P=0.1$ and $P=0.57$) from both visits.

Table 31: Comparison of MRI sequences between the inflamed bowel and normal bowel.

Visit 1				
	N	Inflamed_v1	Normal_v1	P
T2 (ms)	13	71(48-80)	60(52-70)	0.49
MTR	10	0.28±0.4	0.29±0.06	0.67
D (x10 ⁻³ mm ² /s)	11	1.33±0.26	2.76±0.82	0.0001
PF	11	0.24±0.06	0.43±0.14	0.0015
D* (x10 ⁻³ mm ² /s)	11	14.9(10.4-21.5)	22.3(17.2-24.8)	0.1
ADC (x10 ⁻³ mm ² /s)	11	1.61±0.23	3.22±0.72	<0.0001
Visit 2				
	N	Inflamed_v2	Normal_v2	P
T2 (ms)	13	65(50-79)	57(52-76)	>0.99
MTR	10	0.26±0.09	0.29±0.05	0.54
D (x10 ⁻³ mm ² /s)	11	1.38±0.50	2.60±0.87	0.0007
PF	11	0.32±0.14	0.49±12	0.014
D* (x10 ⁻³ mm ² /s)	11	21.3(9-34.5)	21.5(14.7-37.2)	0.57
ADC (x10 ⁻³ mm ² /s)	11	1.78±0.53	3.20±0.71	<0.0001

7.7 Discussion

This study aimed to investigate the performance and responsiveness of T2, MT and IVIM-DWI imaging to biological therapy as well as the association of these quantitative markers to more traditional disease measures, such as, serological markers, patient-reported outcomes, and MR T2 based radiological reporting.

Our most important finding was that in patients undergoing biological therapy, no significant differences after treatment were observed in MRI parameters, CDMI activity score and clinical indexes. There was no correlation among these MRI parameters, and nor between the change in MRI parameters and clinical indexes following treatment. One possible explanation for the discrepancy is that a relatively small number of patients was eligible in the group analysis ($n = 19$). The inter-relationship of T2 and MTR in inflamed and normal segments needs to be investigated further in larger cohorts.

To the best of our knowledge, the performance and responsiveness of quantitative T2 relaxometry have not been studied in CD. T2 weighted images allow CD characterisation that is critical to disease management. This sequence may be a surrogate of gut permeability and inflammation. A low T2 signal of the pathologic bowel wall was correlated with the presence of fibrosis with the amount of fibroblasts and collagen in the muscular propria and submucosal and layers associated with shorter T2 relaxation times exhibited by the bowel wall while an increase in T2 signal has been

shown to correlate with the presence of oedema in the submucosa layer, thereby related to active disease^{162, 163}. T2 relaxometry could be used as a promising non-invasive assessment tool of fibrosis and inflammation of the bowel wall.

In a dextran sodium sulphate (DSS) mouse IBD model, T2 relaxometry was able to differentiate between bowel wall inflammation and fibrosis²⁷⁴. In a conference abstract, the same group attempted to evaluate the value of T2 relaxometry in CD patients by comparing the rectum of healthy participants with the CD-patient group²⁷². The findings in their previous animal study were consistent with the CD study. When comparing the mean T2 within the rectal walls, between healthy volunteers (118.3 ms (115.4-121.3) and CD patients (113.0 ms (109.0-120.4)), no significant difference was observed ($p=0.119$). However, the T2 map of the CD rectum showed that histogram broadened to lower values in comparison with healthy volunteers ($p=0.002$).

In our study, although we did not have a histological read-out of inflammation nor fibrosis, other biochemical or symptom markers of intestinal inflammation were used. We observed no difference in T2 before and after treatment.

MT has been identified as a non-invasive technique for effective assessment of intestinal fibrosis as fibrotic tissues are rich in macromolecules due to the presence of collagen that affects the MTR²⁰,

and, hence, a high MTR at baseline may predict a non-response to biological anti-inflammatory therapy. The quantitative parameters of MT (normalised MTR and MTR) were found to be able to describe the severity of bowel fibrosis in human and animal CD^{20, 165, 166, 168, 275}. In an animal study, an increase in the MTR of fibrotic segments was found compared with control non-inflamed tissue¹⁶⁵. In other rat models, higher normalised MTRs were also reported in bowel wall segments affected by mixed histological inflammation and fibrosis versus other segments only affected by an inflammatory response¹⁶⁶. In a few studies, the correlation strength of normalised MTR and MTR with bowel fibrosis was identified^{168, 271, 275}. Li *et al.*¹⁶⁸ investigated the relative enhanced bowel gains between MTRs and ADC values – signal change with MT pulses, and validated in five CD patients against surgical histopathology. They found that MTRs were more accurate in detecting variable degrees of fibrosis followed by ADC¹⁶⁸. Moreover, the accuracy of MTR and normalised MTRs for distinguishing fibrotic from nonfibrotic bowel walls have been reported. A recent prospective study with 31 CD patients, Pazahr *et al.*²⁰ found that the MTR was higher in fibrotic strictures compared with the normal bowel wall and that the MTR was equivalent or slightly lower in inflammatory stenosis than in unaffected bowel segments. However, our results were in agreement with Pazahr *et al.*'s result, where we found MTR in the normal bowel wall was equivalent or slightly higher than in abnormal bowel segments. This may be owing to the presence of intestinal smooth muscle, and its effect on MTR. All previous studies have not investigated the value of MTR following biological therapies and its possible use at the baseline to predict a

response. In our study, we observed no difference in MTR before and after treatment.

DWI derives its image contrast from differences in movement between tissues in water molecules. This sequence is widely used for the identification of abnormal bowel segments, and the evaluation of inflammatory disease activity in patients with CD. This sequence avoids the use of gadolinium injection. The shortcoming of DWI is that it cannot differentiate between multi-compartmental (such as extracellular vs intracellular and perfusion vs diffusion) effects in biological tissues²³. Conversely, the IVIM can separate diffusion properties in various compartments (f , D^* , D). This technique can provide quantitative information about blood perfusion via perfusion coefficient and fractional perfusion without administering contrasts. Studies concerning IVIM-DWI in CD patients are quite limited^{179-181, 276}. To the best of our knowledge, this study is the first study using this technique especially in evaluating treatment in CD patients. Following biological therapy, IVIM values measured in the bowel wall of CD patients were not significantly different from the baseline.

Our findings revealed that bowel wall inflammation induced restricted diffusion, and that IVIM-DWI yielded quantitative (decreased ADC and D values) data that could be used for the assessment of bowel inflammation. The explanation as to why an inflamed bowel has restricted diffusion presented a difficult problem, and further studies are needed to tackle it. In previous studies, a significant reduction in ADC was found in inflamed bowel

segments^{172, 173, 180, 276}. Our findings showed a significant reduction in ADC value, which was in accordance with prior studies. The water restriction might be related to high cellularity and viscosity as Oto *et al.* suggested¹⁷³. The lamina propria and submucosa are infiltrated in the early stages of CD by inflammatory cells. Other factors contribute to the restricted diffusion of water molecules, such as, the development of granulomas, dilated lymphatic channels, aggregated lymphocytes, and hypertrophied neuronal tissue in the bowel wall can further narrow the extracellular space.

In IVIM characterisation in paediatric CD, previous authors found significantly lower fractional perfusion, a higher perfusion coefficient, and no significant difference in the diffusion coefficient of the enhancement compared with that of the non-enhanced bowel wall¹⁸⁰. In another study, Hectors *et al.* found a significantly lower PF, D, ADC, and a non-significant increase in D* in abnormal versus normal bowel segments in 17 CD patients¹⁸¹. Our results were consistent with Hectors *et al.* study with all IVIM parameters significantly lower in PF, D, ADC, and a non-significant difference in D*. Also, similar findings to the paediatric study¹⁸⁰ in some of these parameters were found in, such as, PF, ADC, but not in D* and D. The reasons for the inconsistency between our results and Freiman *et al.* might be related to the method of DWI measurements and acquisitions, they used axial scanning with 8 different b values = 5, 50, 100, 200, 270, 400, 600, 800 s/mm².

A recent study found that mean D, PF, D*, and ADC values of normal bowel were significantly higher than those of inflamed bowel ($p < 0.05$)²⁷⁶. Overall our findings, also, were in accordance with findings reported by Yang *et al.*²⁷⁶ except for D*. In their results, they found a significant decrease in inflamed bowel compared with normal bowel in PF (33.58% vs 69.59%), D (1.31 vs 1.06), D* (29 vs 13) and ADC 1.57 vs 33.5) mm²/s.

Moreover, we have validated previous findings by suggesting that ADC values were significantly different between normal vs inflamed segments^{180, 270}.

In our study, IVIM-DWI parameters did not show any significant correlation with any of the clinical data (delta HBI, FCP and CRP values). Our results were in line with the previous study by Hectors *et al.* Another study had shown a significant negative correlation between ADC and CRP¹⁷². In our study, an explanation for the lack of correlation with clinical characteristics might be as a result of the relatively long delay between the MRI examination and some of these clinical measurements.

In our study, a few participants were unable to consume the amount of mannitol which might have had an effect on our image quality and results as the bowel did not distend very well, especially, for diffusion.

This study had several limitations. First, the number of patients included in this study was relatively small. Second, only a single slice of MT

and T2 sequences was acquired, and, hence, did not cover the whole abnormal region, and might therefore not be representative of all the diseased tissue. As a result, it might be difficult to interpret the change in value of the MTR and T2 images for the overall assessment in CD. This might be improved if these sequences were obtained by taking multiple slices to incorporate the whole disease area. Moreover, the T2 and MTR measures did not take into account the heterogeneity of intestinal inflammation and fibrosis in these patients as no histological data were available. Due to a limitation of time and manpower no repeatability assessments in this CD cohort were undertaken within this thesis.

We did not have standard references, such as, pathological specimens, so the comparative analysis between MRI parameters and pathology was lacking. Although we have made every effort by magnifying images by drawing ROIs to try to cover the bowel wall exclusively, we were unable to eliminate the possibility of a partial volume effect on parameter measurements, particularly, from the normal bowel wall. Furthermore, the clinical index FCP was not available for all patients.

Conclusion: These parameters have not indicated any change before and after biological treatment, and no correlation between these measurements was observed too. The exact role of these parameters for CD management should be assessed in a larger cohort with histopathological validation.

Chapter eight: Summary, implications and conclusions

8.1 General summary

The work presented in this thesis represents, the application of MT, T2 relaxometry measurements, dynamic contrast enhancement, diffusion weighted imaging for the assessment treatment of Crohn's disease, and pCD. Both MAP (chapter 5) and MOTILITY plus (Chapter 7) studies were more advanced studies, which examined more at the reversibility of the signal, and early clinical utility while the REPEAT study (chapter 6) was a technical validation study for the MOTILITY plus study.

Perianal Crohn's disease (pCD) is a potential complication of CD. Absence of reliable disease measurements makes disease monitoring unreliable. MRI has been, increasingly, used for diagnosing and monitoring pCD patients, and has shown potential for assessing a therapeutic response and inflammation. In **Chapter 5** an exploratory study was undertaken to assess changes in quantitative MRI sequences (DWI, DCE, MT and T2) before and after treatment with biological agents in a group of patients with active pCD, the correlation between these parameters and clinical indices were assessed, and the correlation between the MRI parameters was investigated too (MAP study).

Results showed that the mean and SD of MRI sequences were not significantly different before and after treatment while a significant difference before and after treatment in clinical indicators was observed. Also, no

significant correlations between MRI parameters and clinical indices were observed. A significant negative correlation was found between MTR and T2, DWI and DCE parameter k^{trans} .

Our major finding was that in the MRI assessment of perianal CD, the repeatability of acquired data sets was relatively poor on 1.5T MRI platforms, and for fistula volumes less than 2 mL (albeit indicated from MTR data) irrespective of which MRI platform was used. This finding may be of some clinical significance as at present most worldwide health care systems use 1.5T MRI systems to monitor the response of biological treatment in pCD rather than 3T systems. However, for quantitative measurements our data suggested that 3T systems would be more accurate instead of 1.5T. Having said this, the non-quantitative measure of activity (Van Assche Score) which was measured from the 1.5 T data, did show good correlation with the clinical indicators (CRP and PDAI).

Quantitative MRI datasets based on relaxometry, MT and IVIM-DWI sequences could be used as potential biomarkers of disease activity in inflammatory bowel disease. T2- and T1-weighted sequences are used as disease activity biomarkers in inflammatory CD. Also, the MT and IVIM-DWI imaging might play a role in the imaging of fibrosis in CD. Knowledge of measurement repeatability is essential to differentiate between changes that could be attributable to measurement variability, and those that are the result of therapeutic intervention or disease progression. Evaluating the repeatability and variability of these types of sequence in the bowel is

important before embarking on disease-specific outcome studies. In **chapter 6** we determined the test-retest repeatability and variability of these MRI parameters in the small bowel segment following bowel preparation and administration of an anti-spasmodic agent before we used them in the clinical study (REPEAT study).

An excellent CoV= 5, 7, 8 and 10% was calculated for ADC, MTR, T1 and D respectively across visits, while PF(ICC=20%) had good repeatability, and acceptable for T2 (CoV 21%), however, the D* data repeatability was poor CoV=31%).

An almost perfect correlation between both observers was found for T2 (ICC=0.89) and D (ICC=0.86). A good correlation was calculated for ADC (ICC=0.76) and moderate for both T1 and PF (ICC=0.55 and 0.41, respectively), and poor ICC was found for D* (ICC=0.14) and MTR (ICC=0.08).

The test-retest repeatability appeared to be very high in most of these biomarkers except for D*, which also demonstrated the poorest repeatability and variability. Intra-observer variability data will be needed. As a matter of fact, these analyses are presently underway, and will be available once this workstream has been submitted for publication. Moreover, these sequences will need to be assessed in a reproducible study with a large number of volunteers, and with a wide spread of ages as this variable can affect both repeatability and reproducibility. Finally, a fully- or semi-automated

methodology to identify bowel wall regions will be needed through a development of custom-written software as present procedures have been exceedingly time consuming, and prone to variability, hence, limiting the progression of this important work.

Validation of an imaging biomarker in predicting therapeutic response involves a different methodology. The advent of biological therapy is a major development in CD treatment to improve symptoms and long-term outcomes as well as to reduce hospitalisation and abdominal surgery. However, in CD, when assessing a therapeutic response to biological therapy is challenging as clinical indices are subjective and time-consuming. Since conventional colonoscopy is not an ideal modality for a transmural assessment of the small bowel, in the last two decades several non-invasive tools have been developed for investigating CD. MRI can be used to address these issues and limit the data that evaluate changes in MRI biomarkers with treatment, and, if present, whether changes reflect treatment response or whether they can help to differentiate responders from non-responders. In **chapter 7**, we performed a prospective study with 25 participants with active CD, who were scanned before and approximately 6 months after biological therapy by using the same sequences, which have been described in chapter 4 without using a T1 sequence (MOTILITY plus study). We assessed the change in these MRI biomarkers before and after treatment with biological therapy, the correlation between these parameters and clinical indices, and correlation between the MRI parameters was investigated.

Results showed that no significant change was observed before and after treatment. No correlations were found either between these MRI parameters, or between MRI parameters and clinical indices. Plausible explanations for this outcome are that, as we did not have a histological read-out of inflammation or fibrosis, other biochemical or symptom markers of intestinal inflammation were used.

8.2 Implications

Fibrosis detection is critical for the assessment of CD patients since its identification can have therapeutic consequences since most affected segments have both fibrotic and inflammatory components. Endoscopy or histological analysis of endoscopic biopsy are not suitable for characterising the mix between inflammation and fibrosis. However, these new MRI sequences, such as, MT, T2 and IVIM-DWI are able to evaluate transmural lesions, and to discriminate between severely and fibrotic and inflamed lesions. Additional sequences, such as, motility may be helpful in differentiating between fibrosis and inflammation. Data from this thesis has started to shed some light on the technical validation of these sequences in the normal small bowel wall, the reversibility of these MRI signals in luminal and pCD may provide early pilot data on the role of these MRI sequences in predicting response to therapy.

In general, there are several limitations to these studies. Our cohorts were relatively small. A larger number of participants would enable more robust conclusions. We used PDAI score to define treatment response. This

score has several limitations, such as, being a subjective independent score. A more objective, reliable, non-invasive method is needed to define treatment response. Moreover, the definition of the regions is changeable in these luminal and pCD studies after treatment, many other relaxometry studies would have involved imaging a whole organ (e.g. liver, kidney, or prostate) and therefore it is far easier to see changes in these parameters if the same tissue is sampled each time.

8.3 Research impact

The MAP study was an exploratory study to explore the nature of pCD and how MRI quantitative sequences reflect the results of treatment. The initial result showed a significant negative correlation between these sequences but did not correlate with the clinical outcomes at the 12-week measurement point. By developing novel MR disease activity indices for pCD. It is expected to have a positive impact on clinical decision making (including treatment escalation, timely onset and optimization among the various available schemes and follow-up) as well as on patient's quality of life and disability. Subsequently, this may lead to better allocation of the existing healthcare resources and to the reduction of the economic burden of living with IBD (e.g. reduce preventable hospitalizations, reduce cost of treatment, and increase patient productivity).

Besides this, it may improve knowledge of the relationship of MRI data with other treatment responses and could help scientists to design their research using these sequences.

Imaging biomarkers have the advantage of being non-invasive approaches which are spatially resolved and can be repeated to give temporal information. Development and validation of these biomarkers is important to ensure these biomarkers are robust and fit for purpose. Using these biomarkers in healthy volunteers increases our knowledge about the structure of normal healthy tissue. This will help us to improve our understanding of the effect of physical changes to the tissue when compared to diseased tissue and the technical issues and errors involved in the measurement process. Consequently, this could determine a specific cut-off value between normal and disease areas, which is of value in guiding decision-making in patients.

8.4 Future directions

We now plan to assess responsiveness to medical therapy in a larger sample cohort with significant pCD, with a longer disease follow-up using 3T MRI platforms. We aim to complete the technical validation for these sequences in the small bowel and investigate further the use of these sequences as predictors of response to medical therapy. Moreover, we aim to undertake now a biological validation of DWI, MTR, T2 and motility as imaging biomarkers of fibrosis using histological fibrosis as a gold standard in CD patients needing intestinal resections.

Finally, colleagues and myself aim to better investigate the role of MRI as an imaging tool in colonic inflammation. Early optimisation work in healthy volunteers is presently being undertaken to investigate the effect of

oral contrast on colonic motility and to identify the best time for data acquisition after contrast ingestion. We aim now to investigate these changes against more standardised markers of colonic inflammation.

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