The Application of Novel Imaging Modalities in the Monitoring of Inflammatory Activity in Crohn’s Disease

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ABSTRACT

Clinical scores, serum and faecal markers, and endoscopy all have limitations in their use as instruments to monitor disease activity in Crohn’s disease (CD). Recently, $^{18}$F-FDG-PET and novel MRI techniques have been proposed as sensitive and specific methods to quantify the inflammatory load. The aim of this work was to assess the reliability, responsiveness and, to an extent, the validity of outcome measures in these modalities, in monitoring inflammatory activity over a 12-week interval. In addition, two receptors, TSPO and IL-2R were assessed on tissue specimens ex-vivo for their potential to act as alternative targets for molecular imaging in CD.

Three distinct groups of patients were recruited, 2 of which participated in the clinical imaging study, and one to donate tissue for the laboratory work. Dual timepoint FDG-PET and MRI scanning was performed within 1 week (Group 1) to assess the test-retest reliability of the imaging outcome measures, and before, and twelve weeks into anti-TNFα therapy (Group 2) to assess their responsiveness indices. The third group contributed tissue during scheduled intestinal resection for assessment of TSPO and IL-2R interactions with their corresponding radioligands. To support the latter study, stored tissue sections were also obtained for immunohistochemical assessment of target receptor expression.

Results on 22 patients show that PET endpoints such as SUV$_{\text{MAX}}$ and SUV$_{\text{MEAN}}$ have high responsiveness and reliability indices and demonstrated significant differences in anti-TNF responders compared to non-responders. The finding of luminal FDG signal may affect the face validity of the scan. MRI modalities appeared less responsive at three months. Analysis on ex-vivo specimens showed increased abundance of TSPO in normal bowel, but a relative over-expression in inflamed specimens which was not statistically significant. IL-2R appeared more abundant in transmural sections containing severe CD, but autoradiographic corroboration was not achieved for technical reasons.
DECLARATION

I declare that the work in this thesis is my own.

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Evangelos Russo
London
March 2015
DEDICATION

To Eleni, Aurelia and Aris
With Love
ACKNOWLEDGEMENTS

This thesis would not have been possible without the help of many of people, and it is a pleasure to acknowledge their contributions:

Firstly, I would like to express my gratitude to my primary supervisor, Prof Tim Orchard for his support, guidance and infinite patience throughout the last few years. I would also like to thank my co-supervisor Prof Paul Matthews for introducing me to the concept of PET and MRI inflammatory imaging, for providing valuable assistance with the development of both the clinical and laboratory protocols, as well as for teaching me the basic principles of scientific methodology and approach.

I am obliged to the whole clinical team at Imanova for their help with the imaging study, from inception to protocol design, to scanning and data analysis. In particular, I am very grateful to Ilan Rabiner, Courtney Bishop and Roger Gunn for always being available to offer advice and constructive criticism. Moreover, I would like to thank the Academic Imaging group at University College London (Prof Stuart Taylor, Dr Doug Pendse, Dr Jesica Makanyanga and Nikos Dikaios for their tremendous help in setting up the MRI protocol, proofreading the analysis and reviewing the draft of the relevant thesis chapter. I would also like to thank Dr Sameer Khan, a nuclear medicine physician from Imperial Healthcare NHS Trust who helped me with the analysis of PET scans and reviewed the draft of the relevant chapter.

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I thoroughly enjoyed the company of fellow doctoral students Drs Lucy Hicks, Sam Powles and Nowlan Selvapatt who were reliable research companions, offering much support during the most stressful periods.

Recruitment would not have been possible without the help of the Inflammatory Bowel Disease nurse specialists Santosh Chadda and Lyn Evans at Imperial Healthcare NHS Trust, and Research nurse Susie Marriott at Royal Devon and Exeter NHS Trust to whom I am truly indebted. I am also grateful to the IBD team at West Middlesex University Hospital who also contributed with three participants.

Finally, and most importantly I would like to thank my wife Eleni and my children Aurelia and Aris for their unceasing love, and endurance and for being a constant but much-needed distraction throughout the duration of this project.
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<tbody>
<tr>
<td>ADC</td>
<td>Apparent Diffusion Coefficient</td>
</tr>
<tr>
<td>AIS</td>
<td>Acute Inflammatory Score</td>
</tr>
<tr>
<td>ARSCAC</td>
<td>Administration of Radioactive Substances Advisory Committee</td>
</tr>
<tr>
<td>AU</td>
<td>Arbitrary Units</td>
</tr>
<tr>
<td>CD</td>
<td>Crohn's Disease</td>
</tr>
<tr>
<td>CDAI</td>
<td>Crohn's Disease Activity Index</td>
</tr>
<tr>
<td>CDAS</td>
<td>Crohn's Disease Activity Score</td>
</tr>
<tr>
<td>CDEIS</td>
<td>Crohn's Disease Endoscopic Index of Severity</td>
</tr>
<tr>
<td>CED</td>
<td>Cumulative Effective Dose</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CRP</td>
<td>C –Reactive Protein</td>
</tr>
<tr>
<td>CT</td>
<td>Computerised Tomography</td>
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<tr>
<td>DCE</td>
<td>Dynamic Contrast- Enhanced</td>
</tr>
<tr>
<td>DSS</td>
<td>Dextran Sulfate Sodium</td>
</tr>
<tr>
<td>DWI</td>
<td>Diffusion Weighted Imaging</td>
</tr>
<tr>
<td>ECCO</td>
<td>European Crohn’s and Colitis Organisation</td>
</tr>
<tr>
<td>eGFR</td>
<td>estimated Glomerular Filtration Rate</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked ImmunoSorbent Assay</td>
</tr>
<tr>
<td>FBP</td>
<td>Filtered-back projection</td>
</tr>
<tr>
<td>FC</td>
<td>Faecal Calprotectin</td>
</tr>
<tr>
<td>FDG</td>
<td>Fluorodeoxyglucose</td>
</tr>
<tr>
<td>FFPE</td>
<td>Formalin-Fixed Paraffin-Embedded</td>
</tr>
<tr>
<td>FL</td>
<td>Faecal Lactoferrin</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half-maximum</td>
</tr>
<tr>
<td>GETAID</td>
<td>Groupe d'Etude Therapeutique des Affections Inflammatoires du Tube Digestif</td>
</tr>
<tr>
<td>GFD</td>
<td>Gluten-free Diet</td>
</tr>
<tr>
<td>GI</td>
<td>Gastro-intestinal</td>
</tr>
<tr>
<td>GLG</td>
<td>Global Lesion Glycolysis</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical User Interface</td>
</tr>
<tr>
<td>HBI</td>
<td>Harvey-Bradshaw Index</td>
</tr>
<tr>
<td>H &amp; E</td>
<td>Haematoxylin and Eosin</td>
</tr>
<tr>
<td>HMPAO</td>
<td>Hexamethylpropyleneamine oxime</td>
</tr>
<tr>
<td>hr-IL2</td>
<td>Human Recombinant Interleukin-2</td>
</tr>
<tr>
<td>Hpf</td>
<td>High-power field</td>
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<tr>
<td>HU</td>
<td>Hounsfield Units</td>
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<tr>
<td>IBD</td>
<td>Inflammatory Bowel Disease</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
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<tr>
<td>IL-2</td>
<td>Interleukin-2</td>
</tr>
<tr>
<td>IL-2R</td>
<td>Interleukin-2 Receptor</td>
</tr>
<tr>
<td>IQR</td>
<td>Inter-Quartile Range</td>
</tr>
<tr>
<td>LDA</td>
<td>Linear Discriminant Analysis</td>
</tr>
<tr>
<td>MaRIA</td>
<td>Magnetic Resonance Index of Activity</td>
</tr>
<tr>
<td>MICD</td>
<td>MRE score of activity in ileal Disease</td>
</tr>
<tr>
<td>MEGS</td>
<td>Magnetic Enterography Global Score</td>
</tr>
<tr>
<td>MH</td>
<td>Mucosal Healing</td>
</tr>
<tr>
<td>MRE</td>
<td>Magnetic Resonance Enterography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MVD</td>
<td>Micro-Vascular Density</td>
</tr>
<tr>
<td>NK cells</td>
<td>Natural Killer cells</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative Predictive Value</td>
</tr>
<tr>
<td>OSEM</td>
<td>Ordered Subset Expectation Maximisation</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
</tr>
<tr>
<td>R&amp;D</td>
<td>Research and Development</td>
</tr>
<tr>
<td>REC</td>
<td>Research Ethics Committee</td>
</tr>
<tr>
<td>RCE</td>
<td>Relative Contrast Enhancement</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver Operating Characteristic</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>RRG</td>
<td>Responsiveness Ratio of Gyatt</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse Transcriptase Polymerase Chain Reaction</td>
</tr>
<tr>
<td>SBFT</td>
<td>Small Bowel Follow-Through</td>
</tr>
<tr>
<td>SES</td>
<td>Standardised Effect Size</td>
</tr>
<tr>
<td>SI</td>
<td>Signal Intensity</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computerised Tomography</td>
</tr>
<tr>
<td>SUV</td>
<td>Standardised Uptake Value</td>
</tr>
<tr>
<td>TI</td>
<td>Terminal Ileum</td>
</tr>
<tr>
<td>TIC</td>
<td>Time Intensity Curve</td>
</tr>
<tr>
<td>TIV</td>
<td>Total Inflammatory Volume</td>
</tr>
<tr>
<td>TLG</td>
<td>Total Lesion Glycolysis</td>
</tr>
<tr>
<td>TNBS</td>
<td>2,4,6, TriNitroBenzeneSulfonic acid</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour Necrosis Factor</td>
</tr>
<tr>
<td>TSPO</td>
<td>Translocator Protein</td>
</tr>
<tr>
<td>UC</td>
<td>Ulcerative Colitis</td>
</tr>
<tr>
<td>UCL</td>
<td>University College London</td>
</tr>
<tr>
<td>VIBE</td>
<td>Volumetric Interpolated Breath-hold Examination</td>
</tr>
<tr>
<td>VOI</td>
<td>Volume of Interest</td>
</tr>
<tr>
<td>Voxel</td>
<td>A value on a regular grid in three-dimensional space</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cell</td>
</tr>
<tr>
<td>WSI</td>
<td>Wall Signal Intensity</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

1.1 EPIDEMIOLOGY, CLINICAL FEATURES AND PATHOPHYSIOLOGY OF CROHN'S DISEASE

Crohn’s disease (CD) is a chronic idiopathic disorder affecting the gastro-intestinal (GI) tract and, less frequently, extra-intestinal tissues. Its characteristic features are those of segmental inflammation affecting any part of the gastrointestinal tract, with lesions extending across the full thickness of the bowel.

The highest incidences of CD have been observed in the USA, the UK and Northern Europe, as well as New Zealand with up to 16.5 new cases per 100,000 population each year. Moreover, there is an increasing incidence from regions with a very low prevalence such as the Far-East, South-East Asia and Eastern and Northern Mediterranean. The disease most commonly presents in the 3rd decade, with up to 20% of cases occurring in children 1.

The pathogenesis of the condition has not been fully elucidated. The current pathogenetic paradigm proposes the disturbance of the mucosal barrier and microbiota balance in response to environmental triggers in genetically susceptible individuals. These events lead to the disruption of intestinal mucosal homeostasis 2,3.

Study into the immunogenetics of IBD, predominantly through the latest genome-wide association studies has identified at least 163 associated loci. Analysis of these findings offers insight into the biological mechanisms of the inflammatory bowel diseases, such as autophagy, barrier defence and T-cell differentiation signalling, as well as provide a focus for the discovery of new therapeutic pathways 4,5.
The natural history of the disease has been thoroughly characterised. Even though a hallmark of the disease is its potential to involve any region of the GI tract from mouth to anus, in a large majority its distribution follows one of three characteristic patterns. Patients typically present with either isolated small bowel disease, exclusive colonic disease or ileocolonic disease, which occur in approximately equal proportions (37%, 22% and 33% respectively)\textsuperscript{6,7,8}. While the disease distribution at presentation tends to remain stable in approximately 80% of patients over time \textsuperscript{9,10}, the individual lesions themselves behave in a highly dynamic manner.

Several macroscopic histopathological abnormalities are encountered in CD. The disease typically affects the gut in a transmural fashion and the lesions are discontinuous, separated by uninvolved or ‘skip’ areas. Mucosal ulcers start as punctiform superficial erosions termed ‘aphthoid’ ulcers, which, in time evolve into much larger confluent ‘serpiginous’ ulcerations, which run transversely and longitudinally along the involved mucosa giving it its characteristic ‘cobblestone’ appearance. Transmural extension of these ulcers can occur, typically on the mesenteric aspect of the intestine, which penetrate through the serosa producing fistulae or abscesses. Moreover, tissue oedema causing widening of the submucosa is observed, a sign of active disease. Strictures is another typical macroscopic complication of CD, characterised by bowel wall thickening and luminal narrowing, expansion of the muscularis secondary to smooth muscle cell hyperplasia, and deposition of collagen, laminin and tenascin. Expansion of the mesenteric fat which extends circumferentially around the affected bowel loops is also seen, commonly termed ‘fat wrapping’, which results in separation of diseased loops from uninvolved adjacent ones. Finally, prominent blood vessels are seen in the serosal surface, and swollen lymph nodes can be observed in association with diseased segments \textsuperscript{11}. 

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On a microscopic level the characteristic feature of CD is non-caseating granulomata, collections of macrophages and monocytes, as well as giant cells, lymphocytes and occasionally fibroblasts. Villous or crypt architectural distortion is observed as well as an increase in both acute inflammatory (neutrophils, macrophages) especially around the base of ulcerations and along fistular tracts, and chronic inflammatory cell types (lymphocytes and plasma cells) seen diffusely across all layers.

The macroscopic features of ulceration, fistulisation, abscesses, bowel wall oedema, and prominence of serosal vasculature are discussed again later in this thesis, as they form the basis of the identification of diseased segments by cross-sectional imaging techniques. However, with the exception of ulcers, these are indicators of transmural pathology in CD. While the response of mucosal lesions is a well-described endpoint of treatment, the potential reversibility of these features of transmural inflammation in CD remains to be determined. Moreover, acute and chronic inflammatory infiltrates underpin functional imaging modalities that can potentially quantify disease load, such as FDG-PET, and are also examined in detail later in this work.

De novo lesion development from normal mucosa has been studied in patients following intestinal resection and anastomosis. The process is initiated shortly after the resection by the development of an inflammatory cellular infiltrate proximal to the anastomosis, followed by aphthous ulcers within a few months. At presentation, a majority of patients with ileal disease and almost all with colonic distribution only exhibit inflammatory lesions. Over time, an increasing proportion of lesions develop a stricturing and/or penetrating character, and after five to seven years more than half the patients have one of these phenotypes, at least those with small bowel distribution. This pattern of development has not changed significantly over the last decade. Strictures and fistulae are the major contributors in the overall morbidity of CD, have significant impact in quality of life, and result in
hospitalisation, surgery and a significant cost burden \textsuperscript{16,17}. This pattern of progression is not necessarily reflected in patients’ symptoms, with many patients progressing to structuring or fistulising disease despite minor or relapsing and remitting symptoms. The currently accepted paradigm on the predominant natural history of CD is that of progressive and accumulating bowel damage irrespective of symptomatology. This has now been demonstrated in large studies from both referral centres\textsuperscript{9} and population based cohorts\textsuperscript{10}.

Despite this progress in understanding the pathogenesis the prognosis of CD is very variable and monitoring the disease can be challenging. A number of different modalities have been considered, outlined below, but there still remains significant unmet need.

1.2 DISEASE MONITORING IN CROHN’S DISEASE

Appropriate instruments that monitor disease activity in CD are essential for a number of reasons: Firstly, symptom perception and reporting are often not in keeping with the evolving course of underlying pathology, and, as such, they are poor predictors of worsening morbidity and complications.

Secondly, novel biologic therapies are becoming a mainstay in the management of CD due to their disease-modifying potential\textsuperscript{19,20}, but a significant proportion of patients either fail to respond at induction, or lose their response after a variable period of successful therapy\textsuperscript{21,22}. In addition to being associated with considerable toxicity and side effects\textsuperscript{23}, these agents pose a significant cost burden to health services with annual rates of therapy estimated in the tens of thousands\textsuperscript{24}. A monitoring strategy that would objectively measure response would be useful for better decisions regarding changes of treatment in non-responding patients or to justify ongoing therapy in those with objective indicators of benefit.
Finally, the development of appropriate outcome measures can prove valuable in the field of new drug development. There is a growing pipeline of candidate drugs in IBD\textsuperscript{25}, and, as a result, a great need for suitable biomarkers, which are reproducible, validated and responsive to change. These can enhance the efficacy of enrolment, by ensuring recruitment of patients with objective activity rather than functional symptoms, a well-highlighted issue in past studies\textsuperscript{26}. As biomarkers become more sophisticated, the selection can become even more focused, to include patients with a certain phenotype or a specific dominant inflammatory pathway targeted by the corresponding drug. Just as importantly, they can enable the tracking of the biologic effects of new agents, and even act as benchmarks for the definition of response and remission\textsuperscript{27}.

The following review critically assesses monitoring tools, which either have an established clinical role, or show some potential in research studies.

\textbf{1.2.1 Symptoms and clinical scores as monitoring instruments in CD}

A number of symptom-based scoring systems have been developed for the monitoring of CD activity both in clinical practice and in the context of clinical trials. The Crohn's Disease Activity Index (CDAI) combines 8 independent factors, which correlate best to the physician's overall evaluation of clinical activity\textsuperscript{28}. The simpler Harvey Bradshaw Index (HBI) was proposed shortly afterwards\textsuperscript{29} and was shown to have an excellent correlation with CDAI\textsuperscript{30}. These scores provide a gauge for general wellbeing along with common GI symptoms, as well as extra-intestinal manifestations and complications. While they are very simple tools, which offer themselves for serial monitoring of the condition, the main point of criticism is that of their inherent subjectivity. This is of vital importance when a clinical symptom score provides the only benchmark of success in assessing new therapies in clinical trials. Also, in keeping with the empirical disconnect between symptomatology and
inflammatory activity, recent studies demonstrate a lack of correlation between clinical scores and more robust endpoints of inflammatory activity such as endoscopic or histological assessments. A study testing agreement between CDAI and endoscopic scores 12 months following ileo-caecal resections reported a very poor relationship ($r=0.12, p=0.68$)\textsuperscript{31}. In a different context following anti-TNF\(\alpha\) therapy, a separate group from Scandinavia examined the relationship between endoscopy and clinical scores (HBI and CDAI), and reported positive but modest correlations\textsuperscript{32}.

Common symptoms in IBD are non-pathognomonic, and shared with a variety of gastrointestinal conditions. According to a recent population-based study in over 700 people with established IBD, diarrhoea is the most common complaint in CD (63\%) followed by fatigue (54\%), abdominal pain (47\%) and arthralgia (42\%) while symptoms like bleeding, which are more suggestive of ongoing inflammatory activity, are experienced by a minority of patients (17\%)\textsuperscript{33}.

A well-recognised phenomenon in clinical practice is the ongoing reporting of GI symptoms in patients despite the lack of evidence of disease activity. These symptoms are then commonly attributed to a functional origin. A well-designed, exhaustive meta-analysis of 11 studies collectively examined a cohort of patients with IBD in documented remission for functional symptoms, which were strictly defined by validated criteria. This reports a prevalence of 35\% for such symptoms in IBD patients. When CD patients were looked at in isolation, the percentage was higher at 46\%. Moreover, the two case-control studies comparing 264 CD patients with 414 non-IBD controls revealed irritable symptom prevalence of 26.4\% vs. 6.3\% respectively\textsuperscript{34}.

At the other end of the spectrum there is increasing evidence that patients who are in clinical remission may continue to have progressive subclinical bowel inflammation,
which ultimately culminates to the formation of a stricture or fistula. Recent epidemiological studies independently demonstrated that this paradigm of (often silent) disease evolution from pure inflammatory to a stricturing or fistulising phenotype is applicable to the majority of patients over time\textsuperscript{8,15}.

In summary, the achievement of adequate symptom control is crucial in terms of patient care. However, poor association between symptomatology and underlying activity or even bowel damage\textsuperscript{8} is becoming increasingly recognised. Consequently, the use of symptoms in isolation to monitor disease activity is inaccurate and ineffective. Major effort has therefore been directed in the development of alternative biomarkers to monitor disease activity. This will facilitate both the optimisation of patient care as well as the assessment of therapeutic efficacy and safety in the context of clinical trials.

1.2.2 C-Reactive Protein

C-Reactive protein is a non-specific marker of inflammation, infection or tissue injury. Its name derives from the observation that it reacted with the cell wall C polypeptide of \textit{Streptococcus} \textsuperscript{35}, before its cross-reactivity with a wide range of antigens was subsequently demonstrated. It is predominantly produced by the liver and its short half-life of only 19 hours makes it a very responsive tool in monitoring disease activity.

In CD management, CRP has been shown to be useful in variety of contexts. At first presentation, an elevated CRP has some potential in making a differentiation between IBD and other functional bowel disorders\textsuperscript{36}. It has very little accuracy however in differentiating IBD from other causes of colitis\textsuperscript{37}. 
Several studies have been performed to assess the capacity of CRP to monitor disease activity. Some investigators have used the marker in parallel with a clinical activity score such as CDAI or HBI, and the correlation has been moderate at best (summarised in\textsuperscript{38}). One explanation of this is that clinical indices are frequently elevated by functional symptoms and do not always represent increased inflammatory activity.

Other studies have attempted to correlate CRP levels with a more objective marker of inflammation such as endoscopic activity, or radiological parameters, and the results are again very inconsistent. A typical result is from a study in Belgium, in which 28 consecutive patients with a high CDAI and normal CRP underwent colonoscopy, which demonstrated that 92% had lesions on endoscopy, albeit predominantly mild\textsuperscript{39}. In a similar study by the Mayo clinic on a larger cohort of 104 patients, 62% of patients with raised clinical scores and a normal CRP had active disease on endoscopy\textsuperscript{40}. These studies suggest a low sensitivity of CRP in predicting endoscopically active disease.

More recently, studies have hinted towards a significant correlation between mesenteric fat hyperplasia on imaging and CRP levels. A group from France in a recently published series of elegant experiments confirmed that mesenteric adipocytes are a significant source of CRP in response to bacterial translocation and local cytokines\textsuperscript{41}. This could potentially explain the discrepancies between CRP levels in CD and UC, as well as the variable correlations between mucosal activity and CRP levels. This study has opened a significant avenue of new research, to establish the significance of mesenteric fat involvement in triggering raised CRP levels in CD. However, at present, the frequency of the observed disconnect between CRP and endoscopic or radiological reference standards suggests that the marker is not robust enough to be used in isolation for the monitoring of activity.
1.2.3 Faecal markers of activity

Faecal biomarkers have recently been introduced in the diagnosis and monitoring of IBD. These rely on the fact that in active IBD, there is a 10-fold increase in neutrophil migration towards the diseased intestinal wall and subsequent shedding into the lumen\textsuperscript{42}. Faecal Calprotectin (FC), the most effective of these, was first isolated by Fagerhol in 1980. It is one of the major components of neutrophils, accounting for more than 60\% of their total cytosolic protein, and has a role as one of the phagocytic S100 proteins. These are endogenous molecules released by activated or damaged cells under conditions of cell stress\textsuperscript{43}. As such, it can be obtained and measured in stool specimens from patients with a variety of intestinal inflammatory disorders including IBD, neoplastic conditions, GI infections and others. Its stability at room temperature for up to a week, small intra-subject variability and its resistance to metabolism by gut microflora render it a suitable test from a practical perspective\textsuperscript{44}.

Tibble et al. produced the seminal paper on its use in IBD, demonstrating good correlation of FC with \textsuperscript{111}Indium-labeled white cell excretion over 4 days, and significant differences in FC concentrations between CD patients and normal subjects. The group went on to examine 602 consecutive patients with abdominal symptoms and found that a cut-off of 50\(\mu\)g/g has a sensitivity of 89\% and specificity of 79\% in detecting organic disease\textsuperscript{45}. Several more studies placed its positive predictive value (PPV) and negative predictive value (NPV) in that context between 70-100\% and 70-90\% respectively, and in a large meta-analysis which included over 1200 IBD patients and 3300 controls, a cut-off value of 100\(\mu\)g/g of stool was proposed as more optimal in differentiating IBD from IBS at first presentation\textsuperscript{46}.

Having successfully overcome the first hurdle of identifying IBD, FC was then assessed as a quantitative tool. Initially, the lack of correlation between FC and clinical scores, a common
theme with most biomarkers, was demonstrated by a group from Finland, who reported that as many as 56% of patients in clinical remission had a persistently elevated FC. A much better correlation with endoscopic and histologic scores severity was shown\textsuperscript{32}. In addition, several studies have examined the role of FC in monitoring response to therapy in CD. The same research group reported significantly larger reductions in responders versus non-responders in a small cohort (n=19) treated with corticosteroids and a group (n=15) treated with anti-TNFα, findings which were corroborated by other studies\textsuperscript{47}.

While these demonstrate significant differences in FC levels before and after therapy in responders, they also reveal the biomarker’s main weaknesses in monitoring disease progress. Firstly, despite the significantly different post-treatment values in responders, there is still a large inter-subject variability, with several subjects in this cohort exceeding the threshold for remission, which in itself varies from study to study. Moreover, an unequivocal inferiority in the test’s accuracy in quantifying ileal versus colonic inflammation has also been demonstrated\textsuperscript{32}. As ileal Crohn’s is mostly inaccessible to colonoscopic monitoring, it is the subset that is most in need of an alternative reliable biomarker, and these data suggest that FC is likely not sufficient in filling that gap in this subgroup.

Lactoferrin is an iron-binding protein also present in neutrophil cytoplasm, which appears to have bacteriostatic properties\textsuperscript{48}. Increased concentrations of faecal lactoferrin (FL) in stool samples was first demonstrated in relation to shigellosis \textsuperscript{49}. Similar to FC, several studies have since appeared that demonstrate the ability of FL to differentiate between IBD and functional bowel disease \textsuperscript{50-52}. Moreover, FL has also demonstrated significant correlations with clinical, endoscopic, and histologic markers of disease severity (reviewed in \textsuperscript{53}).
Studies examining FC and FL show comparable diagnostic accuracy and disease monitoring potential for the two markers. As FC has been more extensively investigated it has been preferentially adopted in clinical guideline protocols pending larger studies comparing directly these and other newer faecal markers.

### 1.2.4 Endoscopic Assessment

Ileo-colonoscopy and biopsy has been a long established diagnostic and assessment tool in the management of CD. 25 years ago the CDEIS score was put forward by the GETAID group in an attempt to standardise endoscopic reporting, and introduce it as an endpoint in clinical trials. Initial uptake of CDEIS was significant, but following some criticism on its reproducibility, other more simplified indices were proposed. Currently, formal endoscopic scoring systems are not widely used outside a clinical trial context.

More recently, mucosal healing (MH) has been described by several studies as a robust surrogate marker of successful therapy. It was first proposed a decade ago as a new marker of efficacy in scheduled anti-TNF therapy. Several large studies assessed it both retrospectively, prior to the anti-TNF era as well as prospectively in the setting of anti TNF therapy. All these studies converge on the conclusion that MH is a very powerful tool in assessing response to treatment, and, to that end, it has acted as a benchmark for the validation of several other disease assessment instruments mainly from the field of imaging.

As compelling as the evidence in support of MH may be, there are three major limitations preventing it from standing alone as a gold-standard reference in the monitoring of CD. Firstly, most of the small bowel is beyond the reach of a conventional colonoscope, while it very often harbours the majority of disease bulk. Moreover, in cases of stricturing disease within the colon or ileo-caecum, proximal segments, which are normally within range, may
be inaccessible to endoscopic assessment. Secondly, a key pathologic hallmark of CD is its trans-mural nature, and indeed several characteristics such as oedema, fibrosis and fistulisation involve deeper layers on which colonoscopy cannot inform. Finally, poor patient tolerability is another major drawback that limits the role of colonoscopy as a monitoring instrument.

1.2.5 Imaging

1.2.5.1 Traditional Imaging – Barium studies

Small bowel follow through (SBFT) following ingestion of a barium meal, or enteroclysis of barium and air, have been the mainstay of small bowel assessment in CD for several decades. Excellent visualisation of the bowel mucosa is achieved. Moreover, the relative position of bowel loops can also provide some limited information on mesenteric changes, although the latter cannot be directly observed. In recent years however, newer techniques such as computerised tomography (CT) and magnetic resonance imaging (MRI) have gradually superseded barium studies, mainly due to their higher sensitivity extra-luminal complications such as abscesses and fistulae.

1.2.5.2 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) has gradually been introduced as an assessment tool in Crohn's disease over the last 20 years. It rapidly gained popularity and found a role in clinical practice, due to its two obvious major advantages over competing imaging modalities such as CT and SBFT. Firstly, it does not involve ionising radiation, which is of vital importance in young patient cohorts with chronic illness requiring recurrent
assessments. Secondly, it has the potential of providing information on the entirety of the bowel wall thickness, as well as mesenteric and extra-intestinal tissues in one sitting.

Initial studies examined the modality's accuracy in segments previously identified as diseased, and looked at correlations between MRI and other clinical, pathological and endoscopic markers of disease. One of the first such studies was by Koh et al. who investigated 23 patients with active and 7 patients with quiescent disease and demonstrated a ‘per patient’ sensitivity and specificity values of 91% and 71% respectively when compared to an endoscopic or a histo-pathological reference. The sensitivity was significantly lower on a ‘per segment’ analysis at 59%. This study described bowel wall thickness over 4mm, bowel wall hyper-enhancement and focal mesenteric vascularity (Comb sign) as distinguishing factors between normal and diseased bowel 69. These findings were subsequently replicated by other groups:

Ajaj W et al. focused on colonic segments in 23 patients with IBD and 15 controls having endoscopic and histological assessment. They demonstrated that simultaneously assessing bowel wall thickness and enhancement, loss of mucosal haustrations and mesenteric lymph node enlargement can identify abnormal segments with a sensitivity of 87% and specificity of 100%. These observations have not yet been externally validated70.

A sound argument was subsequently put forward that since MRI is capable of visualising the whole thickness of the bowel wall, or even beyond it, an endoscopic reference standard is not an adequate benchmark for the assessment of its performance. On that basis, three studies from London, Paris and Amsterdam published a more robust methodology of assessing MRI endpoints which involved their correlation to full-thickness histopathological indices, obtained on surgical resection specimens from patients with CD71–73.
The first such study, produced by the University College London group\textsuperscript{71} prospectively examined 18 consecutive CD patients with MRI, prior to scheduled resective surgery. Resected specimens were re-imaged \textit{ex vivo}, to facilitate precise co-localisation of recorded radiological features and histo-pathological scoring of activity. Important findings of this study include a significant correlation between bowel wall thickness as well as a mural signal intensity on fat-suppressed T\textsubscript{2} images (proposed to represent mural oedema), and the acute inflammatory score (AIS), which quantifies elements such as mucosal ulceration, wall oedema and neutrophilic infiltration across the whole thickness of the bowel wall. In addition, a layered pattern of mural enhancement, seen in segments with the highest AIS, was contrasted to a limited mucosal contrast enhancement or homogeneous uptake of the intravenous gadolinium, observed in segments with lower inflammatory activity. Layered enhancement was also the only marker associated with fibrostenosis in this cohort, which reflects an increasingly prevalent hypothesis that the two entities of inflammation and fibrosis co-exist in the most severe of lesions, rather than occurring independently of each other.

Zappa et al. published a similar study correlating MRI features with semi-quantitative scores of inflammation and fibrosis performed on resected intestinal specimens on 53 patients. Each segment was classified as containing no or mild, moderate, or severe inflammation and fibrosis respectively. Interestingly, there was a good correlation between the inflammatory and fibrosis scores in each patient ($r=0.63$ $p=0.0001$), supporting the above hypothesis. Several significant correlations between MR markers and histo-pathologic parameters of inflammation were demonstrated, including bowel wall thickness, retained contrast enhancement in the delayed phase, layered enhancement in the early phase and mural oedema on T\textsubscript{2} (exclusively seen at the severe end of the inflammation spectrum). MRI features associated with fibrostenosis were bowel thickness, blurred wall enhancement, and mural oedema on T\textsubscript{2} (also seen exclusively in severe fibrostenosis). In addition,
extraintestinal findings including mesenteric hypervascularity, abscesses and fistulae were also independent predictors of severe inflammation, the latter also being associated with fibrosis\textsuperscript{72}.

Finally, a group from Amsterdam retrospectively examined MRI data on 39 segments (25 patients), which had been scored using the AIS. Photographs of resected specimens were used to co-localise the histo-pathological findings to the MRI images. Once again, bowel wall thickness was correlated with AIS. In contrast to other published work however, endpoints such as maximal bowel wall enhancement, enhancement layering, mural oedema and the presence of ulcerations did not reach statistical significance \textsuperscript{73}.

In summary, these three studies by Punwani, Zappa and Ziech converged on bowel thickness as the most important marker of inflammation. Mural oedema and layered wall enhancement only reached statistical significance in two of the three studies. Predictors of fibrostenosis were less consistent, with one study which assessed these proposing layered enhancement and the other bowel wall thickness and mural oedema. Interestingly, pre-stenotic dilatation empirically believed to signify the presence of fibrostenosis in clinical practice, did not correlate to the degree of fibrosis observed within pathological specimens.

As investigators strived to obtain maximal benefit from these various observations on the relative weighting of each individual endpoint in reflecting the inflammatory burden, they started organising them together, by applying statistical modelling, in order to construct quantitative scores. These can aid in the better characterisation of disease burden at a particular time-point, but also in the monitoring of the therapeutic effects of various drugs either in clinical practice or in the field of drug development.
The most thoroughly validated score is the Magnetic Resonance Index of Activity (MaRIA), introduced by the Barcelona group in 2009. 50 patients with known CD underwent ileocolonoscopy and estimation of segmental as well as global CDEIS as a reference investigation. Of the 213 segments examined endoscopically, 130 were normal, 43 had mild lesions and 40 contained superficial or deep ulcers. This was compared to T2 weighted and pre and post contrast T1 weighted MR sequences, obtained within 24 hours. Binary logistic regression was used to identify the MR parameters that correlated independently to endoscopic findings. The model revealed that wall thickness, relative contrast enhancement (RCE) and identification of ulcers at MRI were all independent predictors of finding ulcers at endoscopy. In terms of predicting the CDEIS, bowel wall oedema on T2 weighted sequences was added to the above three parameters. The resultant MaRIA index had a highly significant correlation with both segmental (r=0.81) and global CDEIS (r=0.78) as well as HBI (r=0.56) and CRP (r=0.42)\textsuperscript{74}. The same group successfully validated this score in an independent cohort of 48 patients with very similar results\textsuperscript{75}.

Taking into account both these well-designed studies there is little doubt that the MaRIA score can accurately reflect the state of the mucosa in direct comparison to endoscopic scoring, and it could potentially replace endoscopic examinations to assess mucosal activity. One major criticism of this method, however, is that it excludes findings which are well within the scope of MR imaging, though not directly linked to mucosal disease. By its very nature Crohn’s is a transmural process and, often, the condition of the mucosa does not reflect the disease burden in deeper layers. On the other hand, there is evidence that deep bowel layers can themselves act as drivers of the inflammatory process independently of the epithelial activity\textsuperscript{76} and, moreover, they certainly contain targets for effective therapies such as anti-TNF biologics\textsuperscript{77}. Markers such as perimural oedema, mesenteric vascularity, fistulae and lymph nodes are readily identifiable on MR sequences and, in combination with the mucosal markers, can provide a more global overview of disease burden and intestinal
damage that MaRIA does in isolation. Furthermore, a careful look at the MaRIA score makes it obvious that disease extent does not feature as one of the parameters. There is evidence, however, that the length of affected bowel has considerable bearing on long term morbidity associated with CD\textsuperscript{78}. An alternative score, therefore, which incorporates features across the bowel thickness as well as on disease extent could be more representative of the overall disease burden and act as a better benchmark for therapeutic monitoring.

Makanyanga et al. recently published a study on the significantly more inclusive Magnetic Enterography Global Score (MEGS). MEGS was produced by the expansion of MRI Crohn’s Disease Activity Score (CDAS) produced by the same group, which had been validated against histology\textsuperscript{79}. It assesses wall thickness, T\textsubscript{2} signal, peri-mural mesenteric oedema, post contrast T\textsubscript{1} enhancement level and pattern, colonic haustral loss as well as length of disease in each segment. Fistulae, lymphadenopathy, comb sign and abscesses are also evaluated. 71 patients were assessed using MEGS, which was compared to FC, CRP and HBI, showing a positive correlation with the first two (r=46 p<0.001, r=0.39 p=0.002 respectively). MEGS incorporates a variety of non-mucosal factors including peri-mural and extra-enteric complications which partially justifies the lack of attempted correlation with an endoscopic gold standard. Had this been demonstrated, however, it would further strengthen its validity as a marker of inflammatory burden. In contrast to the MaRIA, which probably attempts to substitute endoscopy in the monitoring of CD patients, an absolute indication for MEGS is not directly obvious, but intuitively it is more relevant to the newly proposed natural history paradigm of accumulating bowel damage\textsuperscript{80}. Once it has undergone external validation, it could potentially serve as a tool to trace its progression or regression in response to disease-modifying therapy.

In addition to the various MRI endpoints described above, novel endpoints of inflammatory activity such as assessment of small bowel motility, dynamic contrast-enhanced (DCE) MRI
and diffusion-weighted imaging (DWI) have also been investigated as markers of inflammatory activity in CD.

Small bowel motility can be assessed and quantified by MRI using dynamic ‘cine’ sequences and appropriate software. Briefly, an optic flow registration algorithm estimates deformation of each frame of a given cine loop relative to an initial target frame. The standard deviation of the Jacobian determinant of this deformation acts as a measure of intestinal motility, and it is expressed in arbitrary units (AU). The first report correlating motility with histological inflammation was published in 2012 by the UCL group. The hypothesis was that diseased bowel motility is reduced due to the effect of fibrosis, chronic inflammatory cellular infiltrate and perhaps myenteric plexitis, commonly seen in small bowel resection specimens. The authors reported on 28 patients with known terminal ileal disease who underwent ileoscopy and biopsy, scored using the AIS, as well as MRI within 4 days. A significant difference in the motility indices in inflamed and un-inflamed TIs were demonstrated \((p=0.002)\) as well as a moderate negative correlation between AIS and the motility index \((r=0.57)\). These results were corroborated by a very similar study from Switzerland published a year later. More recently, reproducibility data on MRI-determined small bowel motility in 20 healthy volunteers demonstrated wide inter-segmental variation and poor repeatability over a four-week interval. The significance of this in small bowel segments affected by CD however is still unknown but it suggests a possible limitation of the method.

DCE MRI is a tool that assesses perfusion, by measuring the rate as well as the magnitude of relative contrast uptake by various tissues. The rationale behind its application in inflammatory imaging is that chronic inflammation is typically associated with various degrees of angiogenesis. Angiogenesis, the process of new capillary formation from existing vasculature, has been shown to be a central process in a range of chronic inflammatory
conditions such as psoriasis, rheumatoid arthritis, atherosclerosis etc\textsuperscript{85}. Proposed functions for this altered microvasculature in the immune process are the increased influx of inflammatory cells, enhanced nutrient supply to a metabolically active tissue and local production of cytokines and other pro-inflammatory molecules through endothelial activation \textsuperscript{86,87}.

The evidence base on aberrations in the microvasculature of IBD tissue is scarce and equivocal. On the one hand, there are investigators which suggest there is relative reduction in perfusion in segments with active CD as assessed by various techniques such as endoscopic or intraoperative Doppler \textsuperscript{80,89}, or paucity in end vessels\textsuperscript{90}. Other studies demonstrate increased blood flow in active tissue\textsuperscript{91}. A group from Cleveland carried out a comprehensive study on microvascular changes in IBD. They demonstrated increased microvascular density (MVD) through immunohistochemical expression of the CD31 and von Willebrand/factor VIII markers of endothelium in 8 control and 17 IBD colonic specimens. Moreover, CD31+ve capillaries were shown to express $\alpha_\beta_3$, a specific endothelial marker of angiogenic endothelium\textsuperscript{92}

In another landmark study Taylor et al. recruited 11 patients scheduled for intestinal resection, who underwent prospective DCE imaging of the relevant segment. Histopathological assessment included quantification of vascular elements using a CD34 ligand, as well as the estimation of AIS. The authors demonstrate significant differences in the DCE parameters in normal versus abnormal regions of interests. Surprisingly, there was a strong negative correlation between the MVD and the slope of enhancement ($r=-0.86$), which the authors justified by the hypothesis of predominant ischaemia in CD, i.e. MVD occurs in response to limited tissue flow secondary to arteriolar stenosis. None of the other DCE endpoints correlated to any of the histopathological markers of vascular density or inflammation\textsuperscript{93}.  

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Despite these uncertainties in the pathophysiology of the haemodynamics in diseased tissue, and a knowledge gap concerning the relative significance of increased MVD on the one hand and probable reduced perfusion at the pre-capillary level on the other, several groups have taken the step to evaluate DCE endpoints as a quantitative assessment tool in patients with Crohn’s disease.

Most of the early studies examine the correlation of DCE endpoints to a clinical index of activity rather than a more robust marker of inflammation\textsuperscript{94,95,96}. A group from Chicago examined 51 bowel segments (19 with inflammation and 32 normal as assessed by ileo-colonoscopy and histology or surgery) from 11 patients and reported statistically significant differences in a comprehensive list of parameters. The role of the histological reference standard was merely to differentiate between abnormal and normal segments, and there was no attempt to correlate kinetic parameters with histologic or endoscopic activity\textsuperscript{97}.

This was partially addressed by Rottgen and his group who examined several DCE parameters in a larger cohort of 26 patients\textsuperscript{98}. The slope of enhancement (r=0.59) but not the area under the enhancement curve nor the peak maximum were shown to correlate with endoscopic severity in this retrospective study. More recently the Amsterdam group evaluated DCE parameters in correlation to AIS and FS in 50 intestinal sections from 20 patients undergoing surgical intestinal resection. There were moderate correlations between the AIS and the maximal enhancement as well as slope of enhancement. As predicted by the hypothesis that inflammation will co-exist with fibrosis in the same lesions, significant correlations were also observed when the FS was assessed against the DCE measures\textsuperscript{99}.  

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In summary, even though the pathophysiological basis underpinning the technique still remains dubious, DCE MRI is emerging as a new tool to assess disease activity. This is due to several studies demonstrating that iv contrast appears to be handled differently by inflamed versus normal intestinal wall, a discrepancy which can be formally quantified. How these measures will perform longitudinally, and, in particular, how illustrative they will be of change achieved with therapy remains to be elucidated.

Diffusion-Weighted Imaging (DWI) is a MRI method which allows mapping of the diffusion of molecules, predominantly water, within biological tissues. The movement of water molecules between the intracellular, interstitial and intravascular compartments normally occurs freely, in a random manner, but it is restricted by obstacles such as macromolecules, membranes and fibre. DWI is a technique that assesses the rate of this movement, ultimately producing a map of average diffusion per voxel of tissue. In pathological states where there is increased cellular density (e.g. malignancy or inflammation), or cellular swelling (e.g. cytotoxic oedema), this free movement of water molecules is reduced. DWI was first introduced in the mid-1980s finding applications in the acute imaging of stroke, followed by a wide range of oncological indications and others. Two properties which make it an attractive modality are its quantitative nature and the lack of need for intravenous contrast.

Soon after these reports, several groups examined DWI in the context of inflammatory imaging. Nogushi et al. demonstrated the techniques potential to distinguish inflammatory from malignant processes in the brain. Other investigators have introduced its application in imaging of hepatic inflammation and fibrosis.

The first report of DWI in CD was by Oto et al. who retrospectively assessed 53 segments in 11 patients subsequently undergoing ileo-colonoscopy or resection. The group demonstrated significant differences in the Apparent Diffusion Coefficient (ADC), a
quantitative measure of water diffusivity, between normal and abnormal segments\textsuperscript{105}. Shortly afterwards, a report from Tokyo on a larger cohort of 31 patients in whom DWI was compared to a barium study or surgical pathology revealed strikingly similar results. Moreover, the authors hinted on possible differences between small bowel and colonic ADCs requiring further exploration\textsuperscript{106}.

Oussalah and his group from Nancy, reported clinical–radiological results obtained with DW-MRI in 35 patients with UC and 61 with CD. A segmental magnetic resonance score (MR-score-S) based on DWI values and other MRI parameters was much more successful in detecting endoscopic inflammation in UC, (sensitivity 89\% and specificity of 87\%) than in CD (sensitivity 58 \% and specificity of 84 \%). The authors attributed the differences to the segmental nature of the latter\textsuperscript{107}.

More recently, a different group in France performed DWI imaging in 31 ileal segments using the MaRIA score as a standard of reference for the first time after its validation, and reported a good correlation between it and ADC. ROC analysis was also performed, and a cut-off ADC value of 1.6mm/s\textsuperscript{2} for active ileitis was proposed, with a sensitivity of 83\% and specificity of 100\% (area under ROC curve 0.96). The same unit’s extended experience on 130 patients, inclusive of both small bowel and colonic data was presented in a separate paper. Once again MaRIA was used as a comparator, to determine the 175 out of 848 active bowel segments, and per segment sensitivity and specificity figures were proposed, all being well over 90\%. The ADC threshold was revised to 1.9mm/s\textsuperscript{2}, and, challenging the conclusions of Nancy's group, they advocate the use of DWI in colonic as well as ileal Crohn's monitoring\textsuperscript{108}.

In conclusion, most conducted studies on DWI in CD have supported the hypothesis that the focal hyper-cellularity arising from inflammatory cell influx, and perhaps also the fibrogenic
process, do result in a measurable reduction in water diffusivity in the affected tissue. In the largest of these cohorts MaRIA has been used as the as a gold-standard, or more accurately, a variant of the score proposed by its inventors which does not involve bowel preparation and colonic distension, a strategy which has left the study open to some criticism. Once again the performance of DWI as a monitoring tool, in terms of its responsiveness and its reliability remains undetermined.

1.2.5.2.1 MRI in monitoring disease progress in CD

Whilst there is an increasing evidence base suggesting that several modalities within MRI have a high diagnostic accuracy in demonstrating active segments in patients with CD, only a small number of studies have assessed the responsiveness of MRI qualitative endpoints or quantitative scores over time in such patients. The first study included 8 patients with follow-up MRI at variable times after initiation of therapy (11 days to 4 months), and the authors reported significant changes in T₂ wall signal intensity, T₁ contrast enhancement as well as bowel wall thickness. The prospective multi-centric ACTIF trial exclusively examined terminal ileal segments in 15 patients with active CD prior to, and at two time-points after the introduction of infliximab. The MRE score of severity in Ileal Disease (MCID), a composite score consisting of inflammatory, obstructive and extra-intestinal components (range 1-14), was assessed, which to date lacks external validation. The primary endpoints were a drop in MCID by 2 points at week 26 in combination with a decrease in inflammatory sub-score of at least 50%. These were met by 40% of patients. The authors emphasise the fact that complete resolution of transmural inflammatory features was rarely encountered at 6 months, despite several participants enjoying full clinical remission at that time-point. They propose however further prospective studies to establish the modality’s responsiveness in monitoring the effects of treatment.
In a recent landmark paper, Ordas et al. explored the potential of the MaRIA score, previously proposed and validated by their group, to monitor Crohn's patients with endoscopic evidence of activity and mucosal ulceration, after introduction of corticosteroids or anti-TNF therapy. 48 patients were recruited prospectively before treatment, and received an MRI and an ileo-colonoscopy, which were repeated 12 weeks following introduction of therapy. The aim of this study was to report on the responsiveness and reliability of MRE as a monitoring tool, using the colonoscopy as the gold standard. Appropriately, the primary objective was to investigate the accuracy in the identification of ulcer resolution, as the reference investigation can only provide information on the mucosa. Other components of the MaRIA score were examined, alongside morphological MRE endpoints, however, including bowel wall thickness, oedema and wall contrast enhancement. Using robust statistical methods, the authors demonstrated several important findings: On a ‘per patient analysis’ it was found that a global MaRIA score of <50 was predictive of ulcer healing with sensitivity and specificity of 75% and 80% respectively, as well as of mucosal healing (by CDEIS criteria), with sensitivity and specificity of 83% and 84% respectively. Furthermore, the authors report good agreement between MRE and endoscopy in diagnosing MH ($\kappa =0.71$). For the ‘per segment analysis’ 111 of 265 segments had ulcers at index endoscopy, of which 73 had healed on follow-up. In these, mean CDEIS and MaRIA were significantly different. In the 30 segments without ulcer healing, neither CDEIS nor MaRIA had changed significantly. A MaRIA of <11 was sensitive (94%) but moderately specific for ulcer healing whereas a MaRIA of <7 had a high sensitivity (85%) and specificity (78%) for MH. Segments which demonstrated ulcer healing on the follow-up scan, also had significantly higher rates of resolution of qualitative MRI endpoints such as oedema, lymphadenopathy, peri-enteric vascularisation, and fat stranding, compared to those that did not demonstrate ulcer healing. The score’s responsiveness, according to the formulas described previously was calculated as 1.10 (responsiveness ratio of Guyatt) and 1.72 (standardised effect size ratio), with values over 0.80 signifying good responsiveness.
Finally, Intra-class correlation coefficient was calculated as 0.56 in the subset of segments without ulcer healing, suggesting a good reliability of the score in addition to good responsiveness\textsuperscript{110}.

These data support the use of MaRIA in monitoring primarily the mucosal, but also the transmural effects of therapy. One important issue about the methodology of the MaRIA score is that it involves both bowel cleansing with polyethylene glycol as well as passive colonic distension with rectal catheterisation performed before the exam, a practice which other groups have been reluctant to reproduce because it makes the procedure substantially more invasive, and less acceptable to patients. While the score seems to be a promising monitoring tool, its assessment without the colonic cleansing and subsequent distension component is warranted before it can find a role in clinical practice.

Similar studies are also required to assess reliability and responsiveness of several other quantitative endpoints of MRI enterography, which appear to measure accurately different components of the inflammatory process on ‘snapshot’ observations, such as the MEGS score, Apparent Diffusion Coefficient and DCE MRI parameters.

\textbf{1.2.5.3 CT Enterography}

Computed Tomographic (CT) enterography has also been shown to detect disease activity in CD with accuracy very comparable to MRI. Endpoints which have been highlighted by clinical studies as the most representative of inflammatory activity are, perhaps unsurprisingly, very similar to MRI and are summarised in Table 1.1\textsuperscript{111,112,113,114,115}.
### Table 1.1: Summary of studies on radiographic signs of CT Enterography

<table>
<thead>
<tr>
<th>Radiographic Sign</th>
<th>Studies</th>
<th>Gold-standard + Findings</th>
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</thead>
<tbody>
<tr>
<td><strong>Bowel thickness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bodily Radiology 2006</td>
<td>Correlation between thickness and endoscopic activity (p&lt;0.001) and histologic activity (p=0.01)</td>
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<tr>
<td></td>
<td>Chiorean AJG 2007</td>
<td>Correlation between wall thickness and inflammation score r=0.34 p=0.02</td>
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<tr>
<td><strong>Mural Enhancement</strong></td>
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<tr>
<td></td>
<td>Booya Radiology 2006</td>
<td>Normal jejunum enhancement &gt; Normal ileal enhancement (p=0.001) TI hyperenhancement in pts with definite CD vs. controls (119 vs. 96 HU p&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>Bodily Radiology 2006</td>
<td>TI CONTROL= 96±23HU TI PROBABLE CD=114±28HU TI DEFINITE CD =127±15HU Control vs. Probable p=0.02 Control vs. Definite p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Colombel Gut 2006</td>
<td>TI attenuation + Ratio of ileal/control ileal loop attenuation were significantly correlated to histological score (r= 0.34–38; p&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>Chiorean AJG 2007</td>
<td>Mucosal (p=0.004) but not bowel wall hyperenhancement (p=0.08) were significantly correlated with inflammation score</td>
</tr>
<tr>
<td><strong>Pattern of enhancement (layered / diffuse)</strong></td>
<td></td>
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<tr>
<td></td>
<td>Choi Clin Radiol 2003</td>
<td>Layering enhancement associated with activity and homogeneous enhancement with quiescence p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Chiorean AJG 2007</td>
<td>No significant correlation between mural stratification and inflammation (p=0.30) or fibrostenosis (p=0.68).</td>
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<tr>
<td><strong>Comb sign</strong></td>
<td></td>
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<tr>
<td></td>
<td>Bodily Radiology 2006</td>
<td>Sensitivity 29.35% Specificity 93%</td>
</tr>
<tr>
<td></td>
<td>Booya Radiology 2006</td>
<td>Sensitivity 53.67% Specificity 89-100%</td>
</tr>
<tr>
<td></td>
<td>Colombel Gut 2006</td>
<td>Significantly correlates with histological score (r=0.29 p=0.002)</td>
</tr>
<tr>
<td></td>
<td>Chiorean AJG 2007</td>
<td>Significant association with inflammatory score (OR=5.52 p&lt;0.001)</td>
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<tr>
<td><strong>Mesenteric fat stranding</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bodily Radiology 2006</td>
<td>Low sensitivity (10%) but high specificity for activity (93%)</td>
</tr>
<tr>
<td></td>
<td>Booya Radiology 2006</td>
<td>Low sensitivity (40%) but high specificity (93-96%) for activity</td>
</tr>
<tr>
<td></td>
<td>Colombel Gut 2006</td>
<td>Modest correlation between fat density and histological score (r=0.22 p=0.016)</td>
</tr>
</tbody>
</table>
A major limitation in the application of this modality as a monitoring instrument is the patient exposure to ionising radiation. A team from Ireland showcased the issue by investigating retrospectively the cumulative effective doses (CED) of radiation in 354 patients with CD over 3 5-year periods between 1992 and 2007, aiming to quantify the proportion of these that received a CED over a threshold of 75mSv. This has been shown to be associated with an absolute risk of lifetime all-cancer mortality >7% in a large definitive epidemiological study\textsuperscript{116}. The authors report that more than 15% of patients had exceeded that threshold at a mean age of 39 years. Risk factors associated with increased CED were young age at diagnosis, ileo-colonic distribution and the requirement for steroids, anti-TNF and immune-modulators\textsuperscript{117}. As MRI availability is expanding (data from the centre of disease control and prevention), and clinical guidelines supporting its use over CT are propagated\textsuperscript{118}, it is likely that the role of CT in the management of CD will rapidly diminish, sparing patients from the burden of excessive ionising radiation.

\textbf{1.2.5.4 Functional Imaging}

Molecular imaging has been defined as “the visualisation, characterisation and measurement of biological processes at the molecular and cellular level in humans and other living systems.”\textsuperscript{119} It typically includes 2-dimensional imaging as well as quantification over time \textsuperscript{119}. The techniques involve the incorporation of a radionuclide, detectable by the imaging modality, onto a biologically active molecule which has a known role in the pathway under investigation. The studying of the resulting radioligands’ rate and degree of specific accumulation, as well as rate of metabolism, can provide valuable information on the abundance and kinetics of the biological process.

By far the most broadly studied ligand, with a wide range of established clinical applications is \textsuperscript{18}F-Fluorodeoxyglucose (FDG), an analogue of glucose in which an \textsuperscript{18}F molecule has
replaced the naturally occurring hydroxyl group in the 2’ position. After intravenous administration, FDG is transported intracellularly by the GLUT family of transporters, at a rate proportional to each cell’s glycolysis, and phosphorylated by hexokinase into $^{18}$F-FDG-phosphate. $^{18}$F-FDG-phosphate is trapped intra-cellularly, which gives rise to the hypothesis that the great majority FDG signal originates intra-cellularly, after sufficient time is allowed for the intravascular reserves to be taken up by cells.$^{120}$

It has long been known that malignant cells utilise glucose more rapidly than surrounding tissue. This is a result of an increased metabolic rate in these cells, in combination with a shift of the energy-producing pathway, from oxidative phosphorylation to the less oxygen-dependent glycolysis, as cells adapt to the relative hypoxic conditions within solid tumours.$^{121}$ This property of malignant cells has been the basis of development of FDG-PET scanning as a diagnostic, and subsequently, a monitoring and prognostic tool for a wide range of cancers over the last 30 years.$^{122}$

Shortly after the potential of FDG-PET in oncological imaging began to be realised, a group in Japan, through a series of elegant experiments, proposed its use for the identification of benign inflammatory foci. Stimulated by reports of increased glucose uptake by inflammatory tissue$^{123}$, and incidental FDG foci found within abscesses$^{124}$, Kubota et al. transplanted syngeneic FM3A tumour cells in mice, and followed them up until tumours had fully developed. The animals were then injected with FDG and shortly afterward sacrificed and the tumours were examined using macro and micro-autoradiography, which demonstrated that the majority of the injected FDG had accumulated in macrophage-rich areas as well as young granulation tissue within the tumours.$^{125}$ This was corroborated by another group performing a set of murine studies which showed differential FDG activity in immuno-competent versus immune-deficient mice after xenografting them with an experimental tumour model $^{126}$. The Kyoto group then used turpentine-induced
inflammation, a chronic inflammatory model of granulation tissue in mice to also demonstrate increased FDG affinity in such tissue\textsuperscript{127}, which was completely devoid of a malignant component. Finally, in a similar experiment in a model of concanavalin-A induced lymphoid activation, increased FDG affinity was demonstrated in lymphocytes both in vivo and in vitro, implicating these cells as a source of FDG signal in inflammation\textsuperscript{120}. This latter study also demonstrated that \textsuperscript{18}F-FDG binding in splenocytes of mice that had been inoculated at a remote site with the concanavalin-A 30 minutes previously, was higher than splenocytes of untreated animals. This suggests that the FDG signal is not exclusively related to an increased cellular density that results from cell migration at the site of inoculation, but it also reflects a state of activation of the immune lineages.

These reports gave rise to a large number of studies, which examined the potential of FDG-PET to identify the inflammatory lesions, and measure the inflammatory load in a wide range of conditions. This has been achieved successfully in atherosclerosis, where FDG has been used to quantify the macrophage content in vessel plaques, inflammatory vasculitides, and rheumatoid arthritis among others\textsuperscript{129-132}.

\textbf{1.2.5.4.1 \textsuperscript{18}F-FDG-PET in CD}

The first report on FDG-PET in IBD was published in the Lancet in 1997, where Bicik et al. performed FDG PET alongside ileo-colonoscopy and histology, describing good correlation between the two, as well as between PET and clinical scores for CD and UC respectively\textsuperscript{133}. Two years later, a Canadian paediatric study was published in which 69 intestinal segments in 25 children undergoing FDG-PET alongside colonoscopy and biopsy or small bowel follow through as the reference gold standard. The investigators report an overall ‘per patient’ sensitivity of 81\% and specificity of 85\%, having set the threshold for a positive gut segment
at signal higher than that in the spine. The group’s ‘per segment’ analysis demonstrated a sensitivity of 71% and specificity of 81%.

Neurath et al. published a larger study of 59 patients with established CD comparing FDG-PET with MR enterography, and, in a subset, granulocyte scintigraphy. The authors report a higher incidence of ‘pathological’ segments identified by FDG-PET compared to the other two modalities. In addition, for the 45 segments where endoscopic verification was available, this corresponded to sensitivities of 85% for PET versus 41% for MR and 67% for granulocyte scintigraphy, whereas the specificities for all 3 modalities were over 89%. This study introduced several parameters in the field of FDG-PET imaging in CD: The use of the liver signal as a threshold, above which any bowel signal was considered relevant, was first described in this paper, supported by the fact that 11 of 12 IBS controls as well as 20 controls with no GI pathology, had no bowel FDG accumulation above that threshold. This study was also the first to provide quantitative Standardised Uptake Values (SUVs) for those bowel segments described as abnormal. SUV was henceforth adopted as a measure of activity by the majority of studies in the area.

Two similar studies from India and Wisconsin, published simultaneously in 2007 were the first to introduce the combination of FDG-PET with CT in 17 and 12 patients respectively patients with known IBD. The authors describe sensitivities and specificities similar to previous studies, but the added benefit, if any, of the CT component on the scan was not specifically mentioned.

One of the landmark papers in the literature was published by Edward Louis et al. The authors prospectively recruited 22 consecutive patients with known CD having colonoscopies for clinical reasons and performed FDG-PET/CT scanning as well as faecal calprotectin assay and CRP within 1 week of the endoscopy. The sensitivity of FDG-PET for
the detection of endoscopic lesions was 73% and the specificity of 55%. The group was the first to demonstrate a positive correlation between SUV and endoscopic severity of the lesions. This is a crucial finding as the principal purpose of FDG PET scanning in CD, and its proposed advantage over conventional modalities, is the ability to meaningfully quantify, measure and monitor the inflammatory load at a particular time-point. The concept of expressing SUV as a ratio over the value in liver parenchyma (RSUV) was introduced and a ROC analysis was produced, which demonstrated a predictive value of RSUV ≥ 1.47 as having 100% sensitivity in identifying severely inflamed segments on endoscopy. Finally, the study explicitly addresses the 21 of 95 segments which were positive on FDG PET but negative on endoscopy and demonstrates either CT or histologic features of disease in 16 of them, suggesting that FDG PET can reveal disease better than that achieved by endoscopic mucosal visualization. This latter observation also highlights the shortcomings of endoscopic assessment as an ultimate gold standard of disease activity.

Another important study which significantly contributed to current knowledge was by Jacene et al. who prospectively recruited 12 patients scheduled to have resective surgery for Crohn's disease, and carried out FDG-PET/CT scanning before correlating the findings to histological assessment of inflammation, fibrosis and muscle hypertrophy within resected segments. The investigators demonstrated that the majority of lesions contained features of both inflammation and fibrosis-hypertrophy. They also showed that a $SUV_{\text{MAX}} \geq 8.0$ can select predominantly inflammatory lesions with a sensitivity of 60% but a specificity of 100% and a negative predictive value (NPV) of 78%. This has significant clinical implications in the context of a notorious clinical conundrum in IBD practice, of patients presenting with symptomatic stricturing necessitating a choice of either an aggressive medical approach or an operation in the first instance. The other important contribution of this study was the proposition of Total Inflammatory Volume (TIV) of a lesion as a valuable endpoint. TIV or
Total Lesion Glycolysis (TLG) is calculated as the product of $SUV_{\text{MEAN}}$ of a lesion x Volume of signal and has widespread applications in oncologic monitoring\textsuperscript{139}.

In 2010, a group of investigators from Florida published a very interesting study which examined the potential of a single FDG PET/CT to predict response to medical therapy in patients with CD\textsuperscript{140}. 41 patients were included and 30/41 who demonstrated abnormalities either on the CT or PET component of the scan were subsequently analysed. These were divided into a group of 23 who eventually responded and 7-strong group who failed medical therapy ultimately requiring surgical intervention. 48 abnormal segments were identified on CT, 38 of which also showed increased FDG uptake with a mean $SUV_{\text{MAX}}$ of 4.8. The authors identified CT positive segments without FDG activity as a risk factor for failing medical therapy, found in 6/7 patients of that group and only in 1/23 responders, a finding that reached significance in their performed logistic regression analysis. The authors concluded that while these findings are encouraging, adequately powered studies will be required to establish the predictive role of the investigation.

A recent study by Saboury et al, similar in design to several previous ones inasmuch as it correlates FDG-PET endpoints with clinical, laboratory and endoscopic results in patients with established CD, was, however, the study with the most comprehensive assessment of quantitative endpoints of PET activity. Data from 22 patients undergoing CDAI scoring, CRP, faecal calprotectin and ileo-colonoscopy, was categorized into segmental and ‘per patient’. In the former, segmental $SUV_{\text{MAX}}$, $SUV_{\text{MEAN}}$ and TLG were correlated with segmental CDEIS, the first two reaching statistical significance. In order to derive a meaningful ‘per patient’ assessment of FDG signal, they proposed the Global $SUV_{\text{MAX}}$ (Average $SUV_{\text{MAX}}$ in all abnormal segments), the Global Lesion Glycolysis (GLG), calculated as the sum of TLG in all abnormal segments and the Global $SUV_{\text{MEAN}}$, calculated as the ratio of GLG/ Sum of volumes of abnormal segments. While all of the ‘per patient’ scores correlated with CDAI, GLG
correlated positively with calprotectin only, Global $SUV_{\text{MAX}}$ and $SUV_{\text{MEAN}}$ demonstrated a positive correlation with CRP and CDEIS$^{141}$.

These important clinical studies have also been supplemented by basic science experiments in relevant animal models, which further support the role of FDG-PET as a quantitative measure of inflammatory burden in IBD. Sarah Brewer and her colleagues performed a series of experiments in a range of murine models of mild and severe IBD. She demonstrated that FDG signal in Ga\(i\)2/ mice, a model of severe colitis, was significantly higher than in heterozygote Ga\(i\)2+/ littermates, which was replicated on IL10/−, a model of milder colitis. Moreover, by using piroxicam to augment the inflammation in a subset of the IL10/− animals, she demonstrated the responsiveness of the FDG PET signal, which increased significantly on follow-up scanning in treated mice against controls. In an attempt to determine the cellular source of the FDG signal, the investigators tried correlating the latter with populations of specific immune cells, without result. When individual mononuclear cells were stained for the Glut-1 receptor and subset markers, and examined by flow cytometry, it was revealed that Glut-1 expression was similar in macrophages and CD8+ cells in colitic versus non-colitic mice. CD4+ cells expressed significantly higher Glut-1 expression in all colitic mice. This Glut-1 expression in CD4+ T-cells correlated with the FDG signal in two independent experiments using the IL10/− model$^{142}$.

Yamato et al. performed FDG PET scanning in an indomethacin-induced small bowel ulceration model, in which indomethacin or vehicle solution was injected subcutaneously in male Sprague-Dawley rats, resulting in ulceration within 1 day, granulation tissue at the ulcer edges by day 4, and re-epithelialisation and repair by day 7. This time course was shown to be mirrored by myeloperoxidase activity in intestinal mucosa, an assay which quantifies neutrophilic infiltration, as well as intestinal FDG signal with foci observed exclusively in the indomethacin-treated rodents. The investigators attempted to determine
the cellular source of the signal by performing macro and micro-autoradiography in sacrificed animals shortly after FDG administration. Macro-autoradiography revealed discontinuous, dot-like accumulations on the mesenteric side of the ileal mucosa, which co-localised with the ulcerations. $^{18}$F-FDG micro-autoradiography in combination with H&E staining localized the activity in the ulcer margins, within the mucosa but also in submucosal and smooth muscle layers. In contrast to the Brewer indirect experiments, which revealed CD4+ T helper cells as the probable sole source of the FDG signal, Yamato’s group performed co-staining and direct visualization revealing a much broader range of cells containing $^{18}$F.

On day 1, the majority of cells containing the radioactivity were myeloperoxidase-positive neutrophils and macrophages, whereas on day 4 the foci were mainly α-SMA positive myofibroblasts, CD31-positive endothelial cells and some ED-2 positive macrophages. Moreover, abundant FDG also accumulated in Ki67 stem cells deep inside the crypts.

Finally, a research group from Germany carried out a series of experiments using the Dextran Sulfate Sodium (DSS) model of colitis in C57BL/6 wild type mice. All the animals exposed to DSS for 7 days had an elevated FDG signal compared to control mice, predominantly in the medial and distal colon. Histological activity in the various regions correlated very well with a change in signal before and after DSS, with areas of superficial erosion or deep ulceration demonstrating an up to 400% increase of tracer uptake. Moreover, colonic FDG signal had dropped substantially at day 10, 3 days after discontinuation of DSS, suggesting good responsiveness of the test in reflecting the inflammatory changes. In addition to the intestinal signal, the authors identified an almost 3.5-fold increase of FDG uptake in large joints of exposed mice and suggested the possibilities of a colitis-associated arthropathy versus bone marrow hyperactivation. In an attempt to differentiate between the two, several joints were examined histologically, and there were no features of immune cell infiltrates or cartilaginous damage. Bone marrow histology revealed significant hyperproliferation in keeping with pro-inflammatory
activation. This finding was corroborated with flow cytometry of bone marrow and spleen extracts, which demonstrated a substantial increase in granulocyte precursors\textsuperscript{144}.

In summary, knowledge in the field of FDG-PET scanning in CD has moved forward significantly over recent years. Starting from initial reports qualitatively demonstrating co-localisation of intestinal FDG foci and endoscopic features of disease, we have now identified tools which we can use to measure the signal both in a single segment as well as in the entirety of bowel in each patient. It has also been demonstrated with reasonable confidence that higher FDG activity mirrors more significant inflammation, as measured by the currently accepted gold standards. Evidence from animal experiments suggests that the origin of the FDG signal can be pinpointed in inflammatory but also endothelial and resident intestinal stem cells. Moreover, murine experiments demonstrate that FDG signal intensity readily fluctuates in keeping with the underlying inflammatory cell burden in affected segments in animal studies. For FDG-PET to have a role in the longitudinal monitoring of patients with CD in clinical practice, or as an endpoint of demonstrating early efficacy in the context of new drug development, it is important for this responsiveness to be demonstrated in a human study. Lessons from the rheumatological literature are certainly encouraging to that end, but prospective data with early follow-up are still scarce\textsuperscript{132}.

\textbf{1.2.5.4.2 Alternative targets in inflammatory imaging}

The inflammatory process is triggered, and, subsequently propagated, by a variety of mediators which include cytokines, chemokines and other small molecules, produced by endothelium and resident, as well as newly recruited inflammatory cells. This cascade is commonly accompanied by increased vascular permeability and terminated by mediators of apoptosis of dominant cells.
All of these key cellular components and mediator molecules can potentially function as targets for radiotracers aimed at the imaging of the inflammatory process. The following section summarises current evidence and potential applications appearing in the literature for several such targets. To date, all of the agents outlined below are purely research tools which have not progressed through to clinical translation, some of them however hold great potential of finding a clinical niche in the near future.

1.2.5.4.2.1 Membrane markers of inflammatory cells -TSPO

Translocator Protein (TSPO), previously known as Peripheral Benzodiazepine Receptor (PBR) is a 18kDa trans-membrane receptor consisting of 169 amino acids. It is most commonly expressed on the outer mitochondrial membrane, while some studies have demonstrated the presence of TSPO on erythrocytes which are free of mitochondria, as well as plasma membranes of peripheral cells such as hepatocytes. TSPO has been shown to express throughout the body and brain. Concentrations are relatively higher in steroid-producing endocrine tissues, as opposed to brain, liver and breast. In peripheral blood TSPOs are most commonly found in monocytes and PMNs.

The reason TSPO has been extensively studied in the context of inflammatory molecular imaging is its increased expression in macrophages, neutrophils and lymphocytes. In human atherosclerosis, it has been shown that macrophages express binding sites for PK11195, a selective TSPO ligand, 20 times more abundantly than vascular smooth muscle cells, and autoradiographic experiments demonstrated co-localisation of this ligand with the macrophage-specific CD68. These findings have found applications in in vivo carotid atherosclerotic PET imaging with PK11195, with a high tissue-to-background signal being associated with symptomatic stenoses. Similar increases have also been demonstrated in imaging of synovial inflammation in patients with rheumatoid arthritis, in whom the signal
was also correlated to an influx of macrophages within diseased synovium\textsuperscript{150}. Other studies have demonstrated the efficacy of TSPO PET imaging in inflammatory models of acute lung injury\textsuperscript{151}, as well as non-alcoholic fatty liver disease (NAFLD) \textsuperscript{152}, implicating other cells such as neutrophils and lymphocytes as having a role for the increased tracer uptake.

There is an even more extensive body of evidence supporting the use of TSPO tracers in imaging of brain disease, which is underpinned by a combination of low background expression, which increases sharply in activated microglia, which hold key roles in the pathophysiology in a large array of CNS conditions\textsuperscript{153}.

In addition to TSPO, alternative examples of membrane markers are the somatostatin receptor (sst2) and cortistatin receptor (cst) with applications in the imaging of atherosclerosis \textsuperscript{154}, and the type 2 cannabinoid receptor CB\textsubscript{2}R which holds promise in the field of CNS inflammation \textsuperscript{155}.

### 1.2.5.4.2 Cytokine receptor targets in inflammatory tissue – IL-2R

Interleukin-2 (IL-2) is a pro-inflammatory cytokine produced by activated Th1-cells and it promotes further T-cell proliferation, differentiation of B-cells and Natural Killer and macrophage activation. The effects of IL-2 are mediated through binding to the IL-2 receptor (IL-2R), a heterotrimeric receptor comprising of α (CD25), β and γ subunits \textsuperscript{156,157}.

There have been several studies over the last two decades on applications of IL-2 scintigraphy in the diagnosis and follow-up of a variety of conditions underpinned by abnormal monocytic infiltration in tissue: Signore et al. demonstrated the potential of \textsuperscript{123}Iodine-labelling of the IL2 molecule and subsequent tracing of its accumulation by analysing pancreatic tissue both \textit{in vivo} and \textit{ex vivo} in a murine model of diabetes \textsuperscript{158}. In a different study in diabetic patients the same group used a different radioligand, \textsuperscript{99}Tc-IL2, to
longitudinally assess newly diagnosed Type I diabetics before and after treatment with nicotinamide (NA). IL2 radio-labeling was successful in selecting the subgroup of patients with active insulitis at onset, who benefited from NA therapy as evidenced by significantly lowered insulin requirements at one year

Several other human studies on IL-2 radiolabeling in a variety of auto-immune conditions had been carried out by the same group in the interim. One such study compared 10 patients with celiac disease, 7 of which were re-investigated following gluten-free diet (GFD), with 10 normal controls. Radiographic analysis showed significant differences between untreated coeliacs and coeliacs post GFD in 5/6 regions analysed, as well as between coeliacs and normal controls in 6/6 regions analysed. Importantly, despite clusters of CD25 +ve cells being demonstrated in normal individuals, these did not lead to any appreciable uptake of the radio-ligand to trigger positivity in planar or tomographic γ-camera imaging. Histological data, obtained only in patients with celiac disease demonstrate a changing prevalence of CD25 positive mononuclear cells pre and post GFD (P<0.0001), which is in keeping with the difference of radio-active signals. No immunological marker (e.g. anti-gliadin antibody or serum soluble IL2-R) correlated with 123I-IL2 signal. Finally, immunohisto-staining for CD25 and concurrent micro-autoradiography of biopsied tissue confirms that the 123I-IL-2 signal is actually derived from binding to CD25 cells rather than non-specific binding.

A similar study on carotid atherosclerosis using the 99Tc ligand on IL2 showed similar results with good correlation between signal and CD25+ve cells on histology. However, on this occasion, monocytes accounted for only a proportion of CD25+ cells which in their majority were activated smooth muscle cells (SMCs). This was justified by a ‘synthetic plaque’ paradigm where there is excessive interaction between immune and non-immune cells with a trophic effect in the latter. Regardless of the exact aetio-pathogenesis of CD25
positivity in SMCs, it highlights the observation that IL-2 radio-ligands are not strictly immune-cell selective, in conditions studied 161.

The same group has also performed 123I-IL-2 Single Photon Emission Computed Tomography (SPECT) scanning in Crohn’s disease. The study group comprised of 10 healthy controls and 15 subjects with ileal CD, confirmed by conventional measures, who were not on steroid or immunosuppressive therapy. 10 had active disease and 5 were in remission based on CDAI. Of the 10 patients with active disease, 6 were re-examined following a 12-week interval of treatment with corticosteroids. There were statistically significant differences both in SPECT and planar data between all 3 groups (healthy, CD in remission and active CD). There was also a correlation between activity on IL-2 scanning and clinical deterioration or relapse of disease (r=0.49 P=0.03). Interestingly, there was no attempt to correlate the location of IL-2 accumulation with that of active disease established with conventional methods i.e. the study does not inform if the IL-2 probe reveals the active segments or sub-clinical inflammation elsewhere. On a micro-autoradiography assay which was performed on bowel resected shortly after administration of the radio-ligand, some co-localisation between the radio-activity and CD-25 +ve lineages was demonstrated, as was the case in the coeliac study. 162.

Finally, the same group investigated the prognostic potential of both 99Tc-IL2 as well as 99Tc-HMPAO WBC scintigraphy, in a cohort of 10 controls and 29 CD patients in deep, longstanding remission of over 12 months in duration. Subjects underwent both diagnostic tests at entry, and subsequently clinical and laboratory parameters were monitored for 12 months. Several interesting points arise from this study. Firstly, concordance in the two scans was seen in 17 of 29 patients (12 positive and 5 negative) whereas in the remainder the results were discordant. In addition, out of 12 patients with concordant results, only 1 patient showed co-localisation of monocytic and neutrophilic infiltrates suggesting that the
techniques may be capturing sub-clinical disease at different stages of its natural history.
Finally, both tests had an excellent NPV >90% whereas the Positive Predictive Value (PPV) was around 40%\textsuperscript{161}. Importantly, a complete lack of adverse effects was reported by the authors on all of the aforementioned studies. The study dose is less than 1% of the therapeutic dose of IL2 immunotherapy, licensed for use in end-stage metastatic melanoma and renal-cell carcinoma, and therefore no biologic effects were anticipated.

While IL2 is a well-studied cytokine system in the context of inflammatory molecular imaging, there are several studies of key molecules in alternative inflammatory pathways. Interestingly, the majority of the probes put forward in this category comprise of selective medications already in use in routine clinical practice, with established selectivity profiles, which have been modified by the tagging of appropriate radionuclides which renders them detectable by imaging devices.

One of these is cyclo-oxygenase 2 (COX-2), an enzyme that transforms arachidonic acid into prostaglandins in inflamed tissues. Celecoxib is an established non-steroidal anti-inflammatory selective for COX-2, and several investigators have produced radioligand versions of celecoxib, which until recently had rather poor signal to noise ratios\textsuperscript{163}. A recent\textsuperscript{18F}- celecoxib derivative is likely more specific than its predecessors, which holds some promise as an early marker of pre-malignant lesions.

Another key cytokine in both acute as well as chronic inflammatory cascades is Tumour Necrosis Factor-alpha (TNFa), with chemotactic as well as anti-apoptotic funtions\textsuperscript{164}.
1.2.5.4.2.3 Targets related to angiogenesis

Apart from the interest it has attracted in the field of DCE MRI, research in the process of inflammation-related angiogenesis has provided us with several imaging targets specific to that pathway. One characteristic example is radiolabeled arginine-glycine-aspartic (RGD) peptides specific for a αvβ3 integrins expressed in inflammatory cells in neo-vessels. Interesting positive results have been obtained in murine models of chronic ear infection and atherosclerosis.

Vascular Adhesion Protein-1 (VAP-1), and Vascular Cell Adhesion Molecule-1 (VCAM-1), chemotactic molecules on endothelium which promote leukocyte migration, also show some promise in this area.

1.3 SUMMARY

The last two decades have seen major advances in the diagnostics of Crohn’s disease. Studies that demonstrate the dissociation between symptoms and clinical scores, and even between mucosal activity and underlying bowel damage have had a major impact on the way we program our therapeutic interventions and monitoring strategies. Moreover, as treatments become more sophisticated, as well as costly and potentially toxic, the lack of a gold-standard instrument to quantify and monitor the inflammatory load is more important than ever before. The development of such a gold standard is required in order to fulfill unmet needs such as the timely escalation of therapy before irreversible damage occurs, the early identification of response to treatment as well as the more targeted recruitment of appropriate patients in clinical studies. Intense research effort has gone into thoroughly defining roles for existing modalities, while steps are also being made in investigating functions of novel tools such as molecular imaging in that arena.
1.4 AIMS AND HYPOTHESES

1.4.1 $^{18}$F-FDG Positron Emission Tomography outcome measures in the monitoring of inflammatory activity in Crohn's disease

The study described in Chapter 2 aimed to investigate the monitoring of inflammatory activity in Crohn's disease with $^{18}$F-FDG-PET, for the first time.

i. $^{18}$F-FDG has been shown to accumulate in areas of active inflammation in CD producing higher signal than in unaffected segments. This signal correlates with endoscopic and histopathological markers of severity and it can be quantified in terms of its intensity and extent. It was hypothesised that the magnitude of this signal in each affected bowel segment would remain unchanged in patients with stable disease and on stable therapy, over short periods of time.

ii. It was hypothesized that several measures of this focal FDG activity would change over a 12-week interval in patients responding to anti-TNFα therapy.

iii. It was also hypothesized that several measures of the $^{18}$F-FDG signal would be correlated with clinical and biochemical markers of disease severity both at a single time-point as well as longitudinally.

1.4.2 Magnetic Resonance Imaging outcome measures in the monitoring of inflammatory activity in Crohn's disease

The study outlined in Chapter 3 aimed to investigate the role of MRI scores in monitoring inflammatory activity in Crohn's disease.

i. Several scores have been derived using MRI, which assess anatomical as well as functional aspects of disease severity in actively inflamed segments in patients with CD. These have been correlated with clinical, endoscopic and other
biochemical markers of disease severity. Similarly to the \(^{18}\)F-FDG study, it was hypothesised that these scores would remain unchanged in patients with stable disease, and on stable therapy over short time periods.

ii. It was hypothesised that anatomical and functional MRI scores representing disease activity would change over a 12-week interval in patients responding to anti-TNFα therapy.

iii. It was also hypothesized that anatomical and functional MRI scores would correlate with clinical and biochemical markers of disease severity both at a single time-points as well as longitudinally.

**1.4.3 Exploration of alternative targets for PET radioligands specific to inflammatory pathways**

The study described in Chapter 4 aimed to assess the expression of TSPO and IL2 receptor in normal versus inflamed bowel segments. Moreover it aimed to evaluate the binding properties of TSPO and IL-2R specific radioligands in normal versus inflamed tissue.

i. TSPO has been shown to over-express in a variety of inflammatory conditions. Studies have shown that PET radioligands specific for TSPO can quantify inflammatory load in a variety of inflammatory conditions. Expression of the IL-2 receptor is also increased in Crohn’s mucosa. This has been used in the past in the assessment of activity in CD by SPECT imaging.

ii. It was hypothesised that TSPO and IL2R will be over-expressed in intestinal sections containing inflammation compared to uninvolved controls.

iii. It was also hypothesized that this over-expression will also represent an increase in specific binding sites for first and second generation TSPO as well as IL-2 radioligands.

iv. Finally it was hypothesised that the dissociation constant (K\text{d}) of PBR28, a
second generation radioligand for TSPO, would be similar to that described in brain.
2.1. INTRODUCTION

Recently, there has been a significant interest into the capabilities of the $^{18}$F-FDG PET to delineate Crohn's lesions and quantify their metabolic activity. Early studies suggest that the technique has sensitivity and specificity in lesion identification comparable to other available modalities $^{133,134}$. Methods of quantifying the signal intensity have been explored, with the majority of the early studies focusing on Standardised Uptake Values (SUV) $^{136,138}$ while more recent reports also used composite measures including signal volume, translated from the oncological literature $^{141,168}$. Most of these endpoints correlate reasonably well with respective conventional markers of activity both on a segmental (e.g. CDEIS) as well as on a global level (clinical scoring, blood and faecal inflammatory markers).

There are still several major unknown factors in the role of FDG-PET in monitoring CD patients. There have been no published studies exploring the technique's responsiveness i.e. the potential of FDG-PET to demonstrate change in its outcome measures over time. Several well-designed experiments in appropriate murine models, as well as human studies in other chronic inflammatory conditions have produced encouraging results, but a longitudinal study focused on CD is still lacking$^{142-144}$. Moreover, there is a need for data on the reliability of $^{18}$F-FDG PET in CD, defined as a measure of the consistency of yielded results when the test is administered repeatedly in stable subjects.

Finally, the origin of the FDG signal within intestinal segments has not been accurately ascertained: Murine studies have provided some evidence that the most FDG-avid cellular populations in inflammatory foci are neutrophils, macrophages and lymphocytes, and
inferences have been made in human studies that this also holds true in CD\textsuperscript{144}. On the other hand, work on physiological gut uptake suggests that elements within the intestinal lumen such as colonic flora are also absorbing FDG and, as such, they are able to contribute to signal intensity\textsuperscript{169}.

The aims of this study were:

1) To confirm the correlation of all outcome measures of FDG signal with clinical and biochemical markers of activity

2) To assess 18F-FDG PET’s reliability and responsiveness in monitoring CD and

3) To investigate further the tissue compartments from which FDG intestinal signal originates.

This study tested the following hypotheses: Firstly, it was hypothesised that active CD lesions in patients without changes in their symptoms or treatment regime would produce FDG signal of stable intensity and extent in test-retest scanning over a short period of time. This is because the population and degree of activation of immune cells in CD lesions are believed not to change rapidly in stable disease, and FDG signal is assumed to reflect these two entities. Similarly, it was hypothesised that patients who respond clinically to anti-TNF\alpha will have demonstrable changes in their FDG signal over a period of three months compared to primary non-responders.
2.2. METHODS

2.2.1 STUDY DESIGN - PARTICIPANT SELECTION- POWER CALCULATIONS

To assess responsiveness, patients with active CD (HBI>4) were prospectively recruited, after a decision had been made by the IBD specialist supervising their care to commence anti-TNF therapy. Two PET scans were performed under standardised conditions (outlined in detail in 2.2.3), one baseline before and one follow-up after anti-TNF therapy, with a target interval of three months between the start of therapy and the follow-up scan. Reference investigations performed on the day of the scanning included HBI scoring, CRP and calprotectin analysis as well as the Magnetic Resonance Index of Activity (MaRIA) score \(^{74,75}\) (obtained by MR enterography, see Chapter 3)

With regards to recruitment for the test-retest reliability component of the study, the aim was to select patients with a variety of disease distributions, inclusive of small intestinal as well as colonic segments. We therefore prospectively enrolled patients with active CD (HBI>4, also deemed active by their IBD specialist on the basis of recent endoscopic/radiological examination). The target interval between the two scans was 1 week and a maximum permitted interval of 2 weeks. The HBI score, CRP and a MaRIA score were assessed on both visits, while a calprotectin assay was performed on one of the two visits as additional evidence of active disease.

In addition to active CD at the outset, our inclusion criteria were a minimum age of 18 years, and a post-menopausal status in females or willingness to adhere to contraception advice and avoid unprotected sexual intercourse for the duration of the study in both genders. This was to mitigate the radiation risks of \(^{18}\text{F}-\text{FDG}\) in insemination and early pregnancy.
Participants with alternative GI pathologies, which could produce additional foci of signal (e.g. known polyps, malignancy, GI infection, coeliac disease) were excluded. We also excluded patients with physical disabilities precluding comfortable positioning on the PET scanner. In an order to minimise the risks of ionising radiation, we also excepted patients who were pregnant or who might become pregnant, a history of cancer, either personal or in a first degree relative at an age < 55 years, and also patients who had participated in research which involved ionising radiation in the previous 3 years.

In order to estimate an appropriate number of participants the following were taken into consideration: This was a pilot study with numbers of subjects determined by feasibility. Within our Trust approximately 25-30 patients/year are commenced on anti-TNF therapy for Crohn's disease. Assuming a similar prescription pattern by our specialists during the duration of the study, the aim was to recruit 24 patients (20 for the longitudinal study and 4 for the test-retest component) over 18 months.

I examined studies where bowel segments affected by Crohn's disease were assessed by SUV\textsubscript{MAX} and found that the average SUV\textsubscript{MAX} across all these segments was approx. 5.0 with a standard deviation of 2.2. These figures were used to calculate the power of the study assuming an 80% primary response rate, in keeping with published data\textsuperscript{170}. The potential to detect an effect at 12 weeks over a range of marker sensitivities was estimated, with the use of projected SUV\textsubscript{MAX} drops between 10-50% (see Table 2.1):
Table 2.1: Predicted power for a range of participants and projected ΔSUVMAX pre and post-treatment

<table>
<thead>
<tr>
<th>N (participants)</th>
<th>n (responders)</th>
<th>SD</th>
<th>% drop in SUVMAX</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>8</td>
<td>2.2</td>
<td>10</td>
<td>0.08</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>2.2</td>
<td>20</td>
<td>0.20</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>2.2</td>
<td>30</td>
<td>0.38</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>2.2</td>
<td>40</td>
<td>0.59</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>2.2</td>
<td>50</td>
<td>0.79</td>
</tr>
<tr>
<td>20</td>
<td>16</td>
<td>2.2</td>
<td>10</td>
<td>0.11</td>
</tr>
<tr>
<td>20</td>
<td>16</td>
<td>2.2</td>
<td>20</td>
<td>0.33</td>
</tr>
<tr>
<td>20</td>
<td>16</td>
<td>2.2</td>
<td>30</td>
<td>0.62</td>
</tr>
<tr>
<td><strong>20</strong></td>
<td><strong>16</strong></td>
<td><strong>2.2</strong></td>
<td><strong>40</strong></td>
<td><strong>0.85</strong></td>
</tr>
<tr>
<td>20</td>
<td>16</td>
<td>2.2</td>
<td>50</td>
<td>0.99</td>
</tr>
</tbody>
</table>

With these assumptions, it was estimated that studying 20 patients, (approx. 16 responders) should allow measurement of a significant decrease in signal in the follow-up relative to the pre-treatment scan.

As the MRI component was optional, and radiation exposure the principal risk in this study, only the PET endpoints have been taken into account in the formulation of a power calculation for this study.

2.2.2 SCREENING PROCESS -- PATIENT PREPARATION

Formal approval by a Research Ethics Committee (REC), Research and Development (R&D) departments of participating hospitals and ARSAC had been granted before commencing the study (REC: South East Coast-Surrey 12/LO/1018, R&D: JRCOHH0453, ARSAC Certificate: RPC 630/2892/28817). Informed consent was obtained from all participants. In addition to eligibility checks, screening assessment included a full physical examination with recording of the vital signs. Investigations included routine urine analysis and a pregnancy test.
women of childbearing potential, as well as routine full blood count and biochemical profile performed in advance of the scanning. On the day of the scanning, participants' HBI score was assessed, and blood and stool samples were obtained for blood glucose and CRP, and faecal calprotectin measurements respectively.

2.2.3 SCANNING PROCEDURE

A 20 gauge intravenous cannula was placed and a target dose of 185MBq of $^{18}$F-FDG in 10ml of normal saline was administered intravenously, followed by a saline flush. The patients were then asked to rest semi-recumbent in a quiet room, and were given between 800 and 1200ml of 2.5% mannitol orally for small bowel distention. At 50 minutes following FDG administration, patients were asked to void their bladders, and were positioned on the PET/CT scanner (Siemens Biograph 6 Truepoint, Siemens Healthcare, Enlargen, Germany). 20mg of intravenous hyoscine butylbromide (Buscopan®) was administered to reduce motion artifact, and a low dose CT scan of the abdomen was obtained (130KV, 30mAs, pitch 1.5, 6 slice x 3mm collimation). Subsequently, PET emission data from the gut were acquired in a 3-dimensional model. A maximum of 3 bed positions were used for this component for the baseline scan. The maximal total duration of scanning time was 30 minutes (10 minutes per bed position). ImPACT dose calculator was used, a software for estimation of dose levels in X-ray computed tomography using pre-calculated tables, to estimate our average target effective dosage at 11.2mSv, comprised of 3.5mSv for each of the PET scans and 2.1mSv for each of the CT scans). These procedures were standardised so that they could be accurately replicated on follow-up scanning.
2.2.4 PET SCAN ANALYSIS

PET/CT images were analysed using Inveon Research Workplace (IRW, Siemens Healthcare, USA). Scans were reconstructed in both OSEM (Ordered Subset Expectation Maximisation, 2 iterations, 8 subsets) and FBP (Filtered Back Projection), but the former was used in the analysis. Scans were reconstructed in a 256 x 256 matrix and zoom of 1.3 and 3D Gaussian image filter with 5 mm full-width at half maximum (FWHM).

The attenuation corrected (AC) CT sequence was fused with OSEM PET sequences. Volume of Interest (VOI) derivation and subsequent analysis were carried out by the author and a nuclear medicine expert fully blinded to all clinical data, working independently. In cases of discrepancy in VOI positioning and margins, these were reprocessed in tandem by both investigators until consensus was reached. A VOI of at least 40 cm³ was created inside the liver parenchyma and the mean SUV (LivSUV<sub>MEAN</sub>) was recorded. In keeping with published literature<sup>134,137</sup>, the resulting LivSUV<sub>MEAN</sub> was used as a threshold above which GI signal was deemed abnormal. The entire fused 3-D sequence was adjusted to exclude signal of intensity lower than LivSUV<sub>MEAN</sub>. The bowel was then separated into seven segments (small bowel, terminal ileum, caecum and ascending colon, transverse colon, descending colon, sigmoid and rectum). The distinction between SB and TI was made functionally, with the latter consisting of the most distal unified focus of activity in small bowel irrespective of its length, and SB was classified as the collection of all foci corresponding to regions distal to the pylorus and proximal to TI. The remaining segments were defined anatomically, and special attention was focused on the precise replication of their margins between baseline and follow-up scanning in each patient. Once segments had been identified, a crude ‘parent’ VOI was drawn in those segments which contained visible signal. This parent VOI included individual voxels both above LivSUV<sub>MEAN</sub>, as well as adjacent ones below.
LivSUV_{\text{MEAN}}. In instances where the signal within a segment was not continuous, but interrupted by non-emitting tissue, the latter was included in the parent VOI so that one single parent VOI was drawn for each segment. These parent VOIs were then re-thresholded using a function in the software, to produce 'daughter' VOIs, which only include voxels with signal equal to or higher than LivSUV_{\text{MEAN}}, and therefore, by our definition, abnormal. This process ensured that interposed voxels with FDG activity lower than our pre-defined threshold were excluded form further analysis. The process is illustrated in Figure 2.1.
Figure 2.1: Methodology of VOI derivation

A) Liver VOI drawn and LivSUV_{MEAN} determined (red circle)

B) Sequence thresholded to exclude signal of SUV< LivSUV_{MEAN}

C) ‘Parent’ VOI (green outline) drawn around visible signal focus → Thresholded for LivSUV_{MEAN}

D) ‘Daughter’ VOI produced by software and Volume, SUV_{MEAN} and SUV_{MAX} recorded
Standardised Uptake Value (SUV) is the typical measure of PET signal and is defined as:

\[ \text{SUV} = \frac{r}{\alpha'/w} \]

where \( r \) is the radioactivity concentration in KBq/ml within a VOI, \( \alpha' \) is the decay corrected amount of administered FDG and \( w \) is the weight of the patient.\(^{170}\)

For each ‘daughter’ VOI the software provided the following endpoints: \( \text{SUV}_{\text{MAX}} \) reflecting the SUV of the single voxel of highest intensity, \( \text{SUV}_{\text{MEAN}} \) which represented the average values of all SUVs in the VOI, and its Volume in cm\(^3\). From these three values a number of other endpoints were derived, as described in the literature, on a ‘per segment’ and ‘per patient’ basis. All endpoints are defined in the tables below:

*Table 2.2: Segmental endpoints*

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{SUV}_{\text{MAX}} )</td>
<td>The SUV of the voxel of highest intensity within the daughter VOI</td>
</tr>
<tr>
<td>( \text{SUV}_{\text{MEAN}} )</td>
<td>The average SUV of all voxels within the daughter VOI</td>
</tr>
<tr>
<td>( V )</td>
<td>The volume of abnormal voxels in a daughter VOI (cm(^3))</td>
</tr>
<tr>
<td>( \text{RSUV}_{\text{MAX}} )</td>
<td>( \text{SUV}<em>{\text{MAX}} ) expressed as a ratio over Liv( \text{SUV}</em>{\text{MEAN}} )</td>
</tr>
<tr>
<td>( \text{RSUV}_{\text{MEAN}} )</td>
<td>( \text{SUV}<em>{\text{MEAN}} ) expressed as a ratio over Liv( \text{SUV}</em>{\text{MEAN}} )</td>
</tr>
<tr>
<td>SLG</td>
<td>Segmental Lesion Glycolysis = ( \text{SUV}_{\text{MEAN}} \times \text{Volume of VOI} )</td>
</tr>
<tr>
<td>RSLG</td>
<td>SLG expressed as a ratio over Liv( \text{SUV}_{\text{MEAN}} )</td>
</tr>
</tbody>
</table>

*Table 2.3: Global (‘per patient’) endpoints*

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{GSUV}_{\text{MAX}} )</td>
<td>Global ( \text{SUV}<em>{\text{MAX}} ): The average ( \text{SUV}</em>{\text{MAX}} ) of all segments with abnormal signal</td>
</tr>
<tr>
<td>( \text{RGSUV}_{\text{MAX}} )</td>
<td>( \text{GSUV}<em>{\text{MAX}} ) expressed as a ratio over Liv( \text{SUV}</em>{\text{MEAN}} )</td>
</tr>
<tr>
<td>Total ( V )</td>
<td>Total Volume = ( \Sigma ) of segmental volumes</td>
</tr>
<tr>
<td>TLG</td>
<td>Total Lesion Glycolysis = ( \Sigma ) of SLGs in abnormal segments</td>
</tr>
<tr>
<td>R-TLG</td>
<td>TLG expressed as a ratio over Liv( \text{SUV}_{\text{MEAN}} )</td>
</tr>
<tr>
<td>( \text{GSUV}_{\text{MEAN}} )</td>
<td>Global ( \text{SUV}_{\text{MEAN}} ): TLG / ( \Sigma ) Volumes of all abnormal segments</td>
</tr>
<tr>
<td>R-( \text{GSUV}_{\text{MEAN}} )</td>
<td>( \text{GSUV}<em>{\text{MEAN}} ) expressed as a ratio over Liv( \text{SUV}</em>{\text{MEAN}} )</td>
</tr>
</tbody>
</table>

Most endpoints were expressed as absolute values as well as tissue-to-background ratios (TBR) over Liv\( \text{SUV}_{\text{MEAN}} \) as the liver is always in the field of view and it has been shown to have low within-patient variability.\(^{171}\) Therefore it was chosen as a reference organ for normal tissue activity.
PET endpoints such as $\text{SUV}_{\text{MAX}}$ and $\text{SUV}_{\text{MEAN}}$ in a segment (either as absolutes or as ratios over $\text{LivSUV}_{\text{MEAN}}$) reflect disease severity in that segment, and $\text{GSUV}_{\text{MAX}}$ and $\text{GSUV}_{\text{MEAN}}$ represent global disease severity in each patient. Each daughter VOI’s Volume ($V$) as well as the Total $V$ are measures of disease extent. SLG and TLG are composite measures designed to reflect the metabolic, and as such, the inflammatory activity more globally in each segment or patient respectively.

There were several instances when a segment only expressed signal above threshold on either the baseline or the follow-up scan. In those instances, endpoints for the scan with the active segment were derived as described, but when analysing the scan that contained the segment with no activity, an anatomical VOI of equal volume was placed on that ‘normal’ segment matching the location of the corresponding daughter VOI on the ‘abnormal’ segment as closely as possible. All PET endpoints were then calculated as described above.

The data analysis outlined above was conducted twice in the cohort undergoing longitudinal pre and post-treatment PET. The first time, all intestinal segments expressing FDG signal above the predefined threshold were included. In the second analysis, I excluded all segments which on the concurrent MR enterography had segmental MaRIA scores $<7.0^{73,74}$ or, in the case of small bowel, had normal appearances as judged by an experienced MR radiologist. This allowed us to exclude segments where potentially the FDG-PET signal did not specifically represent Crohn’s disease activity.

2.2.5 DATA ANALYSIS

Correlation between clinico-pathological markers (HBI, CRP and FC) and PET endpoints using the baseline PET scan for each participant from both cohorts were performed
using Spearman rank coefficients. p<0.05 was the threshold of significance. This was not corrected for multiple comparisons. It was hypothesised that in active CD, the clinical score, CRP and FC can be accounted for by (or be representative of) either the global inflammatory activity, or activity within the worst affected bowel segments, so the above correlations were performed using global as well as segmental PET endpoints respectively. The most abnormal segment in each patient was determined on the basis of its \( \text{SUV}_{\text{MEAN}} \).

Subsequently I focused on subjects who completed the longitudinal component of the study by having FDG-PET scanning before and after the introduction of anti-TNF therapy. I assessed correlations between the absolute differences (\( \Delta \)) in clinico-pathological endpoints and with those of corresponding PET endpoints\(^{172} \). The \( \Delta \)-values for each endpoint were defined as follows (exemplified by \( \text{SUV}_{\text{MAX}} \)):

\[
\Delta \text{SUV}_{\text{MAX}} = \text{SUV}_{\text{MAX(POST-TREATMENT)}} - \text{SUV}_{\text{MAX(PRE-TREATMENT)}}
\]

For all these correlations, the Spearman rank correlation coefficient was also used.

I then segregated the patients into responders and non-responders on the basis of a clinically demonstrable response 3 months into therapy. This was defined as a decrease on the HBI scale of 3 points or more in line with previous studies\(^{173} \). Baseline and follow-up PET measures were compared for responders and non-responders. This distinction between responders and non-responders was used to calculate the Responsiveness ratio of Guyatt (RRG)\(^{174} \), as well as the standardized size effect (SES)\(^{109} \). RRG for each endpoint is defined as the (mean \( \Delta \) endpoint in responders /Standard deviation of endpoint in non-responders). The SES is defined as (mean \( \Delta \) endpoint in responders/Standard deviation of endpoint in responders). These two values were calculated, as they constitute direct statistical measures of an endpoint's
responseveness. A value greater than 0.80 in these parameters is suggestive of a high responsiveness.

To assess test-retest reliability of PET-endpoints I measured the % variability (%VAR) for each endpoint using all segments with signal > LivSUV\text{~mean} as previously described\textsuperscript{175}.

%VAR = \frac{\text{Value(2) - Value(1)}}{\text{Value(1)}} \times 100\%

2.2.6 WORKLOAD DISTRIBUTION – PERSONAL INVOLVEMENT

Sequential drafts of the study protocol were produced by me and amended following consultation with my supervisors and the multi-disciplinary team at the Imaging Centre. I subsequently defended the protocol at a REC meeting, and drafted and submitted all subsequent amendments. I also carried out participant recruitment, including obtaining informed consent, by attending IBD clinics at participating hospitals. During scanning visits, I acted as Study Physician, which involved the preparation and administration of oral contrast, administration of the radio-pharmaceutical, and ensuring patient safety from admission to discharge. For the purposes of the analysis, I was trained on the IRW software by a PET physicist, following which I produced all VOIs on all 38 scans. These were individually corroborated or amended by a PET radiologist. Finally, I also performed statistical analysis and interpretation.
2.3. RESULTS

2.3.1 DEMOGRAPHICS

22 patients (13 male) with a mean age of 40 years (range 22-59) were recruited, 17 for the longitudinal component and 5 for the test-retest reliability arm of this study. Demographic details and disease characteristics of these two cohorts are outlined in Table 2.4.
Table 2.4A: Demographic data and disease characteristics in the LONGITUDINAL cohort

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Disease duration (years)</th>
<th>Disease Distribution</th>
<th>Previous resections</th>
<th>HBI baseline</th>
<th>HBI follow-up</th>
<th>Response (by HBI criteria)</th>
<th>CRP baseline (mg/L)</th>
<th>CRP follow-up (mg/L)</th>
<th>FC baseline (mcg/g)</th>
<th>FC follow-up (mcg/g)</th>
<th>Anti-TNF</th>
<th>Completed F/U</th>
<th>Treatment start-F/U scan interval (weeks)</th>
<th>Reason for not completing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>25</td>
<td>4</td>
<td>L3</td>
<td>No</td>
<td>4</td>
<td>1</td>
<td>Y</td>
<td>3.4</td>
<td>0.5</td>
<td>934</td>
<td>23</td>
<td>IFX</td>
<td>Y</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>57</td>
<td>30</td>
<td>L1</td>
<td>2</td>
<td>7</td>
<td>8</td>
<td>N</td>
<td>0.9</td>
<td>0.2</td>
<td>74</td>
<td>33</td>
<td>ADA</td>
<td>Y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>36</td>
<td>4</td>
<td>L3</td>
<td>No</td>
<td>7</td>
<td>6</td>
<td>N</td>
<td>34.5</td>
<td>42.5</td>
<td>1340</td>
<td>850</td>
<td>ADA</td>
<td>Y</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>54</td>
<td>14</td>
<td>L1</td>
<td>No</td>
<td>5</td>
<td>4</td>
<td>N</td>
<td>6.2</td>
<td>2.9</td>
<td>173</td>
<td>116</td>
<td>IFX</td>
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<td>13</td>
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<tr>
<td>5</td>
<td>F</td>
<td>39</td>
<td>13</td>
<td>L3</td>
<td>1</td>
<td>5</td>
<td>---</td>
<td>---</td>
<td>5.3</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>ADA</td>
<td>N</td>
<td>---</td>
<td>Discontinued anti-TNF after 1 dose</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>53</td>
<td>11</td>
<td>L1</td>
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<td>11</td>
<td>---</td>
<td>---</td>
<td>2.6</td>
<td>75</td>
<td>---</td>
<td>---</td>
<td>ADA</td>
<td>N</td>
<td>---</td>
<td>Ca diagnosis during screening</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>41</td>
<td>4</td>
<td>L3</td>
<td>1</td>
<td>8</td>
<td>2</td>
<td>Y</td>
<td>7.8</td>
<td>2.2</td>
<td>443</td>
<td>59</td>
<td>ADA</td>
<td>Y</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>40</td>
<td>13</td>
<td>L1</td>
<td>1</td>
<td>10</td>
<td>7</td>
<td>Y</td>
<td>1.6</td>
<td>0.2</td>
<td>178</td>
<td>33</td>
<td>ADA</td>
<td>Y</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>47</td>
<td>3</td>
<td>L1</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>N</td>
<td>5.7</td>
<td>10.3</td>
<td>237</td>
<td>595</td>
<td>IFX</td>
<td>Y</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>22</td>
<td>1</td>
<td>L3</td>
<td>No</td>
<td>14</td>
<td>6</td>
<td>Y</td>
<td>51</td>
<td>0.4</td>
<td>252</td>
<td>33</td>
<td>ADA</td>
<td>Y</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>40</td>
<td>2</td>
<td>L1</td>
<td>No</td>
<td>8</td>
<td>2</td>
<td>Y</td>
<td>17.6</td>
<td>3.5</td>
<td>2100</td>
<td>850</td>
<td>ADA</td>
<td>Y</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>27</td>
<td>3</td>
<td>L1</td>
<td>1</td>
<td>10</td>
<td>---</td>
<td>---</td>
<td>0.3</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>ADA</td>
<td>Y</td>
<td>---</td>
<td>Lost to F/U</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>51</td>
<td>1</td>
<td>L3</td>
<td>1</td>
<td>9</td>
<td>4</td>
<td>Y</td>
<td>5</td>
<td>6.9</td>
<td>91</td>
<td>6</td>
<td>IFX</td>
<td>Y</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>30</td>
<td>2</td>
<td>L2</td>
<td>No</td>
<td>5</td>
<td>---</td>
<td>---</td>
<td>0.2</td>
<td>89</td>
<td>---</td>
<td>---</td>
<td>ADA</td>
<td>N</td>
<td>---</td>
<td>Lost to F/U</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>32</td>
<td>26</td>
<td>L1</td>
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<td>5</td>
<td>1</td>
<td>Y</td>
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<td>1.0</td>
<td>70</td>
<td>64</td>
<td>IFX</td>
<td>Y</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>44</td>
<td>13</td>
<td>L3</td>
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<td>13</td>
<td>5</td>
<td>Y</td>
<td>6.4</td>
<td>2.5</td>
<td>186</td>
<td>1515</td>
<td>IFX</td>
<td>Y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>36</td>
<td>5</td>
<td>L1</td>
<td>No</td>
<td>9</td>
<td>6</td>
<td>Y</td>
<td>46.4</td>
<td>13.8</td>
<td>377</td>
<td>---</td>
<td>IFX</td>
<td>Y</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>
**Table 2.4B: Demographic data and disease characteristics in the TEST-RETEST cohort**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Disease duration (years)</th>
<th>Disease Distribution*</th>
<th>Previous resections</th>
<th>HBI baseline</th>
<th>CRP baseline (mg/L)</th>
<th>CRP follow-up (mg/L)</th>
<th>FC (mcg/g)</th>
<th>Completed both scans</th>
<th>Baseline-F/U scan interval (days)</th>
<th>Reason for not completing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>55</td>
<td>22</td>
<td>L1</td>
<td>2</td>
<td>7</td>
<td>14.8</td>
<td>7.9</td>
<td>655</td>
<td>Y</td>
<td>7</td>
<td>Declined MRI on repeat visit</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>41</td>
<td>12</td>
<td>L3</td>
<td>No</td>
<td>5</td>
<td>5.8</td>
<td>10.3</td>
<td>325</td>
<td>Y</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>59</td>
<td>23</td>
<td>L3</td>
<td>No</td>
<td>10</td>
<td>--</td>
<td>22.5</td>
<td>839</td>
<td>N</td>
<td>--</td>
<td>Declined repeat scanning</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>24</td>
<td>3</td>
<td>L3</td>
<td>No</td>
<td>7</td>
<td>7</td>
<td>13.5</td>
<td>257</td>
<td>Y</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>31</td>
<td>11</td>
<td>L3</td>
<td>No</td>
<td>10</td>
<td>10</td>
<td>2.8</td>
<td>1.7</td>
<td>Y</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

*Disease distribution: L1: small bowel only L2: colonic only L3:ileo-colonic*
2.3.2 SEGMENT ANALYSIS

145 segments were assessed by PET with MaRIA score, which was obtained on MRI enterography performed on the same day, as a reference standard. This was on the basis of its close correlation with endoscopic disease scores. The MaRIA threshold for a bowel segment to be deemed inflamed was >7.0. Since MaRIA has not been validated in small bowel disease, presence of activity in small bowel proximal to the TI was assessed on the basis of an expert opinion by an MR radiologist with 10 years experience in abdominal MR, blinded to the clinical details of the patients. Of these 145 segments, MaRIA (or MR enterography in the case of small bowel) was positive in 51 and negative in 94. Segmental FDG signal equal or higher than LivSUV_{mean} correctly identified 47 of 51 segments resulting in a sensitivity of 92%. 67 of the 94 negative segments had signal of intensity less than LivSUV_{mean}, therefore the specificity was 71%. Figure 2.2 outlines the various segments and their MRI and PET positivity on the baseline scan.

*Figure 2.2: Distribution of PET and MRI +ve segments*
2.3.3 ‘SNAPSHOT’ CORRELATION OF BASELINE PET ENDPOINTS WITH CLINICO-PATHOLOGICAL SCORES

Spearman rank correlation coefficients were performed to assess the degree of correlation between each of the clinico-pathological variables and the FDG-PET endpoints. These are reviewed in Tables 2.4A-D. The closest and more consistent correlations are observed between CRP and FDG-PET measures of intensity. HBI and FC correlations with PET endpoints were weaker and less consistent.
Tables 2.5A, 2.5B, 2.5C, and 2.5D: Correlations between clinico-pathological (Harvey Bradshaw Index, CRP and Faecal Calprotectin) and PET endpoints (segmental and global SUV<sub>MAX</sub>, SUV<sub>MEDIAN</sub> and lesion glycolysis). Statistical significance highlighted in pink.

A) All PET-positive segments, absolute values

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>SUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>SUV&lt;sub&gt;MEDIAN&lt;/sub&gt;</th>
<th>SLG</th>
<th>GSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>GSUV&lt;sub&gt;MEDIAN&lt;/sub&gt;</th>
<th>TLG</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBI</td>
<td>r=0.40 p=0.06</td>
<td>r=0.40 p=0.06</td>
<td>r=0.23 p=0.3</td>
<td>r=0.46 p=0.03</td>
<td>r=0.24 p=0.2</td>
<td>r=0.32 p=0.1</td>
</tr>
<tr>
<td>CRP</td>
<td>r=0.55 p=0.00</td>
<td>r=0.54 p=0.00</td>
<td>r=0.29 p=0.1</td>
<td>r=0.57 p=0.00</td>
<td>r=0.51 p=0.01</td>
<td>r=0.52 p=0.01</td>
</tr>
<tr>
<td>FC</td>
<td>r=0.36 p=0.1</td>
<td>r=0.15 p=0.5</td>
<td>r=0.13 p=0.5</td>
<td>r=0.31 p=0.1</td>
<td>r=0.01 p=0.95</td>
<td>r=0.37 p=0.1</td>
</tr>
</tbody>
</table>

B) All PET-positive segments, TBR

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>RSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>RSUV&lt;sub&gt;MEDIAN&lt;/sub&gt;</th>
<th>RSLG</th>
<th>RGSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>RGSUV&lt;sub&gt;MEDIAN&lt;/sub&gt;</th>
<th>RTLG</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBI</td>
<td>r=0.46 p=0.03</td>
<td>r=0.43 p=0.04</td>
<td>r=0.25 p=0.2</td>
<td>r=0.47 p=0.02</td>
<td>r=0.51 p=0.01</td>
<td>r=0.28 p=0.2</td>
</tr>
<tr>
<td>CRP</td>
<td>r=0.63 p=0.00</td>
<td>r=0.58 p=0.00</td>
<td>r=0.32 p=0.1</td>
<td>r=0.63 p=0.00</td>
<td>r=0.57 p=0.00</td>
<td>r=0.44 p=0.04</td>
</tr>
<tr>
<td>FC</td>
<td>r=0.51 p=0.0</td>
<td>r=0.42 p=0.06</td>
<td>r=0.27 p=0.2</td>
<td>r=0.47 p=0.03</td>
<td>r=0.40 p=0.0</td>
<td>r=0.46 p=0.0</td>
</tr>
</tbody>
</table>

C) PET-positive MRI-positive segments, absolute values

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>SUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>SUV&lt;sub&gt;MEDIAN&lt;/sub&gt;</th>
<th>SLG</th>
<th>GSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>GSUV&lt;sub&gt;MEDIAN&lt;/sub&gt;</th>
<th>TLG</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBI</td>
<td>r=0.36 p=0.1</td>
<td>R=0.40 p=0.064</td>
<td>R=0.3 5</td>
<td>R=0.46 p=0.031</td>
<td>R=0.3 8</td>
<td>R=0.46 p=0.031</td>
</tr>
<tr>
<td>CRP</td>
<td>r=0.49 p=0.01</td>
<td>R=0.48 p=0.024 0</td>
<td>R=0.1 0</td>
<td>R=0.56 p=0.007</td>
<td>R=0.50 2</td>
<td>R=0.49 p=0.022</td>
</tr>
<tr>
<td>FC</td>
<td>R=0.41 p=0.070 7</td>
<td>R=0.44 R=0.05 9</td>
<td>R=0.2</td>
<td>R=0.0 2</td>
<td>R=0.3 4</td>
<td>R=0.3 4</td>
</tr>
</tbody>
</table>

D) PET-positive MRI-positive segments, TBR

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>RSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>RSUV&lt;sub&gt;MEDIAN&lt;/sub&gt;</th>
<th>RSLG</th>
<th>RGSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>RGSUV&lt;sub&gt;MEDIAN&lt;/sub&gt;</th>
<th>RTLG</th>
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</thead>
<tbody>
<tr>
<td>HBI</td>
<td>r=0.43 p=0.04</td>
<td>r=0.40 p=0.06</td>
<td>r=0.26 p=0.2</td>
<td>r=0.45 p=0.03</td>
<td>r=0.53 p=0.01</td>
<td>r=0.39 p=0.0</td>
</tr>
<tr>
<td>CRP</td>
<td>r=0.57 p=0.00</td>
<td>r=0.55 p=0.00</td>
<td>r=0.32 p=0.1</td>
<td>r=0.57 p=0.00</td>
<td>r=0.46 p=0.03</td>
<td>r=0.41 p=0.0</td>
</tr>
<tr>
<td>FC</td>
<td>r=0.47 p=0.03</td>
<td>R=0.39 p=0.085</td>
<td>R=0.2 5</td>
<td>R=0.41 p=0.071 4</td>
<td>R=0.2</td>
<td>P=0.41 p=0.07</td>
</tr>
</tbody>
</table>
2.3.4 LONGITUDINAL ASSESSMENT -- RESPONSIVENESS

2.3.4.1 CLINICO-PATHOLOGICAL –PET ENDPOINT CORRELATIONS

The strongest and most consistent longitudinal correlations were between segmental and global SUV<sub>MAX</sub> and ΔHBI and ΔCRP and both segmental and global ΔSUV<sub>MAX</sub>. These were present but less strong with ΔSUV<sub>M</sub>/ΔGSUV<sub>M</sub>. Similarly to the static relationships outlined above, there was no correlation between ΔHBI and ΔCRP and lesional glycolysis, while ΔFC correlated with none of the PET endpoints. These observations changed little whether values were absolute (Tables 2.5A, 2.5C) or expressed as TBR (Tables 2.5B, 2.5D) or whether all PET+ve segments (Tables 2.5A, 2.5B) or PET+ve / MRI +ve subsets were examined (Tables 2.5C, 2.5D).
Tables 2.6A, 2.6B, 2.6C, 2.6D: Correlations between Δclinico-pathological and ΔPET endpoints (statistical significance highlighted in pink if corresponding snapshot correlation in Table 2.4 was not significant or purple if snapshot correlation was also significant)

A) All PET-positive segments, absolute values

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Δ SUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>Δ SUV&lt;sub&gt;M&lt;/sub&gt;</th>
<th>Δ SLG</th>
<th>Δ GSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>Δ GSUV&lt;sub&gt;M&lt;/sub&gt;</th>
<th>Δ TLG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ HBI</td>
<td>r=0.63</td>
<td>p=0.0</td>
<td>r=0.27</td>
<td>r=0.91</td>
<td>p&lt;0.000</td>
<td>r=0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ CRP</td>
<td>r=0.64</td>
<td>p=0.0</td>
<td>r=0.35</td>
<td>r=0.74</td>
<td>p=0.000</td>
<td>r=0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ FC</td>
<td>r=0.25</td>
<td>p=0.43</td>
<td>r=0.02</td>
<td>r=0.06</td>
<td>p=0.8</td>
<td>r=0.17</td>
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</tbody>
</table>

B) All PET-positive segments, TBR

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Δ RSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>Δ RSUV&lt;sub&gt;M&lt;/sub&gt;</th>
<th>Δ R SLG</th>
<th>Δ RGSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>Δ RGSUV&lt;sub&gt;M&lt;/sub&gt;</th>
<th>Δ RTLG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ HBI</td>
<td>r=0.60</td>
<td>p=0.0</td>
<td>r=0.24</td>
<td>r=0.77</td>
<td>p=0.000</td>
<td>r=0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ CRP</td>
<td>r=0.64</td>
<td>p=0.0</td>
<td>r=0.41</td>
<td>r=0.79</td>
<td>p=0.000</td>
<td>r=0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ FC</td>
<td>r=0.33</td>
<td>p=0.3</td>
<td>r=0.00</td>
<td>r=0.15</td>
<td>p=0.6</td>
<td>r=0.10</td>
</tr>
</tbody>
</table>

C) PET-positive MRI-positive segments, absolute values

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Δ SUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>Δ SUV&lt;sub&gt;M&lt;/sub&gt;</th>
<th>Δ SLG</th>
<th>Δ GSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>Δ GSUV&lt;sub&gt;M&lt;/sub&gt;</th>
<th>Δ TLG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ HBI</td>
<td>r=0.75</td>
<td>p=0.005</td>
<td>r=0.57</td>
<td>r=0.83</td>
<td>p=0.00007</td>
<td>r=0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ CRP</td>
<td>r=0.74</td>
<td>p=0.005</td>
<td>r=0.50</td>
<td>r=0.79</td>
<td>p=0.002</td>
<td>r=0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ FC</td>
<td>r=0.28</td>
<td>p=0.3</td>
<td>r=0.12</td>
<td>r=0.08</td>
<td>p=0.7</td>
<td>r=0.18</td>
</tr>
</tbody>
</table>

D) PET-positive MRI-positive segments, TBR

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Δ RSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>Δ RSUV&lt;sub&gt;M&lt;/sub&gt;</th>
<th>Δ R SLG</th>
<th>Δ RGSUV&lt;sub&gt;MAX&lt;/sub&gt;</th>
<th>Δ RGSUV&lt;sub&gt;M&lt;/sub&gt;</th>
<th>Δ RTLG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ HBI</td>
<td>r=0.66</td>
<td>p=0.0</td>
<td>r=0.64</td>
<td>r=0.65</td>
<td>p=0.0</td>
<td>r=0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ CRP</td>
<td>r=0.71</td>
<td>p=0.00</td>
<td>r=0.58</td>
<td>r=0.74</td>
<td>p=0.00</td>
<td>r=0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ FC</td>
<td>r=0.32</td>
<td>p=0.3</td>
<td>r=0.25</td>
<td>r=0.15</td>
<td>p=0.6</td>
<td>r=0.18</td>
</tr>
</tbody>
</table>
2.3.4.2 PERFORMANCE OF FDG-PET ENDPOINTS IN RESPONDERS VERSUS NON-RESPONDERS

9 of the 13 patients that completed PET scanning before and 3 months after treatment had a clinically demonstrable response, as demonstrated by a $\Delta$HBI ≥ 3. The median (range) $\Delta$HBI in responders was 5 (3.9) and in non-responders was 1(-1.2). While this dichotomy was made on clinical criteria, tracking CRP and FC between the two visits largely corroborates the allocation of patients in each group. CRP was abnormal (>5.0mg/L) in 5/9 responders, and normalized in 4, and in 3/4 non-responders and it increased in 2. FC was abnormal (>100mcg/g) in 7/9 responders and normalized in 5, and in 3/4 non-responders and normalized in none (Table 2.3A)

Differences before and after treatment were statistically significant in all PET endpoints in responders versus non-responders (Table 2.6 A). Shifting the focus to segments which were also MRI +ve did not have a significant impact except for a non-significant result for TLG (Table 2.6B). This was also the case when values were expressed as TBR (results not shown). Figure 2.3 shows characteristic appearances of baseline and follow-up segmental signal in responders and non-responders.
Figure 2.3 (A) Characteristic PET signal in a terminal ileal segment of a non-responder at baseline (top) and follow-up (bottom). (B) Typical PET signal in a descending colon of a responder at baseline (top) and follow-up (bottom)
Table 2.7: Magnitude of change for each endpoint in PET +ve (Table 2.7 A) and PET+ve MRI +ve segments (Table 2.7 B). The number of segments or patients showing a reduction (improvement) of the endpoint in each of the responder and non-responder groups is shown. The mean absolute reduction and a mean % reduction between index and follow-up scan are also given. Finally the p-value from Mann-Whitney test comparisons for each ΔPET endpoint in responders versus non-responders is shown (p-values derived from Figures 2.4 and 2.5)

### A

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>All PET +ve Segments</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ in responders</td>
<td>Δ in Non-responders</td>
</tr>
<tr>
<td></td>
<td>(n=9 patients, 26 segments)</td>
<td>(n=4 patients, 14 segments)</td>
</tr>
<tr>
<td></td>
<td>n improving</td>
<td>Absolute Δ</td>
</tr>
<tr>
<td>ΔSUV(_{\text{MAX}})</td>
<td>22/26</td>
<td>-3.1</td>
</tr>
<tr>
<td>ΔSUV(_{\text{MEAN}})</td>
<td>23/26</td>
<td>-1.0</td>
</tr>
<tr>
<td>ΔSLG</td>
<td>22/26</td>
<td>-80</td>
</tr>
<tr>
<td>ΔGSUV(_{\text{MAX}})</td>
<td>9/9</td>
<td>-3.1</td>
</tr>
<tr>
<td>ΔGSUV(_{\text{MEAN}})</td>
<td>7/9</td>
<td>-0.9</td>
</tr>
<tr>
<td>ΔTLG</td>
<td>8/9</td>
<td>-230</td>
</tr>
</tbody>
</table>

### B

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>All PET +ve MRI +ve segments</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ in responders</td>
<td>Δ in Non-responders</td>
</tr>
<tr>
<td></td>
<td>(n=9 pts, 26 segments)</td>
<td>(n=4 pts, 14 segments)</td>
</tr>
<tr>
<td></td>
<td>n improving</td>
<td>Absolute Δ</td>
</tr>
<tr>
<td>ΔSUV(_{\text{MAX}})</td>
<td>18/20</td>
<td>-3.6</td>
</tr>
<tr>
<td>ΔSUV(_{\text{MEAN}})</td>
<td>18/20</td>
<td>-1.2</td>
</tr>
<tr>
<td>ΔSLG</td>
<td>17/20</td>
<td>-94</td>
</tr>
<tr>
<td>ΔGSUV(_{\text{MAX}})</td>
<td>8/9</td>
<td>-3.2</td>
</tr>
<tr>
<td>ΔGSUV(_{\text{MEAN}})</td>
<td>8/9</td>
<td>-1.1</td>
</tr>
<tr>
<td>ΔTLG</td>
<td>8/9</td>
<td>-210</td>
</tr>
</tbody>
</table>
Figure 2.4: Baseline and follow-up values of all PET endpoints in all PET+ve segments. ΔPET endpoints in responders vs. non-responders: A) SUV\text{MAX}/ GSUV\text{MAX}  B) SUV\text{MEAN}/ GSUV\text{MEAN} and C) SLG/TLG.
C

**SLG in Responders vs Non-Responders**

- Responders
- Non-Responders

**ΔSLG in Responders vs Non-Responders**

- p=0.0004 *

**TLG in Responders vs Non-Responders**

- Responders
- Non-Responders

**ΔTLG in Responders vs Non-Responders**

- p=0.033 *
Figure 2.5: Baseline and follow-up values of all PET endpoints in all PET+ve MRI+ve segments. ΔPET endpoints in responders vs. non-responders: A) $\frac{SUV_{\text{MAX}}}{GSUV_{\text{MAX}}}$ B) $\frac{SUV_{\text{MEAN}}}{GSUV_{\text{MEAN}}}$ and C) $\text{SLG}/\text{TLG}$. 

**A** 

SUV$_{\text{MAX}}$ in Responders vs Non-Responders 

- Responders
- Non-Responders

Global SUV$_{\text{MAX}}$ in Responders vs Non-Responders 

- Responders
- Non-Responders

**B** 

SUV$_{\text{MEAN}}$ in Responders vs Non-Responders 

- Responders
- Non-Responders

GSUV$_{\text{MEAN}}$ in Responders vs Non-Responders 

- Responders
- Non-Responders

ΔSUV$_{\text{MAX}}$ in Responders vs Non-Responders 

- Responders
- Non-Responders

ΔSUV$_{\text{MEAN}}$ in Responders vs Non-Responders 

- Responders
- Non-Responders

ΔGSUV$_{\text{MEAN}}$ in Responders vs Non-Responders 

- Responders
- Non-Responders

* p-values indicate statistical significance.
C

**SLG in Responders vs Non-Responders**

Baseline   Follow-up

**TLG in Responders vs Non-Responders**

Baseline   Follow-up

**ΔSLG in Responders vs Non-Responders**

Responders   Non-Responders

\[ p = 0.006 \]

**ΔTLG in Responders and Non-Responders**

Responders   Non-Responders

\[ p = 0.07 \text{ (ns)} \]
2.3.4.3 RESPONSIVENESS RATIOS

RRG and SES, for each outcome measure are summarised in Table 2.7. Values over 0.80 are considered as indicative of good responsiveness for an evaluative instrument. \(^{162}\)

Table 2.7: Responsiveness ratios for FDG-PET outcome measures

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>RRG</th>
<th>SES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All PET+ve Segs</td>
<td>PET+ve MRI +ve Segs</td>
</tr>
<tr>
<td>SUV(_{\text{MAX}})</td>
<td>0.85</td>
<td>0.95</td>
</tr>
<tr>
<td>SUV(_{\text{MEAN}})</td>
<td>1.14</td>
<td>1.63</td>
</tr>
<tr>
<td>SLG</td>
<td>0.27</td>
<td>0.59</td>
</tr>
<tr>
<td>GSUV(_{\text{MAX}})</td>
<td>1.29</td>
<td>1.20</td>
</tr>
<tr>
<td>GSUV(_{\text{MEAN}})</td>
<td>3.22</td>
<td>2.35</td>
</tr>
<tr>
<td>TLG</td>
<td>0.21</td>
<td>0.49</td>
</tr>
</tbody>
</table>
2.3.5 TEST-RETEST RELIABILITY

5 patients (1 female) with active CD were recruited for this arm of the trial, and 4 completed the two scans within a median of 7 days (range 4-12 days). Demographics and clinico-pathological scores are listed in Table 2.1B. %Variability for each endpoint was measured using segments with a signal focus > LivSUV\text{MEAN}. Results are summarised in Table 2.8.

*Table 2.8: % Variabilities in SEGMENTAL and GLOBAL endpoints of FDG-PET scanning in CD*

<table>
<thead>
<tr>
<th>Segmental Endpoints</th>
<th>(n=20)</th>
<th>Global Endpoints</th>
<th>(n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean %VAR</td>
<td>St. dev</td>
<td>Mean %VAR</td>
</tr>
<tr>
<td>SUV\text{MAX}</td>
<td>20</td>
<td>16.6</td>
<td>GSUV\text{MAX}</td>
</tr>
<tr>
<td>RSUV\text{MAX}</td>
<td>20</td>
<td>15.8</td>
<td>RGSUV\text{MAX}</td>
</tr>
<tr>
<td>SUV\text{MEAN}</td>
<td>9</td>
<td>6.0</td>
<td>GSUV\text{MEAN}</td>
</tr>
<tr>
<td>RSUV\text{MEAN}</td>
<td>9</td>
<td>6.0</td>
<td>RGSUV\text{MEAN}</td>
</tr>
<tr>
<td>Volume</td>
<td>64</td>
<td>6.9</td>
<td>TLG</td>
</tr>
<tr>
<td>SLG</td>
<td>66</td>
<td>54.4</td>
<td>RTLG</td>
</tr>
<tr>
<td>RSLG</td>
<td>67</td>
<td>55.5</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.6: An example of 18F-FDG PET signal in the neo-terminal ileum of patient 5 of the test-retest cohort, receiving baseline (left) and follow-up (right) scans 4 days apart. $SUV_{\text{MAX}}$ and $SUV_{\text{MEAN}}$ were similar between the two scans while Volume (and SLG) was significantly more variable.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV_{MAX}</td>
<td>5.9</td>
<td>4.3</td>
</tr>
<tr>
<td>SUV_{MEAN}</td>
<td>2.6</td>
<td>2.3</td>
</tr>
<tr>
<td>Volume</td>
<td>87 cm$^3$</td>
<td>43 cm$^3$</td>
</tr>
</tbody>
</table>
2.3.6 ORIGIN OF SIGNAL

During our initial analysis, it became apparent that the precise origin of the signal foci in various intestinal segments was difficult to ascertain. Sequentially increasing the signal threshold in fused PET-CT images demonstrated that voxels with the highest SUV values are concentrated in the centre of VOIs, typically representing the intestinal lumen, rather than the peripheries, which represented the intestinal wall. This is illustrated in figure 2.6. It was therefore hypothesised that a proportion of signal in affected segments is not produced by FDG trapped intracellularly in mural cells, rather by diffused FDG inside the bowel lumen.
Figure 2.7: As signal threshold was increased (red circles), the voxels of highest signal (yellow areas) concentrated in the centre of the VOI which overlaid the intestinal lumen. A: Longitudinal section B: Transverse section.

We therefore set out to differentiate the mural and luminal components of the signal in three subjects with recto-sigmoid disease having a scan as part of the pre and post treatment arm of our study. Our hypothesis was that we can eliminate recto-sigmoid luminal radioactivity by means of a phosphate enema, and we can quantify it as a proportion of the total radioactivity of the segment.
After completing the original 30-minute period of PET signal acquisition, the images were reconstructed and qualitatively reviewed for the presence of a signal focus in the recto-sigmoid region. Once this was visually confirmed, and without any quantitative measurements being obtained at that stage, the patient was administered a phosphate enema, and following evacuation of bowel contents, he was asked to return on the scanner. A repeat limited low-dose CT scan of the pelvis, using the parameters described above, followed by an additional 10-minute PET acquisition over a single pelvic bed position was obtained (patient 1). For the latter two patients, we measured directly the radioactivity (RA) in the eliminated bowel contents, instead of performing a repeat scan, and following decay correction, expressed it as a proportion of the original total RA in the recto-sigmoid.

The total radioactivities (RA) in KBq in the whole of the recto-sigmoid in the pre and post enema scans were compared. In addition, in the latter two subjects, the eliminated bowel contents were also placed in the scanner for 10 minutes and their total radioactivity quantified. All measured radioactivities for the post enema scan and bowel contents were decay-corrected to the time of the pre-enema PET acquisition.

Table 2.9: Rectosigmoid radioactivities pre and post PO₄ enema and faecal radioactivities

<table>
<thead>
<tr>
<th>Patient</th>
<th>Rectosigmoid RA (KBq) pre-enema</th>
<th>Rectosigmoid RA (KBq) post-enema</th>
<th>RA (KBq) in bowel contents</th>
<th>% of RA in bowel contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>568</td>
<td>467</td>
<td>Not measured</td>
<td>Estimated 17.8%</td>
</tr>
<tr>
<td>2</td>
<td>989</td>
<td>Not measured</td>
<td>300</td>
<td>30.3</td>
</tr>
<tr>
<td>3</td>
<td>373</td>
<td>Not measured</td>
<td>38.9</td>
<td>10.4</td>
</tr>
</tbody>
</table>
2.4. DISCUSSION

2.4.1 SUMMARY OF FINDINGS

Key results from this study are the following:

- CRP closely correlates with FDG-PET endpoints of intensity (segmental and global $SUV_{\text{MAX}}$ and $SUV_{\text{MEAN}}$) both in the static as well as the longitudinal assessment.
- There was no correlation between faecal calprotectin, a marker of intestinal neutrophil migration, and FDG-PET outcome measures.
- Segmental and Global $SUV_{\text{MAX}}$ and $SUV_{\text{MEAN}}$ are significantly more responsive than SLG and TLG in demonstrating change in patients responding to anti-TNFα within 12 weeks.
- Segmental and Global $SUV_{\text{MAX}}$ and $SUV_{\text{MEAN}}$ are significantly more reliable than SLG and TLG on test-retest assessments in stable patients at short time intervals.
- A significant proportion of intestinal FDG signal appears to originate intra-luminally, rather than trapped intra-cellularly in cells within the bowel wall.
- If the significant presence of luminal FDG is not confirmed in larger studies, then $(G)SUV_{\text{MAX}}$ and $(G)SUV_{\text{MEAN}}$ should be taken forward with larger studies confirming their suitability as early monitoring markers in Crohn’s disease.

2.4.2 DISCUSSION OF FINDINGS

FDG-PET is a non-invasive method of quantifying inflammatory activity both on a segmental as well as a global level in patients with CD, and it has previously shown good correlation with endoscopic and histo-pathological reference standards $^{133-135,138,141,168}$. Little is known however on the technique’s potential to monitor the inflammatory
lesions over time. To my knowledge this is the first prospective study that evaluates comprehensively the entirety of proposed FDG-PET endpoints’ responsiveness and reliability in demonstrating the variations in disease activity.

Twenty-two patients had at least 1 PET scan and simultaneous clinical (HBI) and biochemical markers of severity (CRP and faecal calprotectin). These baseline data were used for correlations of PET with clinico-pathological outcome measures. In the case of segmental PET endpoints it was hypothesised that the most inflamed segment, judged on the basis of its $SUV_{MEAN}$ value, will have the highest contribution towards symptoms (HBI) and CRP and FC rise, so it was used for the correlation.

$SUV_{MAX}$ correlated significantly with CRP, whereas when expressed as a TBR, $RSUV_{MAX}$, the correlation was significant with all three clinico-pathologic markers. Its longitudinal performance was also satisfactory as both responsiveness ratios were consistently above the pre-defined threshold of 0.80. The effect was maintained when PET+ve -MRI+ve segments were examined. This observation was further strengthened by the fact that $\Delta SUV_{MAX}$ in responders was significantly larger than in non-responders. In addition to the significant responsiveness figures, the test-retest %variability of 20% is favourable, especially when considering the significant difference between that figure obtained in the test-retest cohort, with a % change of -32% in responders. $GSUV_{MAX}$, which represents the average $SUV_{MAX}$ in all PET+ve segments performed very similarly to its segmental counterpart both in its static correlations with HBI and CRP, as well as in its longitudinal performance. Importantly all 9 responders had reductions in their $GSUV_{MAX}$ between baseline and follow-up scans (average % change -40%) versus only 1 of 4 non-responders.
It is important to consider that $SUV_{\text{MAX}}$ only reflects activity in a single voxel within the entire segment, and may not be representative of the totality of inflammatory activity. Intuitively therefore, more inclusive endpoints such as $SUV_{\text{MEAN}}$ should offer more confidence that a true picture of the whole lesion is being obtained. Despite this theoretical limitation, $SUV_{\text{MAX}}$ has had an excellent track record of providing valuable diagnostic as well as prognostic information in oncological practice \(^{122}\), and it is certainly biologically plausible that this will also be the case in inflammatory monitoring as a reduction in the peak of metabolic activity suggests a decrease in the degree of inflammatory cell activation.

$SUV_{\text{MEAN}}$ and $GSUV_{\text{MEAN}}$, similarly to $SUV_{\text{MAX}}$, were also shown to be responsive outcome measures. In addition, they demonstrated good correlation to CRP, and, when expressed as TBR, also to HBI. Highly significant differences were shown in $\Delta SUV_{\text{MEAN}}$, as well as $\Delta GSVU_{\text{MEAN}}$, between responders and non-responders. Moreover, the within-patient test-retest % variabilities were very low at 9% and 2%, indicating excellent reliability of the measure.

The above results on $(G)SUV_{\text{MAX}}$ and $(G)SUV_{\text{MEAN}}$ are in contrast with the performance of SLG and TLG as monitors of CD activity: The snapshot assessment in all 22 patients revealed a modest correlation between TLG only and clinico-pathological parameters. In the majority, these were not maintained in the longitudinal assessment where there was a profound lack of correlation between the evolution of CRP and FC compared to SLG/TLG over time. The $\Delta SLG$ were statistically significant between responders and non-responders, whereas $\Delta TLGs$ were only different when all PET+ve segments were assessed. Moreover, the responsiveness markers for these two endpoints were low. On test-retest analysis, the volume of signal in each segment and, as a consequence, SLG and TLG had much more pronounced variabilities of 66% and 51% respectively. The
thorough standardization of scanning parameters, bowel distention protocols and the significantly better performance of SUV\textsubscript{MAX} and SUV\textsubscript{MEAN} in that assessment suggest that this was not due to methodological factors that can be improved in obvious ways. One explanation is that Crohn’s lesions are more dynamic than originally believed, with the cellular inflammatory influx changing significantly even within days. If these cells have a relatively uniform glucose metabolism, and therefore FDG uptake, this would explain the lower variability in SUV\textsubscript{MEAN} between the two assessments as well as the large variability in the volume of segmental signal. An alternative reason for this phenomenon could be an intra-luminal ‘escape’ of a proportion of FDG, variable from visit to visit. This is less probable as a dilution of the signal resulting from FDG escape into free luminal space, would also have an effect on SUV\textsubscript{MEAN} values, which was not observed. Until these variations are better understood, given the poor reliability of these two endpoints as demonstrated by the test-retest component of the analysis, these results do not support clinical applications of volume-dependent PET endpoints in the quantification and monitoring of CD activity.

The existing evidence base also concurs with the conclusion that SLG and TLG are not likely to be suitable assessment tools in CD. SLG was first introduced by Jacene et al. who attempted to correlate several FGD-PET endpoints obtained pre-operatively with histopathological scores of inflammation in the resected intestinal specimen in a cohort of 12 CD patients. That group also pointed out the large inter-subject variability of SLG and its lack of correlation to lesional inflammatory scores \textsuperscript{168}. In a more recent paper, Saboury et al. also assessed regional and global lesion glycolysis and demonstrated modest correlations between the latter and CDAI as well as FC, but not with CRP or more robust endoscopic scores of severity \textsuperscript{141}. 
The demonstration of an intra-luminal component of the signal is potentially important in interpretations of bowel FDG-PET. There are at least two hypotheses to explain this finding. It is already known that epithelial tight junctions are disturbed in active inflammatory bowel disease lesions. While this has been studied more in the context of bacterial translocation and immune sensitisation, barrier impairment could also result in intra-luminal FDG escape from the extracellular space. The impact for quantitative interpretation arises despite the fact that this would be a direct consequence of the disease process. This is because the luminal FDG does not represent an increased metabolic activity, which underpins the use of the technique in inflammatory imaging, and thus it may reduce the accuracy of quantification. An alternative explanation for the intra-luminal component could be the shedding of mucosal intestinal cells occurring in the interval between FDG administration and image acquisition.

Moreover, while studies on murine models of IBD are suggestive that a very significant component of the signal is produced by inflammatory cell lineages in affected segments, similar studies in humans are still lacking. A potential direct way of testing this in humans would be to administer FDG immediately pre-operatively in subjects undergoing intestinal resection, and then performing a combination of micro-autoradiography and immunohistochemistry in several sections of the resected specimen.

2.4.3. METHODOLOGICAL CONSIDERATIONS

As discussed extensively in the introduction to this thesis, selecting suitable reference comparators to correlate to FDG-PET was a challenging process, as is the case with most studies assessing novel monitoring markers in CD. HBI CRP and FC all have favourable
profiles in demonstrating active disease, but none can be considered as gold-standard in the context of this study, due to a combination of suboptimal sensitivities and specificities, and lack of validation as monitoring tools. Quantitative MaRIA scores and CDEIS analysis are significantly more robust endpoints to use for this purpose, and recent studies suggest that the two are closely correlated. As MRI was routinely performed in patients in this cohort, it was decided to use MaRIA scores as a determinant of normal versus abnormal segments. Encouragingly, a large study attributing a similar role to MaRIA has since been published: Hordonneau et al. compared diffusion weighting in 848 bowel segments from 130 CD patients defining normal versus active segments on the basis of their MaRIA score.

The methodology around the use of reference standard had some limitations. MaRIA was solely used as a surrogate for endoscopy to differentiate ‘true’ from ‘false’ PET-positive segments on the baseline scan. At the time of study design, MaRIA had not been validated as a measure of treatment response so it was not used as a way of differentiating responders from non-responders in the relevant analysis. Moreover, even though MaRIA is expressed as a continuous variable, in my view it behaves more like a categorical one, with distinct clustering of the values around 4-5 in the absence of disease, 11-13 in diseased but non-ulcerated segments and 23-30 in segments with visible ulcerations. For that reason, a direct correlation between quantitative PET endpoints and MaRIA scores in each segment was not deemed appropriate. For lack of an alternative gold-standard monitoring measure it was decided that the most appropriate way of making that distinction was on the basis of a clinically meaningful response, i.e. an HBI drop of 3 or more between the baseline and follow-up visit as defined in the methods of large clinical trials.
When patients are introduced to anti-TNF biologics, this is described to them as a novel, potent and, in most countries, end-of-the-line therapy. Moreover, due to cost limitations, only primary responders are entered into a maintenance phase of therapy. This is a potential, but non-circumventable source of bias in this study, as it is likely to have led to an over-estimation or over-reporting of the therapy’s symptomatic benefit by participants, resulting in larger ΔHBI between pre and post-therapy assessments. Inevitably, this would unduly class more of them as responders. Had a more objective measure of treatment response been available, the measured differences in PET endpoints between responders and non-responders may have been even more pronounced.

The main aim of this study was not to assess FDG-PET scanning exclusively in the context of monitoring the effects of anti-TNF therapy, rather to assess the responsiveness of the test in reflecting disease modification at ‘per patient’ and segmental level. A closer look on the proposed mode of action of anti-TNF agents provide rationale why the particular patient group was selected at the particular time-point. TNFα suppression is not the only effector mechanism of anti-TNFα monoclonals, as evidenced by lack of efficacy of anti-TNF agents directed against soluble TNFα (e.g. etanercept) 178,179. The likely predominant mechanism of action is the induction of apoptosis of lamina propria T-lymphocytes 180 and monocytes 181 via binding of membrane-bound TNFα (mTNFα), antibody-dependent cell-mediated cytotoxicity, and complement- dependent cytotoxicity 182. Regulatory macrophages have also been demonstrated to contribute to mucosal healing in vitro and in vivo. These cells have been shown to inhibit proliferation of activated T cells, produce IL-10, and express the regulatory macrophage marker CD206 183,184. It was presumed therefore that performing the scanning around a period of these major cellular events, which should occur preferentially in responding patients, would give us the highest chance of
demonstrating measurable signal changes both in individual segments as well as globally in each patient.

Determining a working level of FDG signal to define the threshold for PET+ve segments was an additional significant challenge in the design. One option that was entertained was to not use a threshold and to obtain measurements from all segments in all participants. However, a degree of FDG accumulation in the intestine, and in particular the colon, is very frequent and not always pathological. Several studies in the literature have demonstrated the phenomenon of physiological ("artefactual") FDG accumulation in the gut. A number of mechanisms have been proposed including uptake by intestinal smooth muscle, the swallowing of saliva and uptake by glucose-metabolising microbial flora. In addition, the dense population of lymphocytes in the caecum and ascending colon may also justify some of the uptake in these regions \(^{185}\), and in this cohort these segments were certainly the ones with the highest 'false positive' rates (Figure 2.2). While the estimation of segmental SUV\(_{\text{MAX}}\) in each segment would have been possible, as it represents the single voxel of maximal signal, any further measurements such as determination of SUV\(_{\text{MEAN}}\) without a thresholding activity to distinguish physiological from pathological signal would have been practically impossible. I therefore examined the literature for cut-offs used by other groups. One study defined abnormal segments as those with an intestine-to-liver signal of >1.2 \(^{186}\), while the most widely employed strategy, which I also used, was to quantify signal that appeared equal to or larger than the liver \(^{136,137,138}\).

Radiation burden to participants, as well as funding and time constraints, were the main reasons that dictated optimisation of participant numbers in each of the two principal arms of the study. As the test-retest variability of most of the PET endpoints had been predicted to be low in stable active patients, and considering the fact that each patient

99
can contribute data on up to seven bowel segments, it was determined that a small number of 5 patients would be sufficient for this arm of the study.

In determining a suitable target number of patients to be recruited for the longitudinal arm of the study several factors were considered. Firstly, this was a pilot observational study with numbers of subjects determined by feasibility. Within our Trust, approximately 25-30 patients are commenced on anti-TNF therapy for Crohn's Disease each year. Assuming a similar prescription pattern by our specialists during the recruitment period, and an uptake rate in the region of 50%, I estimated to recruit 20 patients over 18 months.

As FDG-PET scanning is not performed routinely for IBD, there was no alternative to prospectively recruiting subjects. Target intervals between scans were decided on appropriateness relevant to the indication. Cellular composition, and therefore metabolic activity, was expected to remain largely unchanged within an interval of 1 week in patients with chronic active disease who have had no changes to their therapy. For the longitudinal cohort a period of 3 months was selected, as by that time the patients have been established on therapy past the loading phase, and any effect at the cellular level within the lesions will have materialised and, furthermore, it is a reasonable juncture at which to make a differentiation between responders and non-responders using clinical criteria.

There were several reasons why I decided against the use of ileo-colonoscopy as an additional assessment of activity and response. Firstly, the technique only provides information on mucosal activity, whereas PET can measure signal through each segments wall (and perhaps even the lumen). Moreover, it was decided, that proposing to perform ileo-colonoscopies on both occasions, in addition to FDG-PET and MRI was
going to act as a deterrent, having a critical negative impact on recruitment rates, and, more importantly, it would cause considerably more inconvenience in patients with an already significant disease burden so it was not included in this study.

The selected dose of 185MBq was selected as the lowest dose of FDG through which meaningful data were obtained in a CD study 187. Optimisation of radiation exposure was the reasoning behind the use of a low-dose CT for attenuation correction and segment localization, instead of a full-dose CT enterography protocol. If FDG-PET scanning does find a clinical niche in the monitoring of CD patients, then more research will be required in an attempt to further reduce the FDG dosage. There is also a suggestion that the CT tube current can be reduced to 10mA in follow-up studies without compromising detection, and while this seems plausible it is not as yet supported by data 188.

This study has several strengths. It is the first study that focuses on the responsiveness and reliability of FDG-PET for the purposes of monitoring inflammatory activity in patients with Crohn's disease. Moreover, it is the first attempt to systematically dissect the origin of the signal in each segment. Patient preparation, scanning procedures and scanning intervals were meticulously standardised. In addition, a comprehensive range of FDG-PET endpoints proposed in the literature on the modality's use in inflammatory imaging was evaluated.

The longitudinal data on a cohort of 13 patients completing the pre and post-treatment scans of my protocol would benefit from external validation in a larger group of patients. Each individual endpoint’s performance can be further probed by ROC analyses in larger cohorts, which can determine sensitivity and specificity values in demonstrating a response. In addition, a longer follow-up and accumulation of
additional data after the follow-up scan could help establish the potential of these endpoints in predicting longer term therapeutic outcomes.

2.5. CONCLUSION

This study confirms good sensitivity and specificity for $^{18}$F-FDG-PET in distinguishing inflamed from normal bowel segments compared to a recently validated MRI index used as gold standard. A thorough assessment of all outcome measures proposed in the literature demonstrated a superiority of (G)SUV$_{\text{MAX}}$ and (G)SUV$_{\text{MEAN}}$ compared to endpoints that depend on the volume of the signal (SLG and TLG) in terms of test-retest reliability, static and longitudinal correlation with clinico-pathological outcome measures and longitudinal responsiveness. More specifically in the context of responsiveness, highly significant differences in most parameters of the FDG signal were demonstrated between responders and non-responders.

However, the finding that a sizeable component of the signal originates in luminal contents is significant and merits further exploration as it can impact on the face validity of the technique as a molecular probe of activated inflammatory cells.

Potential roles of FDG-PET in the assessment of inflammatory activity of Crohn’s disease are further discussed in Chapter 5.
3. MAGNETIC RESONANCE IMAGING OUTCOME MEASURES IN THE MONITORING OF INFLAMMATORY ACTIVITY IN CROHN’S DISEASE

3.1. INTRODUCTION

Magnetic Resonance Imaging (MRI) is an imaging modality that utilises the electromagnetic properties of protons within tissue. Protons are first stimulated by a constant strong magnetic field (B0), which causes them to align with that field. Their processing frequency is proportional with the strength of that field. Subsequently, radiofrequency pulses are emitted in a transverse direction to the original field, disrupting protons accordingly. Once these pulses are removed two relaxation times are described: $T_1$ measures the time taken for $2/3$ of protons to revert to the natural longitudinal direction of B0, whereas $T_2$ measures the time taken for the transverse direction to decay in $2/3$ of protons. $T_1$-weighted imaging is used for the differentiation of anatomical structures on the basis of $T_1$ values; i.e. the scanning parameters are set to minimize $T_2$ relaxation effects. Tissues with high fat content (e.g. white matter) appear bright and compartments filled with water (e.g. CSF) appear dark. This is good for demonstrating anatomy. $T_2$-weighted imaging, on the other hand, is used to differentiate structures on the basis of $T_2$ values. Water-filled compartments appear bright and fat-rich tissue appears dark. $T_2$-weighted imaging is useful for demonstrating pathology since most lesions are associated with an increase in water content.\textsuperscript{189}

The applications of MRI in CD started being explored over two decades ago\textsuperscript{67,190}, and research has since been carried out, providing evidence that the sensitivity and specificity of the modality are superior to more traditional imaging techniques\textsuperscript{191}. Subsequently, important morphological characteristics were identified and grouped into quantitative scores (e.g. MaRIA, MEGS) which show good correlations with endoscopic
and biochemical indices of severity at a single time-point \textsuperscript{71-75,192}. In addition, other functional MRI sequences believed to act as surrogates of the inflammatory load, such as small intestinal motility,\textsuperscript{82,83} DCE MRI \textsuperscript{88-92,97,99} and DWI \textsuperscript{105-108,193,194} have shown similar results in the same context.

The aims of this study were:

1) To confirm the correlation of the above anatomical and functional quantitative MRI scores with clinical and biochemical markers of disease

2) To assess MRI's reliability and responsiveness in monitoring inflammatory load in CD

The hypotheses made for this study were the following: Firstly, it was hypothesised that active CD lesions in patients without changes in their symptoms or treatment regime would produce reproducible MRI scores in test-retest scanning over a short period of time. This is because parameters which make up such scores including mucosal ulceration, tissue oedema, bowel wall thickness and the localised perfusion dynamics are not expected to change significantly in stable lesions over a period of 1 week.

Similarly, it was hypothesised that patients who respond clinically to anti-TNFα will have demonstrable changes in their MRI scores over a period of three months compared to primary non-responders. While the potential of such improvement has been demonstrated for mucosal disease\textsuperscript{60}, the natural history of deeper tissue response to anti-TNF therapy has not been elucidated.
3.2. METHODS

3.2.1 STUDY DESIGN - PARTICIPANT SELECTION

MRI scans was performed on the same patient cohort recruited for the PET study (see Table 2.3 for demographics). The MRI scan was performed on the same visit as the PET on all occasions. Patients that satisfied the inclusion criteria for that study were automatically eligible also to participate in the MRI study. Exceptions were patients with severe liver or kidney disease, documented allergic reactions to gadolinium, and the presence of metallic implants that were not MRI compatible.

3.2.2 SCANNING PROCEDURE

MRI scanning took place either immediately before or after the PET scan. Patients were asked to ingest between 800-1200ml of 2.5% mannitol, before the first scan, and an additional 400-1000ml prior to the second scan according to tolerance. Subsequently, patients were positioned supine on a Siemens Verio 3T Scanner. Two surface receiver coils were placed over the abdomen and pelvis. Acquisition sequences are summarised in Table 3.1.
Table 3.1: MRI acquisition parameters

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Plane</th>
<th>FOV (mm)</th>
<th>Resolution (mm)</th>
<th>TE (ms)</th>
<th>TR (ms)</th>
<th>Flip Angle (°)</th>
<th>Slices</th>
<th>Duration (s / BHs)</th>
<th>iPAT</th>
<th>Additional Info</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrueFISP</td>
<td>Coronal</td>
<td>400</td>
<td>1.8x1.7x5</td>
<td>1.32</td>
<td>669</td>
<td>60</td>
<td>36</td>
<td>48 / 3</td>
<td>1</td>
<td>2 NEX</td>
</tr>
<tr>
<td>TrueFISP</td>
<td>Coronal</td>
<td>400</td>
<td>3.1x2.5x5</td>
<td>1.4</td>
<td>242</td>
<td>60</td>
<td>12</td>
<td>57 / 3</td>
<td>2</td>
<td>20 CINE frames</td>
</tr>
<tr>
<td>HASTE</td>
<td>Coronal</td>
<td>380</td>
<td>2.1x1.5x4</td>
<td>80</td>
<td>1200</td>
<td>-</td>
<td>4</td>
<td>41 / 2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>HASTE</td>
<td>Coronal</td>
<td>380</td>
<td>2.1x1.5x4</td>
<td>80</td>
<td>1200</td>
<td>-</td>
<td>4</td>
<td>41 / 2</td>
<td>3</td>
<td>SPAIR</td>
</tr>
<tr>
<td>HASTE</td>
<td>Axial</td>
<td>380</td>
<td>1.5x1.5x4</td>
<td>80</td>
<td>1100</td>
<td>-</td>
<td>69</td>
<td>76 / 4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>HASTE</td>
<td>Axial</td>
<td>380</td>
<td>1.5x1.5x4</td>
<td>80</td>
<td>1100</td>
<td>-</td>
<td>69</td>
<td>76 / 4</td>
<td>2</td>
<td>SPAIR</td>
</tr>
<tr>
<td>DW-EPI</td>
<td>Axial</td>
<td>350</td>
<td>3.4x2.7x5</td>
<td>71</td>
<td>1120</td>
<td>-</td>
<td>50</td>
<td>5:36 / free breathing</td>
<td>2</td>
<td>3 NEX, 3 Diffusion Directions, b=50,400,800 mm²/s</td>
</tr>
<tr>
<td>3D VIBE</td>
<td>Coronal</td>
<td>420</td>
<td>1.9x1.9x4</td>
<td>1.3</td>
<td>3.0</td>
<td>10</td>
<td>60</td>
<td>14 BHs</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

DCE oblique 3D spoiled gradient echo (VIBE) images were acquired before and at 11 time-points across 6 minutes after IV administration of a single-dose (0.1 mmol/kg) Gd-chelate (Dotarem, Guerbet). Patients received a total of 30 mg of intravenous hyoscine butylbromide in two doses during each scan to reduce motion artifact.
3.2.3 MRI SCAN ANALYSIS

Analysis of the MRI scans was performed using open-source image-viewing and post-processing software (OsiriX 32-bit, v5.6; Pixeo, Bernex, Switzerland). Initial analysis and ROI placement was performed by the author but all regions of interest (ROIs) and score calculation were corroborated by one of two radiologists with experience in abdominal MR imaging, who were blinded to clinical and PET data.

3.2.3.1 Magnetic Resonance Index of Activity (MaRIA)

The MaRIA score was calculated as previously described \(^74,75\): The bowel was divided into six segments (terminal ileum, ascending, transverse, descending and sigmoid colon and rectum). The following parameters were recorded for each segment: bowel wall thickness (in mm), the presence of mural oedema (defined as hyperintensity of the bowel wall in comparison to the psoas muscle on T2 sequences), and the presence of ulceration (defined as deep depressions of the mucosal surface). In addition, wall signal intensity (WSI) within ROIs in areas of maximal wall thickness before and 70 seconds after intravenous gadolinium was measured. Relative contrast enhancement (RCE) was then calculated as follows:

\[
RCE = \frac{(WSI_{POST-GADOLINIUM} - WSI_{PRE-GADOLINIUM})}{WSI_{PRE-GADOLINIUM}} \times 100 \times \left(\frac{SD_{NOISE\ PRE-GADOLINIUM}}{SD_{NOISE\ POST-GADOLINIUM}}\right)
\]

where SD noise pre-gadolinium corresponds to the average of three standard deviations of the signal intensity measured outside of the body before gadolinium injection, and SD noise post gadolinium corresponds to the standard deviation of the same noise after gadolinium administration. Finally, the segmental MaRIA score was calculated using the following formula:

\[
MaRIA_s = 1.5 \times \text{wall thickness} + 0.02 \times \text{RCE} + 5 \times \text{oedema} + 10 \times \text{ulcers}
\]
The global MaRIA_g score was the sum of all MaRIA_s in each patient.

3.2.3.2 Magnetic Enterography Global Score (MEGS)

To calculate MEGS score, the bowel was divided into nine segments (jejunum, ileum, terminal ileum, caecum, ascending, transverse, descending, sigmoid colon and rectum). The following MR features were scored from 0-3, as defined in the original paper: wall thickness, mural and peri-mural T2 signal, post contrast T1 enhancement level, contrast enhancement pattern (homogeneous, mucosal or layered) and loss of colonic haustrations. The total length of disease in each segment was measured using electronic calipers and was used as a multiplication factor for each individual segment score (x1 if <5cm, x 1.5 if between 5 and 15cm, x2 if >15cm). Segmental scores were summed and then 5 points were added for each of lymph nodes (>1cm), abscesses, comb sign and fistulae respectively.

3.2.3.3 Diffusion MRI

Diffusion sequences were first assessed visually and the region of highest signal in each intestinal segment was identified. ADCs were calculated twice for the 23 segments in patients participating in the test-retest arm of the study. An ROI was placed on the bowel wall of relevant segments using b=800s/mm² sequence and then it was automatically propagated through to the b=50s/mm² and b=400s/mm² acquisitions. Occasionally, manual adjustment was required to correct for motion, in order for the ROI to be placed on the exact same part of wall in the latter two acquisitions. Two analysis approaches were explored: In the first, the ROI was 1-pixel wide, and positioned manually over the wall layer with the highest signal intensity within the bowel wall. Effort was made so that the ROI included only intestinal wall tissue, specifically aiming to exclude the lumen. The second set of measurements used larger
ROIs, ranging between 1 and 3 cm$^2$ in surface area, as described previously $^{105,108,194,195}$.

The two types of ROI are illustrated in Figure 3.1

*Figure 3.1: Examples of pixel-sized (top, green) versus larger ROIs (bottom, red) in the same segment*

As normal segments were frequently not producing any signal on the b=800 s/mm$^2$ image, ROI positioning on such segments was frequently not possible as the signal was too weak to discern any anatomical features. Therefore on some occasions (18/117) these segments had to be excluded from the analysis. Following analysis of the two alternative approaches (see results) the former approach was used for the remainder of the cohort.

ADC was subsequently calculated using the ROI’s mean SI for each of the three acquisitions, using a simple mono-exponential model:

$$S(b)/S_0 = \exp(-bx_{ADC})$$
3.2.3.4 Dynamic Contrast-Enhanced (DCE) MRI

For DCE analysis, ROIs of at least one pixel in size were drawn on intestinal wall in all 6 segments used for MaRIA scoring, as well as in small bowel proximal to TI when this was thickened and abnormal. One previous study took a methodologically different approach, where larger VOIs covering the entirety of each segment within the sequence\textsuperscript{196}. Replicating this method in a few segments produced significantly lower mean Slope of Enhancement (SoE), Maximal Enhancement (ME) and Area Under the enhancement Curve at 300 seconds (AUC300) as the lumen of the segments as well as peri-enteric and mesenteric structures were also included, so this technique was abandoned.

These ROIs were propagated across all 14 time-points (3 prior to and 11 post-iv contrast) and manually adjusted to correct for motion between each sequence. Mean signal intensity (SI) in each time point was used to plot a time intensity curve (TIC). The derived curve parameters were calculated, using MatLab, (The MathWorks, Inc., Natick, MA) as previously reported:

1) SoE is the gradient of the SI time curve between the last point of baseline SI and the inflexion point following initial rapid enhancement.

2) ME=(SI\textsubscript{PEAK} - SI\textsubscript{BASELINE})/ SI\textsubscript{BASELINE}, where SI\textsubscript{PEAK} is the peak signal intensity and SI\textsubscript{BASELINE} is the baseline signal intensity

3) Area under the TIC up to t=300s post contrast administration,

\[ AUC_{300} = \int_{t=0}^{t=300} (t) \]
3.2.3.5 Motility

Motility data were processed and quantified according to a previously published and validated method. This uses an optic flow registration algorithm to estimate deformation of each frame of a given cine loop from an initial target frame, thereby serving as a measure of motility (since we expect deformation of images acquired in the same anatomical position to be primarily due to motion). Such motion can be quantified by taking the standard deviation of the Jacobian determinant of this deformation (quantified in arbitrary units, AU). To facilitate this analysis, a graphical user interface (GUI) developed using Matlab was used, which both displays the cine loops and permits the user to define which areas of small bowel should be subjected to motility analysis by encompassing them in a free hand ROI. The ROI was drawn to include the bowel wall and lumen but extra-enteric tissues were excluded. This process was repeated for the post-treatment MRE scans. Care was taken to match exactly the position of the ROI to that placed on the pre-treatment images.

3.2.4 STATISTICAL ANALYSIS

Data was analysed using statistical software (Prism 6.0, Graphpad, San Diego, CA, USA). Correlations between clinico-pathological and MRI endpoints were performed using the index scan for all participants, with the Spearman rank coefficient. Subjects in the longitudinal arm of the study were then segregated into responders and non-responders on the basis of a clinically significant response, described by a change in HBI of ≥3. The difference in each endpoint between responders and non-responders in the longitudinal arm was calculated using the Mann-Whitney-Wilcoxon rank sum test. All comparisons were deemed significant at a p-value ≤0.05 and correction for multiple comparisons was not performed. The responsiveness of each marker was then assessed.
by calculating the responsiveness ratio of Gyatt (RRG) and the standardized effect size (SES) as previously described \(^{175}\). Finally, test-retest reliability was expressed as %variability in each outcome measure \(^{176}\).

### 3.2.5 WORKLOAD DISTRIBUTION – PERSONAL INVOLVEMENT

Similar to the PET study, after receiving training on the Osirix software, I performed the MRI scan analysis, and designed all relevant VOIs. These were corroborated by an MRI radiologist at University College Hospital.
3.3. RESULTS

Data on the same 22 patients (14 male) with a mean age of 40 years (range 22-59) who had participated in the PET study were acquired. 17 patients were recruited for the longitudinal arm and 5 for the test-retest reliability component of the study. Of these 13/17 and 3/5 patients respectively completed both the baseline and the follow-up scans. 1 of the 17 patients in in longitudinal arm did not receive intravenous contrast due to a borderline estimated Glomerular Filtration Rate (eGFR), and as such only contributed DWI data.

3.3.1. ANATOMICAL SCORES

3.3.1.1 Magnetic Resonance Index of Activity (MaRIA)

MaRIA was calculated in 117 segments from 21 patients. There were positive correlations between segmental MaRIA<sub>S</sub> (segment with the highest score) and CRP, as well as between global MaRIA<sub>G</sub> and CRP and FC. HBI did not significantly correlate with either the segmental or the global MaRIA scores.

Table 3.2: Spearman rank correlations between MaRIA<sub>S</sub> and MaRIA<sub>G</sub> scores and clinicopathological reference standards in 21 patients

<table>
<thead>
<tr>
<th></th>
<th>HBI</th>
<th>CRP</th>
<th>FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MaRIA&lt;sub&gt;S&lt;/sub&gt;</td>
<td>r=0.41 p=0.07</td>
<td>r=0.52 p=0.01</td>
<td>r=0.22 p=0.36</td>
</tr>
<tr>
<td>MaRIA&lt;sub&gt;G&lt;/sub&gt;</td>
<td>r=0.12 p=0.61</td>
<td>r=0.56 p=0.007</td>
<td>r=0.50 p=0.02</td>
</tr>
</tbody>
</table>

These correlations were not maintained however when the differences in each marker between baseline and follow-up scans were used (Table 3.3).
Table 3.3: Spearman rank correlations between ΔMaRIA$_{S}$ and ΔMaRIA$_{G}$ scores and the difference (Δ) of clinico-pathological reference standards in 13 patients

<table>
<thead>
<tr>
<th></th>
<th>ΔHBI</th>
<th>ΔCRP</th>
<th>ΔFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔMaRIA$_{S}$</td>
<td>r=0.36</td>
<td>r=0.20</td>
<td>r=-0.22</td>
</tr>
<tr>
<td></td>
<td>p=0.22</td>
<td>p=0.50</td>
<td>p=0.50</td>
</tr>
<tr>
<td>ΔMaRIA$_{G}$</td>
<td>r=0.53</td>
<td>r=0.29</td>
<td>r=-0.03</td>
</tr>
<tr>
<td></td>
<td>p=0.06</td>
<td>p=0.34</td>
<td>p=0.92</td>
</tr>
</tbody>
</table>

The difference in MaRIA$_{S}$ and MaRIA$_{G}$ were compared between responders (22 segments in 9 patients) and non-responders (7 segments in 4 patients) and were not statistically significant (Figure 3.2).

Figure 3.2: Baseline and follow-up MaRIA$_{S}$ (top) and MaRIA$_{G}$ (bottom) in responders vs. non-responders. ΔMaRIA$_{S}$ (top) and ΔMaRIA$_{G}$ (bottom) in responders vs. non-responders

Segmental MaRIA in Responders vs Non-Responders

Δ Segmental MaRIA in Responders vs Non-Responders

p=0.12 (ns)

Global MaRIA in Responders vs Non-Responders

Δ Global MaRIA in Responders vs Non-Responders

p=0.38 (ns)
The observed change in MaRIA$_S$ in responders was modest (mean $\Delta$MaRIA$_S$ was 4.9). Oedema, which accounts for 5 points of the total segmental score, was observed in 19 of 22 segments in the baseline scan, and it had only resolved in 2 of these 19 segments. Ulcers, which account for 10 points, was present in 11 segments at baseline in the same group, and had only resolved in 4 of these in the follow-up scanning.

The responsiveness ratios of Gyatt and standardized effect size for MaRIA$_S$ and MaRIA$_G$ and test-retest variabilities are summarised in Table 3.4.

<table>
<thead>
<tr>
<th></th>
<th>RRG</th>
<th>SES</th>
<th>%VAR (STDEV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MaRIA$_S$</td>
<td>0.71</td>
<td>0.87</td>
<td>8.4 (11.4)</td>
</tr>
<tr>
<td>MaRIA$_G$</td>
<td>0.54</td>
<td>0.58</td>
<td>4.7 (3.5)</td>
</tr>
</tbody>
</table>

3.3.1.2 MEGS

Subsequently, the MEGS was calculated in the 21 patients who had received IV contrast. There was a modest correlation between MEGS and CRP ($r=0.37$, $p=0.04$) but not with HBI ($r=-0.01$, $p=0.67$) or FC ($r=0.37$, $p=0.12$). This significant correlation with CRP was not maintained when the differences in the two markers were examined over time.

The longitudinal behaviour of the MEGS score in responders and non-responders was subsequently assessed. The average $\Delta$MEGS in responders was 13.6. The two patients with the most profound drop in HBI between the two scans also had the highest drop in MEGS. Participants without a clinical response had lower changes in their MEGS score within that interval ($\Delta$MEGS 1.4). The $\Delta$MEGS between the two groups did not reach statistical significance (Figure 3.3)
Subsequently, the score’s responsiveness parameters were assessed. The RRG was (0.50) and the SES was (0.80). The test-retest reliability of the score was satisfactory with a mean %VAR of 2.7%.

In order to ascertain the role of individual parameters of MEGS, the % change in each was assessed collectively in all responders (Table 3.5). This suggests that peri-mural and mural oedema, appeared to be the most responsive components of MEGS, while thickness and length of disease were the least responsive.
Table 3.5: Relative %change in all components of the MEGS score in responders of the longitudinal arm

<table>
<thead>
<tr>
<th>MEGS parameter</th>
<th>Σ of scores in baseline scan (responders)</th>
<th>Σ of scores in F/U scan (responders)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness score</td>
<td>48</td>
<td>40</td>
<td>17%</td>
</tr>
<tr>
<td>Mural oedema score</td>
<td>50</td>
<td>33</td>
<td>34%</td>
</tr>
<tr>
<td>Peri-mural oedema</td>
<td>15</td>
<td>8</td>
<td>47%</td>
</tr>
<tr>
<td>score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt; Enhancement score</td>
<td>53</td>
<td>38</td>
<td>28%</td>
</tr>
<tr>
<td>Enhancement pattern score</td>
<td>20</td>
<td>16</td>
<td>20%</td>
</tr>
<tr>
<td>Haustral loss score</td>
<td>13</td>
<td>10</td>
<td>23%</td>
</tr>
<tr>
<td>Length of disease score</td>
<td>39.5</td>
<td>34.5</td>
<td>13%</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>Comb sign</td>
<td>40</td>
<td>30</td>
<td>25%</td>
</tr>
<tr>
<td>Fistulae</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>Abscesses</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
</tr>
</tbody>
</table>

3.3.2. FUNCTIONAL SCORES

3.3.2.1 DWI

There was excellent correlation between the two sets of ADC measurements using pixel-sized and larger ROIs (r=0.89 p<0.0001) (Figure 3.4) and no significant difference between the two groups on Wilcoxon matched pairs sign ranked test (p=0.19). Moreover, the test-retest %variability was lower using pixel-sized ROIs compared to larger ones (21% vs. 27%), so the former method was used for the analysis of ADCs in the longitudinal arm of the study.
There were no significant correlations between the ADC of the most diseased segment and any of the clinico-pathological parameters, either in the static or the longitudinal assessments. Furthermore, there was no statistically significant difference between ΔADC in segments from responders versus non-responders.

Of the 117 bowel segments that had been scored with MaRIA§ ADC analysis was feasible in 99 due to difficulties in creating ROI in 18 normal segments that did not produce any signal in the b=800 s/mm² acquisition (described in the methods). I first compared the ADC values in 53 normal segments (MaRIA§ <7.0) versus 46 abnormal segments (MaRIA§ >7.0). The mean ADC in normal segments was 2.18 ± 0.56 x10⁻³ mm²/s², significantly higher than that in diseased segments (1.60 ±0.49 x10⁻³ mm²/s²) (P<0.0001) (Figure 3.5).

---

**Figure 3.4: Correlation between ADC using pixel-sized versus larger ROIs**

**Figure 3.5: Baseline ADC in normal versus abnormal (MaRIA+ve) intestinal segments**
ROC analysis revealed that an ADC<1.9 mm/s² has 85% sensitivity and 75% specificity in revealing positive segments.

Focusing on the longitudinal arm of the study, there was a significant difference in ΔADC in abnormal segments between responders and non-responders. It is important to note however that in 10 of the 22 analysed segments in responders, there was an incremental drop of ADC (and therefore diffusivity) between the baseline and follow-up scans. This meant that when responders were looked at in isolation, there was no statistical significance between the baseline and follow-up scans (p=0.24). Despite this, the overall ΔADC in non-responders was statistically lower than in responders (Figure 3.6).

*Figure 3.6: Baseline and follow-up ADC in all abnormal (MaRIA+ve) segments in responders and non-responders. ΔADC in responders vs. non-responders*

In keeping with these observations the responsiveness scores for ADC were low with an RRG at 0.51 and SES of 0.52. Finally, the mean %variability of ADC in abnormal segments in the test-retest cohort was 21%.
3.3.2.2 DCE

All 3 DCE endpoints from 115 segments correlated moderately strongly with the corresponding MaRIA₅ (Table 3.6)

Table 3.6: Spearman rank correlation coefficients between MaRIA₅ and DCE endpoints

<table>
<thead>
<tr>
<th>MaRIA₅</th>
<th>SoE</th>
<th>ME</th>
<th>AUC300</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r=0.63</td>
<td>r=0.66</td>
<td>r=0.65</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Moreover, there were statistically significant differences between DCE values of normal (MaRIA₅<7.0) and abnormal segments (MaRIA₅>7.0) illustrated in Figure 3.7.

Figure 3.7: DCE endpoints in normal and abnormal intestinal segments

The correlation between all three endpoints and disease duration (years) was also examined (Table 3.7)

Table 3.7: Correlation between DCE MR endpoints in the most affected segment (highest MaRIA₅ score) and disease duration (years)

<table>
<thead>
<tr>
<th></th>
<th>SoE</th>
<th>ME</th>
<th>AUC300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration</td>
<td>r=0.16</td>
<td>r=0.33</td>
<td>r=0.59</td>
</tr>
<tr>
<td>(years)</td>
<td>p=0.50</td>
<td>p=0.14</td>
<td>p=0.005</td>
</tr>
</tbody>
</table>
Once again, Spearman rank correlation coefficients between HBI, CRP and FC, and DCE endpoints were not significant (table 3.8). Moreover, none of the three studied endpoints changed differently in responders and non-responders over time (results not shown).

Table 3.8: Correlations between DCE MR endpoints in the most diseased segment (highest MaRIA₅ score) and clinico-pathological markers of activity

<table>
<thead>
<tr>
<th></th>
<th>HBI</th>
<th>CRP</th>
<th>FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SoE</td>
<td>( r=0.19 )</td>
<td>( r=0.30 )</td>
<td>( r=-0.03 )</td>
</tr>
<tr>
<td></td>
<td>( p=0.40 )</td>
<td>( p=0.18 )</td>
<td>( p=0.91 )</td>
</tr>
<tr>
<td>ME</td>
<td>( r=0.14 )</td>
<td>( r=0.27 )</td>
<td>( r=0.01 )</td>
</tr>
<tr>
<td></td>
<td>( p=0.55 )</td>
<td>( p=0.23 )</td>
<td>( p=0.98 )</td>
</tr>
<tr>
<td>AUC₃₀₀</td>
<td>( r=-0.14 )</td>
<td>( r=0.35 )</td>
<td>( r=0.28 )</td>
</tr>
<tr>
<td></td>
<td>( p=0.55 )</td>
<td>( p=0.12 )</td>
<td>( p=0.25 )</td>
</tr>
</tbody>
</table>

Analysis of the longitudinal cohort showed no statistically significant difference between responders and non-responders in the absolute difference of the three DCE endpoints (Figure 3.8). Responsiveness and test-retest variability figures for each are shown in Table 3.9.
Figure 3.8: Baseline and follow-up SoE, ME and AUC\textsubscript{300} in MaRIA+ve segments in responders and non-responders. $\Delta$SoE, $\Delta$ME, $\Delta$AUC\textsubscript{300} in responders vs. non-responders

Table 3.9: Responsiveness and test-retest variability for SoE, ME and AUC\textsubscript{300}

<table>
<thead>
<tr>
<th>DCE Endpoint</th>
<th>Responsiveness</th>
<th>Reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRG</td>
<td>SES</td>
</tr>
<tr>
<td>SoE</td>
<td>0.21</td>
<td>0.14</td>
</tr>
<tr>
<td>ME</td>
<td>0.56</td>
<td>0.31</td>
</tr>
<tr>
<td>AUC\textsubscript{300}</td>
<td>0.47</td>
<td>0.59</td>
</tr>
</tbody>
</table>
3.3.2.3 Motility

Small bowel motility quantitation was achieved in 7 of the 9 responders and all 4 non-responders. Differences in motility at baseline versus follow-up in diseased small intestinal segments, in responders only, were statistically significant (Figure 3.9). However, the difference in motility between responders and non-responders did not reach statistical significance (Figure 3.10)

Figure 3.9: Baseline and follow-up motility in diseased small bowel segments in responders

Figure 3.10: Baseline and follow-up motility in responders and non-responders. ΔMotility in responders vs. non-responders

The RRG for motility was just over the accepted threshold at 0.85 while the SES was poor at 0.46. Motility data were not available for the test re-test cohort so %Variability was not calculated.
3.4. DISCUSSION

3.4.1 SUMMARY OF FINDINGS

Key results of this study were the following:

- MaRIA score shows a good static correlation with CRP but △MaRIA does not correlate with any of the clinico-pathological markers of activity.
- MaRIA had a high reproducibility but its responsiveness was lower in this cohort than that described in published literature\textsuperscript{110}.
- Similar to MaRIA, the MEGS score correlates significantly with CRP in a single timepoint but not longitudinally.
- MEGS is a reproducible score, which however shows low responsiveness. Parameters such as oedema show greater change at 3 months compared to others e.g. length of disease in each segment.
- ADC was significantly lower in abnormal versus normal bowel segments. However it did not correlate with any of the clinico-pathological markers of severity.
- ADC has a good reproducibility at test-retest, but a low responsiveness score. This is because half the examined segments in responders demonstrated an unexpected reduction in water diffusivity within the three-month interval.
- DCE endpoints seemed unsuitable tools for longitudinal assessment of disease in this cohort.
- Terminal ileal motility was significantly higher at follow-up in responders.
- A 3-month interval was likely too short for any of the aforementioned MRI techniques to be utilised as monitoring instruments which assess transmural disease process. MaRIA, MEGS and small bowel motility should be tested over longer intervals in future studies.
3.4.2 DISCUSSION OF FINDINGS

Magnetic Resonance Enterography is a non-invasive method which aims to characterise inflammatory activity across the full thickness of affected segments in the bowel wall. Several studies have demonstrated positive correlations between anatomical as well as novel, functional MR endpoints, with endoscopic or histo-pathological reference standards 71–73. With the exception of MaRIA however, the literature on the longitudinal performance of these outcome measures is scarce. In this study, I examined a comprehensive range of quantitative MRI endpoints over time, assessing their potential of acting as monitoring tools in CD.

The findings on the MaRIA score were, overall, not in keeping with those of the Barcelona group that introduced the score. Rimola et al. described a positive correlation between MaRIA\textsubscript{G} and HBI, as well as between MaRIA\textsubscript{G} and CRP in both their derivation as well as their validation cohorts 74,75. In this study, only the latter result was corroborated. However, I identified a positive correlation between MaRIA\textsubscript{G} and faecal calprotectin, which had not been assessed in the group’s seminal papers. Validation of this observation through a larger study will be required.

In the longitudinal arm of the study I assessed the change over time of both MaRIA\textsubscript{S} in all diseased segments in the baseline scan, as well as the MaRIA\textsubscript{G} in each participant, comparing responders with non-responders. While there was a trend towards a larger decrease in MaRIA\textsubscript{S} between index and follow-up scans in responders compared to non-responders, this did not reach statistical significance (p=0.12). The observed change of MaRIA\textsubscript{S} in responders was modest (average absolute $\Delta$MaRIA\textsubscript{S} was 4.9) with the two major components, namely oedema and mucosal ulceration resolving only in a minority of segments, even in the responder group. This observation was in contrast to the
Barcelona group’s longitudinal assessment of the MaRIA score who reported a mean ΔMaRIAS in responders of 10.1. In order to put these interval differences into context, it is important to refer to the formula for the calculation of the segmental MaRIA score: The presence of any ulceration has the highest weighting (10 points) and the presence of oedema is awarded half of this weight (5 points). These are assessed in a binary fashion. The remaining two parameters, namely bowel wall thickness and RCE are continuous variables, and differences in these between the two scans contributed far less to the observed effect.

The persistence of oedema and ulceration in participants with a clinical response is likely responsible for the responsiveness scores (RRG of 0.5, SES of 0.6) which are considerably more modest than the ones described by the Barcelona study (1.10 and 1.72 respectively). Test-retest variability measures in this cohort show an excellent reliability profile both for the segmental as well as the global MaRIA score.

There were several methodological differences in the power and design of the two studies, which could potentially account for the differences in the measured effects. The original MaRIA score, as proposed and executed by the Barcelona group, involves rectal instillation of luminal contrast for colonic distension in addition to oral contrast for small bowel distension. This has implications for the general acceptability of the test to patients. Hence other groups, including ours, have used MaRIA without rectal instillation of contrast in order to improve the tolerability of the test. However, it is possible that suboptimal distension of a colonic segment can give rise to mucosal invaginations in an otherwise healed mucosa, which can be misinterpreted as ulcers and in turn produce an over-estimation of the MaRIA score in a given segment. I concentrated on terminal ilea of the responders, the distension of which should not be affected by rectal contrast, and found that 7 of the 9 had ulcerations at baseline, and 3 of
these 7 had resolved at follow-up. This further strengthens the hypothesis that colonic segments may have been 'over-read' as ulcerated, as it demonstrates that the three-month scanning interval is adequate for a substantial proportion of ulcers to have healed. Moreover, in their responsiveness analysis, Ordas et al. only examine segments with endoscopic ulceration at baseline, while I included all segments with a baseline MaRIA <sub>S</sub> >7.0. This is because the Barcelona group aimed to assess the test as a surrogate examination for endoscopic mucosal healing, whereas my standpoint was one of a more inclusive analysis of all endpoints that assess the totality of intestinal damage. Finally, the Barcelona study was significantly larger with 111 ulcerated segments analysed, compared to 29 in this study, which may justify the lack of statistical significance between responders and non-responders as well as the modest responsiveness scores.

The MEGS score (the other anatomical score of disease activity) showed a weak but significant correlation with CRP, whereas the correlation with HBI and FC did not reach statistical significance. The latter was the reference standard in the score's derivation study, where a positive and significant correlation was reported. There was a definite observed trend towards a higher drop in MEGS score in responders versus non-responders, which did not however reach statistical significance. As the number of non-responders in this cohort was quite limited (n=4), the fact that only a minority of global endpoints reached statistical significance was probably anticipated.

The responsiveness markers of MEGS were equally modest to the MaRIA <sub>c</sub> despite the fact that ulceration does not feature as a parameter on the MEGS score. A breakdown of the various constituents of MEGS and their %change in responders versus non-responders (table 3.5) reveals which of these are responsive and which are more rigid to change, at least within the three-month interval. Mural and peri-mural oedema appeared to be the most responsive endpoints. These are evaluated by the intensity of
T₂ signal inside the bowel wall and peri-mural tissue respectively, in comparison to the corresponding signal inside the psoas muscle. The higher the T₂ signal in these compartments, the greater the quantity of interstitial fluid within them. This is certainly biologically plausible as excess interstitial fluid is a central feature in both acute and chronic inflammation which reduces readily as the latter improves 198. Bowel wall thickness and length of disease were the least responsive components of the MEGS score. If we refer to the original studies validating MRE endpoints against histopathology 71–73, we see that bowel wall thickness correlates both with AIS as well as fibrostenosis, and that the two most commonly co-exist within the same segments. Whilst the inflammatory infiltrate is expected to reduce upon introduction of anti-TNF therapy, the fibrostenotic element remains unaffected and therefore a modest response of thickness scores to therapy is not surprising. Through the MEGS score a very low % reduction in overall disease length can be observed. To understand this, it is important to consider that full resolution of all the mucosal, mural and perimural signs had to take place for the lesion to be deemed shorter on the follow-up scan. As it has already been demonstrated in the ACTIF study199, this is a rare occurrence even at 26 weeks following introduction of therapy.

Analysis of DWI sequences also produced some interesting results. ADC values were significantly lower in diseased versus normal bowel segments. These differences would have been even more pronounced if ADC values could have been produced for the 18 normal segments which did not produce a signal at the b=800s/mm², as ADCs in these would have been at the higher end of the spectrum, causing larger separation of the medians of the two groups. Moreover, ROC analysis revealed that an ADC value of 1.9 mm/s² is 85% sensitive and 75% specific in identifying bowel segments with a MaRIA>7.0, and an area under the curve of 0.79. The largest study to date which examines ADC values in CD in a single time-point proposed a similar figure as a
threshold, but the resulting sensitivity and specificity figures were far superior at well over 90% \(^{108}\). As the methodology of image acquisition between this and the Hordonneau study were very similar, this disparity likely arises form the tighter confidence intervals in the much larger French study (848 versus 117 analysed segments).

Despite this, correlation between ADC in the worst affected segment and clinico-pathological parameters were all non-significant. In the longitudinal arm of the study I identified increases in \(\Delta\)ADC in abnormal segments in responders, which reached statistical significance compared to non-responders. There was a high variability in this response, with almost half of the affected segments in responders demonstrating a reduction in diffusivity. As a consequence, the responsiveness markers for this endpoint were modest. The combination of these with an appreciable test-retest variability of 21% render ADC a sub-optimal monitoring tool for inflammatory load, at least for a 12-week interval.

There are several methodological challenges presented by the DWI sequences that merit special mention. Even at a low b-value of 50s/mm\(^2\) the image definition is significantly reduced compared to other T\(_1\) or T\(_2\) sequences. ROI position frequently required the simultaneous use of an anatomical sequence obtained several minutes earlier and hence any motion between the two could not be easily corrected for. Moreover, in poorly distended segments the interface between bowel wall and luminal content can be very hard to determine in DWI images, a problem most commonly encountered in normal segments. For that reason, ADC calculation was obtained in 99 out of the 117 segments that had been scored with MaRIA.
While DWI has attracted an increasing amount of interest in the recent years, current knowledge on the causative factors that underpin the reduction of diffusivity in diseased segments is scarce and can permit at best a speculative discussion. The most commonly proposed element responsible for the reduction in diffusivity is the increased tissue density produced by the cellular influx associated with the inflammatory process. Lymphatic dilatation\textsuperscript{200} and neuronal hypertrophy\textsuperscript{201} occurring at later stages of the pathogenic process can further contribute to the contraction of extra-cellular space. Moreover, the fibrogenic process has also been proposed as a causative agent in the reduction of free water movement, independently of inflammation. In a study of 23 patients with chronic hepatitis and 7 controls, Taouli et al. demonstrated a significant correlation between ADC and fibrotic score\textsuperscript{103}. A similar study in IBD that focuses specifically on the role of fibrosis in ADC reduction has not yet been performed. With such a multifactorial aetio-pathological profile for the reduction of diffusivity in CD-affected gut tissue, the variable behaviour of this outcome measure in responders, non-responders and test-retest cohort was not unanticipated. More work will be required towards the dissection of the relative roles that cellularity, fibrosis and other less important elements of the chronic tissue injury play in ADC behaviour before the marker can be used with any confidence in the monitoring of CD activity.

DCE endpoints seemed the least suitable for longitudinal monitoring of the inflammatory load, at least within my defined interval of three months. The correlations of all three endpoints in each segment with the reference standard, MaRIA\textsubscript{S}, were significant, and moderately strong. This is also demonstrated by significant differences in all three endpoints between normal (MaRIA\textsubscript{S} < 7.0) and diseased (MaRIA\textsubscript{S} >7.0) segments. There was no correlation, however, between any of the DCE endpoints in the most-diseased segment in each patient and HBI, CRP or FC. In addition, a focus on the longitudinal cohort reveals no differences in the evolution of these scores in responders
versus non-responders. This is also reflected in the low responsiveness ratio. Moreover, all of these endpoints showed very high test-retest variabilities.

There are two conclusions that can be drawn from the DCE data. Firstly, all three endpoints are extremely variable and their quantification, at least within pixel-sized ROIs, is prone to a high degree of error. This is the first set of published data on the test-retest reliability of these measures, and despite its limited size, the low reliability is quite convincing. Considering the fact that regional perfusion of a tissue segment is physiologically subject to a wide range of neuro-humoral influences, all of which can be altered within a small period of time, the observed test-retest reliability figures are not surprising.

The second major conclusion is that even though there are undoubted differences in all three endpoints between normal and diseased segments, these do not demonstrate any signs of improvement within the three months of therapy. Referring back to the literature there is evidence that a major determinant of increases in DCE endpoint is disease chronicity93. This was corroborated by a second group, which, at the same time, found no correlation between DCE variables and endoscopic severity 196. I found a highly significant correlation between disease duration and AUC300, a composite measure dependent on both the slope and maximal enhancement. There is limited evidence from the rheumatology literature that anti-TNFα treatment, through the suppression of vascular endothelial growth factor (VGEF) has an effect solely on early immature blood vessels but not on mature established vasculature 202. This may justify the lack of a clear response of DCE endpoints to 3-months of anti-TNFα therapy in the subset of patients demonstrating an obvious benefit from the treatment as judged by clinical and other radiological markers.
Results on small bowel motility were somewhat more encouraging. There seems to be a trend towards improved motility of small bowel in responders. This is reflected in the RRG, which, unlike all other outcome measures, was greater than the threshold of 0.80. It is possible that a moderate increase in the scanning interval may have resulted in statistical significance between the two groups, as was a case in a separate retrospective cohort of 35 patients with a median scanning interval of 55 weeks (p =0.0002) (Plumb A, unpublished data).

Reduction in intestinal motility has been well-documented in CD, though the mechanism underpinning this pathophysiological phenomenon is not well understood. However, active CD is accompanied by inflammatory involvement of the submucosal or myenteric plexuses (i.e. plexitis) which likely disrupts the normal neuronal control of gut motion. The natural history of this process and its potential for improvement following treatment or remission of CD, has not been investigated to date, partly because of the difficulty and inconvenience of taking such measurements using manometry. Available studies both on gastric emptying and intestinal motility offer comparisons between CD patients and controls rather than a longitudinal assessment of patients over time. However, unlike manometry-based techniques, MRE is widely available and better tolerated. Incorporating dynamic 'cine'-sequences in MRE protocols is not a considerable burden, as it requires no contrast and the scanning time is approximately 3-4 minutes. Further studies on the evolution of this marker are feasible and pertinent.

This study has several strengths. It is the first study that focuses on the longitudinal performance of a very comprehensive range of proposed quantitative MR endpoints that measure various aspects of disease activity in CD. The prospective design allowed optimal standardisation processes in patient preparation and MR acquisition protocols.
On the other hand, similarly to the PET study, the cohort of completed pre-and post treatment scan pairs was quite limited, particularly for global or ‘per-patient’ outcome measures. Once again, the choice of MaRIA$\text{S}$ instead of colonoscopy as a reference standard to classify segments into diseased and normal may invite some criticism.

3.5 CONCLUSION

Magnetic Resonance Enterography is becoming increasingly popular tool in the assessment of Crohn’s disease. This is a result of its widening availability, the lack of ionising radiation and an expanding evidence base supporting its potential to evaluate disease activity through measures which are in close correlation with endoscopic and histo-pathological reference standards.

Whilst scores such as MaRIA, MEGS and small bowel motility show good static correlation with disease activity, the longitudinal assessment suggests that a 12-week interval is probably too short even for those more strongly performing endpoints to track changes in disease load across the full thickness of the bowel wall. Larger longitudinal studies over longer follow-up intervals will be required in the future and are discussed further in Chapter 5
4. THE EXPRESSION OF MOLECULAR TARGETS FOR NOVEL PET RADIOLIGANDS IN CROHN'S LESIONS

4.1. INTRODUCTION

In the previous chapters the potential of the most current FDG-PET and MRI methods to quantify and monitor the inflammatory load in Crohn's disease was explored. Some of the investigated endpoints, particularly from PET, show some promise in this context, but several limitations have been identified: Firstly, the presence of a significant amount of luminal FDG activity related to inflamed gut segments suggests that this signal does not reflect exclusively the inflammatory activity in the bowel wall. More importantly, while activated inflammatory cells are responsible for a proportion of FDG activity in tissue, the glycolytic activity in examined bowel segments can increment for a variety of reasons, with increased activity of leiomyocytes as well as relative stasis and bacterial overgrowth being prime examples.

As discussed in the introduction of this thesis, there have been significant advancements in the PET scanning of inflammatory tissue, with a focus on the development of radioligands more specific to the inflammatory pathway. Examples of such receptors that act as targets to novel radioligands are the Translocator Protein (TSPO) and the Interleukin-2 receptor (IL-2R).

The occurrence of TSPO in gut tissues has been assessed by Bribes et al. using an immunohistochemical probe, which showed heterogeneous expression. More specifically, expression seemed weak in the colon, and moderate in the small intestine. Han et al. demonstrated a significant and measurable difference of TSPO expression between malignant colorectal specimens and normal colonic tissue. More recently, Ostuni et al. confirmed the distribution of TSPO in biopsies of normal ileum and colon.
and suggested an over-expression in areas affected by Crohn’s disease and ulcerative colitis. The number of samples was not declared, however, and there was no attempt to quantify this over-abundance.

TSPO in being explored as a target in molecular imaging of inflammation as high levels have been demonstrated in activated macrophages sampled from a diverse range of peripheral inflammatory processes.

First generation TSPO radioligand PK11195 has now been largely superseded by the far more specific PBR28 and others. While these have significantly better signal-to-noise profiles, they also exhibit significant between-subject variability. This has been accounted for by a genetic polymorphism (rs6971) in the gene encoding TSPO. Homozygote high-affinity (HAB) and low-affinity binders (LAB) as well as heterozygote mixed-affinity binders (MAB) have been described, and the dissociation constant (Kd) has been estimated for each of the three phenotypes, at least for TSPO extracted from brain tissue. Genotyping for binding status of subjects has been shown to form an integral part of the clinical scanning process using these ligands.

IL-2 is a pro-inflammatory cytokine produced by activated Th1-cells, which promotes further T-cell proliferation, differentiation of B-cells and NK and macrophage activation. The effects of IL-2 are mediated through binding to the IL-2 receptor (IL-2R), a heterotrimeric receptor comprising of α (CD25), β and γ subunits.

Several studies suggest a key role of IL-2 in the pathophysiology of CD. These show that patients with active Crohn’s disease have elevated serum IL-2 and soluble IL-2R concentrations. Moreover, patients with active disease who respond to cyclosporin and anti-TNFα therapy are more likely to have decreases in serum IL-2 and soluble IL-
2R levels than are treatment-refractory patients\textsuperscript{215,216}. In addition, there have been reports of quiescent Crohn's disease re-activitation shortly after therapeutic administration of IL-2 in two patients with renal cell carcinoma\textsuperscript{217}. More recently, genetic polymorphisms in the region of the II2/IL21 receptor have been linked with increased susceptibility to IBD\textsuperscript{218}. Intestinal regional IL-2R expression has also been investigated. In healthy controls there is very little expression on the lamina propria (LP), however, there is some CD25 detected in Peyer's patches. LP as well as subepithelial segments from CD affected small and large bowel on the other hand, demonstrated abundant CD25\textsuperscript{219}.

Similarly to TSPO, the IL2R has found a role as an imaging target in a variety of conditions underpinned by a lymphocytic infiltrate in tissue. Most studies originate from the same group from the University of Rome, that investigated the receptor as a target for imaging using the SPECT technique. Following two seminal, proof of concept studies in a murine model of diabetes \textsuperscript{220,221}, the group produced a series of papers correlating \textsuperscript{123}I or \textsuperscript{99}Tc–tagged IL2 with clinical and histological markers of activity in a variety of conditions including coeliac disease\textsuperscript{160}, atherosclerosis\textsuperscript{161} and Crohn’s disease\textsuperscript{162,222}.

These studies are very suggestive that TSPO and IL-2R are potentially suitable, receptors for use in inflammatory molecular imaging of Crohn's disease. In the bench-to-bedside pathway of a new radioligand, or an established radioligand proposed for a new indication, several characteristics of its interaction with its target receptor need to be characterised. Key features of this interplay are the specificity of the tracer for its receptor, as well as the binding potential between the two. Specificity refers to the distribution of activity at target versus non-target sites, or, also described as specific
versus non-specific binding. Binding potential is defined by the availability or density of the receptor ($B_{\text{MAX}}$) and the affinity of the ligand towards that receptor ($K_d$)

This study had two main purposes. Firstly, I set out to investigate the differential expression of the two target receptors, TSPO and IL2R in normal versus inflamed segments. Secondly, I wanted to examine such key characteristics of radioligand binding such as the density of available receptors ($B_{\text{MAX}}$) and affinity of radioligand binding ($K_d$) in the inflamed versus normal gut.

The hypotheses underpinning this study were that there is a measurable difference in TSPO and IL2-R expression between inflamed and uninvolved segments in patients with CD. Moreover these differences will translate into increased binding of TSPO and IL-2R specific radiotracers in the relevant segments. Finally, it was hypothesised that the binding affinity of the second-generation TSPO radiotracer, PBR28, in peripheral tissue will be comparable to that described in brain specimens.
4.2 METHODS

4.2.1 PATIENT- SPECIMEN SELECTION

Two separate sources of specimens were used for this work and approval by Research Ethics Committees (REC) was obtained for both (South West London REC 1, Ref No 10/H0801/59 and NRES Committee London-Wandsworth Ref No 11/LO/0380). The first study (immunohistochemical assessments of the two targets) involved the use of stored specimens, whereas the second (autoradiographic and homogenate binding studies) required prospective recruitment of patients undergoing intestinal resection.

For immunohistochemical (IHC) analysis of TSPO and IL-2R expression the histopathology database of St Mary’s hospital was searched and appropriate mucosal biopsies and resection specimens were retrieved. These met the following eligibility criteria:

Inclusion criteria:
1) Specimens from patients collected before September 2006 (for the purposes of compliance with the Human Tissue Act).
2) Specimens from patients over 18 years of age at the time of endoscopy/ surgery.
3) Biopsies/ resection specimens containing normal bowel.
4) Biopsies/ resection specimens containing quiescent Crohn’s disease.
5) Biopsies/ resection specimens containing active Crohn's inflammation.

Exclusion criteria
1) Samples from patients with a diagnosis of gastro-intestinal dysplasia or cancer.
2) Samples derived from patients who meet inclusion criteria, but not adequately preserved or technically suitable for analysis.

For the autoradiography and homogenate binding studies I prospectively recruited adult patients with proven Crohn’s disease who had failed medical therapy, and a decision had been made for intestinal resection to take place. The study was approved by the NIHR portfolio and recruitment took place in 4 hospitals. All participants gave informed consent, authorising the use of 2 tissue sections, one from the resection margin, which appeared disease-free macroscopically at the time of operation, and one from the diseased area in the middle of the resected specimen, which the surgeon deemed abnormal intra-operatively (Figure 4.1). I excluded participants having intestinal resection for reasons other than symptomatic CD. Following the publication on the rs6971 polymorphism ethical permission was sought and the binding status of the latter 5 participants was determined on a blood sample.

*Figure 4.1: Schema representing the sites sampled following intestinal resection*
4.2.2 TISSUE PREPARATION - FREEZING

Stored tissue:
Formalin-fixed paraffin-embedded (FFPE) mucosal biopsies and tissue blocks were used to produce sequential 1μm-thin sections, which were mounted onto glass slides. These were then stored at room temperature until staining. 1 section from each block was stained with Haematoxylin and Eosin (H&E) for histological assessment of inflammatory activity, and the others were stained with IHC probes.

Fresh tissue:
Immediately following surgical resection, the specimen was transferred to the department of histopathology in each participating hospital and two 5mm-thick rings of tissue, one from the disease-free edge, (acting as control) and one from the diseased segment were cut. The two specimens were washed in phosphate-buffered saline (PBS), excess moisture was removed, and were snap-frozen by immersion into a mixture of 2-methylbutane (Sigma Aldrich, Dorset, UK) at circa. -30 to -40°C. Snap-frozen specimens were immediately stored at -80°C until further use.

4.2.3 HISTOLOGICAL ASSESSMENT

Following sectioning and H&E staining, stored formalin-fixed paraffin embedded gut slides were assessed and Global Inflammatory Score (GIS) of each section was calculated168. This consisted of the sum of the acute neutrophilic and chronic lymphoplasmatocytic infiltrates, each being given a score of either 0 (absent), 1 (mild), 2 (moderate) or 3 (severe). The GIS on each section ranged from 0 to 6.
4.2.4 IMMUNOHISTOCHEMISTRY

Section staining was carried out at Pathology Diagnostics Ltd, Cambridge, UK. This was performed in 3 phases:

Validation Phase 1: Validation of staining using tissue samples or FFPE control tissue samples as appropriate and adapting the commercial antibody data sheet supplied method for use on the automated DAKO Autostainer-Link platform.

Analytical Phase 2 Staining on xenograft tumour samples to determine optimal antibody dilutions.

The final optimised antibody dilutions were as follows:

- CD3 (Dako, M7254, 20007999): 1:50
- CD25 (Abcam, 128955, YJ092903CS): 1:100
- TSPO (Abcam, ab109497, GR113098-6): 1:400
- CD45 (Dako 15751, 10005827): Ready to use
- CD68 (Dako M0876, 20002540): Ready to use

Phase 3

Following completion of the validation phase, staining of all the tissue samples was performed using the method developed for each of the five markers from Phase 1. Histological assessment and IHC scoring was performed by a histopathology research fellow with 3 years experience, and were corroborated by a senior histopathologist with more than 30 years experience in GI pathology.
4.2.4.1 TSPO

TSPO was assessed semi-quantitatively with a score of 0–9 derived by the product of the increase in staining intensity compared with the negative controls (0, no labeling; 1, faint staining; 2, moderate staining; 3, strong staining) and the frequency of stained cells (0: less than 10%, 1: 10–25%, 2: 25–50%, 3: more than 50%)\(^{224}\). In a small subset of 4 sections, co-staining with either a leukocyte-common antigen CD45 antibody or a macrophage-specific CD68 antibody was performed to qualitatively assess for co-localisation with TSPO.

4.2.4.2 IL-2RECEPTOR (CD25)

CD25 expression in the gut mucosa was quantified as follows: Using light microscopy the epithelium was identified and marked with 1mm graduations. At each graduation high power fields (hpf, x400 magnification) were sequentially examined and the number of CD25 positive cells within the mucosa manually counted. The average number of CD25 positive cells /hpf was recorded. To assess expression across the whole thickness of bowel wall on the other hand, light microscopy was used to select well-orientated, full-thickness sections of bowel wall, and 1-mm thick zones were marked. For each zone the whole of the mucosa, submucosa and muscularis propria were recorded using a Vernier scale. Expression was recorded as CD25+ve cells/mm\(^2\). Due to significant regional variability in CD25 expression even within a single slide, more than one 1mm-wide, well-orientated bowel wall regions were scored on each slide. Finally, in a small subset of 4 sections, co-staining with a CD45 antibody or a T-cell specific CD3 antibody was performed to qualitatively assess for co-localisation with CD25.
4.2.5 AUTORADIOGRAPHY

15μm-thick sections from fresh gut specimens were serially sectioned using a cryostat microtome (Leica, Wetzlar, Germany; CM1900), and thaw-mounted onto slides before being re-stored at -80°C.

One the day of the bind, slides were thawed to room temperature and washed for 15 mins in assay buffer (50 mmol/L Tris Base, 140 mmol/L NaCl, 1.5 mmol/L MgCl₂, 5 mmol/L KCl, 1.5 mmol/L CaCl₂, pH 7.4, room temperature) to remove the endogenous ligand.

4.2.5.1 TSPO

To determine the total binding signal, sections were incubated in assay buffer containing either [³H]PBR28 (0.5 nmol/L) for 60 minutes or [³H]PK11195 (1 nmol/L) for 120 minutes. The non-specific binding component was determined on adjacent sections by blocking TSPO receptors with unlabelled PK11195 (10 μmol/L).

4.2.5.2 IL-2R

To determine the total binding signal, sections were incubated in assay buffer containing ¹²⁵I-IL2 (0.3nmol/L)(3 x Kd of the high affinity CD25 subunit) (PerkinElmer, Boston, MA, USA). The non-specific binding component was determined on adjacent sections in the presence of 100nmol/L of unlabelled human recombinant interleukin-2 (hr-IL2) (Life Technologies, Paisley, UK) used to saturate all specific sites. The hr-IL2 concentration of 100nM correlates with 1000 x Kd of the high-affinity CD25 subunit.
After incubation, slides were washed twice in ice-cold wash buffer (50 mmol/L Tris Base, 1.4 mmol/L MgCl₂, pH 7.4, 4°C; 60 seconds) followed by a final wash in ice-cold distilled water (4°C; 60 seconds). Slides were dried in a cool airstream before exposure to tritium and ¹²⁵I-sensitive film (Kodak Biomax MS film, Hemel Hempstead, UK) with [³H] and [¹⁴C] microscale standards (GE Healthcare, Amersham, UK) for [³H]-PBR28/ [³H]-PK11195 and ¹²⁵I-IL-2 respectively, in X-ray cassettes at room temperature (12 weeks for [³H]-PBR28/ [³H]-PK11195 and 2 weeks for ¹²⁵I-IL2). After development of the radiograms, the films were quantified using microcomputer imaging device analysis software (MCID Core 7.0; Interfocus Imaging Ltd, Linton, UK). Circular regions of interest (ROIs) with an internal diameter of 10 pixels were manually placed in quadruples for each area of mucosa, submucosa and muscularis/serosa and their average value recorded. In addition, a larger quadrangular ROI along the whole axis of the specimen was placed, to measure the mean radioactivity across the whole section (Figure 4.2). The total and non-specific binding values were obtained from the conversion of Relative Optical Density (ROD) units to fmol [³H]ligand/mg wet tissue equivalent using a standard curve derived from the calibrated [³H]microscale standards which had been positioned alongside the tissue slides at the time of laying down to film. The specific binding (fmol/mg tissue) was determined by subtracting the non-specific binding signal from the total binding signal.
Figure 4.2: ROI placement on autoradiograms. 4x10 pixel–wide ROIs were positioned on each of the three layers and their average was recorded (blue in mucosa, yellow in submucosa and red in muscularis). Finally, a larger quadrangular ROI was placed across the full thickness of the section (green), which represents average receptor density.
4.2.6 HOMOGENATE BINDING STUDIES

Following slide sectioning, residual tissue (when available) was homogenised in 10 times weight for volume buffer (0.32 mmol/L sucrose, 5 mmol/L Tris-Base, 1 mmol/L MgCl₂, pH 7.4, 4°C) which contained protease inhibitor cocktail tablets (1 tab/ 50ml of buffer) (Roche, Branford, CT, USA). Homogenates were centrifuged (1,000× g, 20 minutes, 4°C) and the supernatant was removed and re-centrifuged (32,000 × g, 20 minutes, 4°C). High-spin pellets were re-suspended in at least 10 times w/v (weight for volume) buffer (50 mmol/L Tris-Base, 1 mmol/L MgCl₂, pH 7.4, 4°C) followed by two washes by centrifugation (32,000 × g, 20 minutes, 4°C). Membranes were suspended in buffer (50 mmol/L Tris-Base, 1 mmol/L MgCl₂, pH 7.4, 4°C). Protein concentration in homogenates (μg/mL) was determined using the bicinchoninic acid assay (BCA Kit, Sigma-Aldrich, Gillingham, UK) and absorption was read at 562nm. Membrane suspensions were finally stored at -80°C until further use.

Aliquots of membrane suspension were prepared using assay buffer (50mmol/L Tris-Base, 140mmol/L NaCl, 1.5mmol/L MgCl₂, 5mmol/L KCl, 1.5mmol/L CaCl₂, pH 7.4, 37°C). This was incubated with [³H]PBR28 in a final volume of 500μL for 60 minutes. For the saturation binding analysis, triplicates of 8 concentrations of [³H]PBR28 were used. In HABs, these ranged between 100pM and 300nM, and it MABs and LABs the range was between 500pM and 1μM. To calculate the non-specific binding component of [³H]PBR28, unlabeled PK11195 (100μM) was added in half of the binding wells. At 60 minutes, assays were terminated by filtration through Whatman GF/B filters (Whatman, Maidstone, UK), followed by 3 x 1ml washes using ice-cold buffer (50mmol/L Tris-Base, 1.4mmol/L MgCl). During incubation, Whatman GF/B filters (Whatman, Maidstone, UK) were pre-incubated in 0.05% polyetheleneimine.
The filters were then placed in scintillation vials and scintillation fluid (3mL/vial, Perkin Elmer Ultima Gold MV) was added, and were counted using a Perkin Elmer Tricarb liquid scintillation counter.

The specific binding values were plotted; \( B_{\text{MAX}} \) (fmol/mg protein) and \( K_d \) (nmol/L) were determined for each membrane preparation using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA) by using a non-linear regression fit for either one binding site or two binding sites.

**One site fit:**
\[
Y = \frac{B_{\text{MAX}} \times X}{(K_d + X)}
\]

**Two-site fit:**
Site 1 = \( \frac{B_{\text{MAX}} \times X}{K_{d1} + X} \)
Site 2 = \( \frac{B_{\text{MAX}} \times X}{K_{d2} + X} \)

\( Y \) = specific binding (fmol/mg protein)
\( X \) = concentration of radioligand.

I compared the \( K_d \) between normal and diseased sections from each patient to establish whether TSPO in inflamed tissue has different affinity towards its ligands. Moreover, affinities in low affinity binders and high affinity binders (and low and high affinity receptors in mixed affinity binders respectively) were compared with the ones reported in the published literature.

**4.2.7 DATA ANALYSIS**

Distribution results are expressed diagrammatically as median± Inter Quartile Range (IQR). CD25 expression between sections of different GIS was compared by Mann-
Whitney U-test. Wilcoxon rank sum test was used to compare specific binding for PK11195 and PBR28 on autoradiograms from normal and diseased sections of tissue in each patient. Similarly, PBR28 affinities (K_d) in normal and diseased membranes from each patient were also compared using Wilcoxon rank sum test. Finally, saturation binding data was analysed using iterative non-linear regression curve-fitting software (Prism 6.0, Graphpad, San Diego, CA, USA).

4.2.8 WORKLOAD DISTRIBUTION – PERSONAL INVOLVEMENT

Sequential drafts of the study protocols were produced by me and amended following consultation with my supervisors and consulting with a senior histopathologist at St Mary’s Hospital. I subsequently defended the protocol at a REC meeting, and drafted and submitted all subsequent amendments. I also carried out the database interrogation to select appropriate sections for the immunohistochemical experiments. Sectioning and staining was outsourced at Pathology Diagnostics Ltd and histological and immunohistochemical scoring was performed by a Histopathology research fellow in conjunction with her supervisor.

I also carried out recruitment and consenting for 7 of the 12 participants who offered tissue prospectively for the autoradiographic and homogenate binding studies; the remainder were recruited at a remote site by a local research nurse. I subsequently carried out tissue snap-freezing, sectioning, autoradiographic and homogenate binding studies, including film apposition and read-outs as well as operated the scintillator, after a period of training on the techniques using porcine tonsillar specimens.
4.3. RESULTS

4.3.1 TSPO

4.3.1.1 HISTOLOGICAL ASSESSMENT OF SEVERITY

33 stored mucosal biopsies were obtained, 5 from normal controls and 28 from patients with CD. 18 transmural sections from 7 CD patients who had undergone intestinal resections were also assessed. H&E slides from each of these sections revealed the following distribution of Global Inflammatory Scores:

*Table 4.1: Distribution of GIS in mucosal biopsies and transmural sections assessed with TSPO IHC*

<table>
<thead>
<tr>
<th></th>
<th>Mucosal biopsies</th>
<th>Transmural sections</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls GIS 0</strong></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><strong>Crohn's disease GIS 0-1</strong></td>
<td>13</td>
<td>GIS 0-2</td>
</tr>
<tr>
<td><strong>GIS 2-4</strong></td>
<td>8</td>
<td>GIS 3-6</td>
</tr>
<tr>
<td><strong>GIS 5-6</strong></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>33</td>
<td>18</td>
</tr>
</tbody>
</table>

4.3.1.2 IMMUNOHISTOCHEMICAL EXPRESSION

*Figure 4.3: Mucosal TSPO expression index in normal controls and CD mucosal sections sub-categorised according to GIS score*
Figure 4.4: Transmural TSPO index in sections with mild-moderate (GIS 0-2) versus severe (GIS 3-6) inflammation.

Figure 4.5: Characteristic TSPO expression in (a) epithelial and (b) transmural specimens in my cohort. The yellow circle in (a) corresponds to an area of epithelial inflammation, which demonstrates similar expression to the rest of the un-inflamed epithelium. The red circle in (b) corresponds to an area of denser staining without any features of inflammation. (c) shows the negative control slide.
Figure 4.6: Another example of TSPO expression across the full thickness. An area of ulceration with inflammatory cell infiltrate also staining for TSPO is demonstrated (blue arrow)

When co-staining for TSPO and CD45, as well as TSPO and CD68 was performed, this demonstrated moderate-strong TSPO staining throughout the mucosal glands in both the control and the inflamed slides and a lack of co-localisation between TSPO and either of the two immune cell markers. More specifically, CD45 was heavily concentrated within lymphoid aggregates in the diseased section, whereas some sparse transmural positivity was seen in the healthy section. CD68 expression was also transmural and appeared more concentrated in the diseased section.
4.3.1.3 AUTORADIOGRAPHIC BINDING

The 12 pairs of tissue which were collected prospectively from intestinal resection specimens were assessed qualitatively by consultant histopathologists at participating hospitals. Representative sections from the specimen edges were reported as normal in 11/12 specimens, with 1/12 showing evidence of quiescent ileitis. All 12 specimens from the diseased core contained inflammation and features of fibrosis.

4.3.1.3.1 $^3$H-PK11195

$^3$H-PK11195 autoradiography was successfully performed on 8 sections from uninflamed margins and 8 sections from inflamed gut (6 matched pairs from the same patients). Comparison was possible on a 7th pair for submucosa and muscularis only. The remainder 4 and 4 specimens respectively were not examined due to poor tissue sectioning (see discussion). Specific binding achieved using 1nM $^3$H-PK11195 ligand and 10μM unlabeled PK11195 block was low (mean ± SD = 41.5±17.5%) (Figure 4.7).

*Figure 4.7: Characteristic PK11195 binding (a) total and (b) non-specific binding. This is illustrated in black-white (top) and pseudo-colour (bottom) modes. In the latter, high radioactivity counts is represented by brown-range whereas background staining is represented by green. The non-specific binding is obviously reduced, compared to the total, but still clearly visible.*
The differences in receptor density (specific binding, fmol/mg of tissue) between the normal and inflamed sections were not statistically significant (Figure 4.8).

Figure 4.8: TSPO specific binding (SB) (fmol/mg or tissue) as determined by $^3$H-PK11195 autoradiography on normal un-inflamed (blue) versus diseased sections (red). Comparisons for each layer separately is shown in graphs (a), (b) and (c). Comparisons of transmural expression are shown in (d)
4.3.1.3.2 $^3$H-PBR28

$^3$H-PBR28 autoradiography was successful on 10 sections from uninflamed margins and 10 sections from inflamed gut (8 complete pairs). Comparison was possible for a 9th pair for submucosa and muscularis only. The specific binding achieved using 0.5nM of $[^3]$H]PBR28 and 41μM of unlabeled PK11195 ligand, as a block to saturate specific sites, was high (mean ± SD 91±11%)(Figure 4.9).

*Figure 4.9: Characteristic $^3$H-PBR28 binding (a) total and (b) non-specific binding shown in pseudo-colour mode. Non-specific binding is far less prominent than with PK11195 in Figure 4.7*

A trend was demonstrated towards a higher specific binding in diseased sections in the transmural and submucosal ROIs but this did not reach statistical significance (Figure 4.10)
Figure 4.10: TSPO specific binding (fmol/mg or tissue) as determined by $^3$H-PBR28 autoradiography on normal, un-inflamed (blue) versus diseased sections (red). These are compared with Wilcoxon rank sum test and p-values are demonstrated. Comparisons for each layer separately is shown in graphs (a), (b) and (c). Comparisons of transmural expression are shown in (d).

Given the lack of statistical significance between specific binding in normal and diseased sections, I plotted all specific binding in LABs, MABs and HABs separately (when genotype was known) and observed the appropriate clustering both in each individual layer, as well as transmurally (Figure 4.11). This observation confirms that the specific binding observed with LABs is lower than that seen in MABs and HABs. In a setting of
human PET scanning with this radioligand, the subject genotype needs to be determined in advance and be factored in in the quantification of the PET signal.

Figure 4.11: Specific binding in LABs MABs and HABs in mucosa (top left) submucosa (top middle) muscularis (top right) and transmurally (bottom).
4.3.1.4 HOMOGENATE BINDING STUDIES

Tissue homogenates from 5 pairs of tissue from patients genotyped for the rs6971 polymorphism (1 LAB, 2 MABs, 2 HABs) were used to estimate the binding affinity ($K_d$) and density of available binding sites for PBR28.

*Figure 4.12: Matched LAB and HAB binding affinities from membrane homogenates in 5 subjects. There was no statistically significant difference between the $K_d$ for LAB and HAB sites from healthy and diseased specimens.*

As there was no statistical significance in $K_d$ in membranes from normal and diseased specimens, high affinity sites and low affinity sites were grouped from both normal and diseased specimens and measured. Low affinity $K_d$ was $68.7 \pm 16.5$ nmol/L and high affinity $K_d$ was $10.24 \pm 4.0$ nmol/L (Figure 4.13)

*Figure 4.13: Distribution of low affinity and high affinity $K_d$ in gut tissue*
Figure 4.14: Binding curves for the 5 pairs of normal and diseased membrane preparations. (a) MAB (b) HAB (c) LAB. $B_{\text{MAX}}$ is denoted by the plateau of the signal. As available receptors in membrane homogenates are much more concentrated than in whole tissue laid on film, the resulting $B_{\text{MAX}}$ in fmol/mg is much greater than in autoradiography. Moreover, the differences between healthy and diseased $B_{\text{MAX}}$ appear more pronounced.
25 stored mucosal biopsies were obtained, 2 from normal controls and 23 from patients with CD. 12 transmural sections from 5 CD patients who had undergone intestinal resections were also assessed. H&E slides from each of these sections revealed the following distribution of Global Inflammatory Scores:

Table 4.2: Distribution of GIS in mucosal biopsies and transmural sections assessed with CD25 IHC

<table>
<thead>
<tr>
<th></th>
<th>Controls GIS 0</th>
<th>Crohn’s disease</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GIS 0-1</td>
<td>GIS 2-4</td>
</tr>
<tr>
<td>Mucosal biopsies</td>
<td>2</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Transmural sections</td>
<td>0</td>
<td>18</td>
<td>12</td>
</tr>
</tbody>
</table>

4.3.2.2 IMMUNOHISTOCHEMICAL EXPRESSION

Figure 4.15: Mucosal CD25+ve cells per hpf according to GIS. There were no statistically significant differences between any of the groups.
In order to assess CD25 expression across the full thickness of the bowel wall, 12 sets of slides were produced from 5 resection specimens from patients with CD. A median of 3 (range 2 - 6) 1 mm-wide, well-orientated bowel wall regions were scored on each slide (total 41) (see Methods 4.2.4.2). Of these, 18 (44%) had no/mild inflammation (GIS 0-1), 12 (29%) showed moderate inflammation (GIS 2-4), and 11 (27%) had severe inflammation (GIS 5-6).

Figure 4.16: Transmural CD25 expression in the various groups. There was statistically significant differences in CD25 expression between mild and moderate, and mild and severe GIS. Differences between moderate and severe GIS were borderline non-significant.

Figure 4.17: Area with significant lymphocytic infiltrate (a) H&E, circle, (b) CD25 stain (brown)
Co-localisation studies of CD25 and CD3 or CD45 suggest that the majority, but not all CD25 expression occurs on leukocytes (CD45 positive cells) and specifically T-lymphocytes (CD3 positive cells). It was difficult to distinguish CD25 staining in CD3 stained cells due to strong intensity of staining.

4.3.2.3 AUTORADIOGRAPHIC BINDING

Several attempts were made to corroborate the immunohistochemical data, which suggested an enhanced expression of CD25 in severely inflamed segments with autoradiography binding studies. A combination of $^{125}$I-IL-2 at a concentration of 0.3nM (3 x $K_d$ of high affinity sites expressed on T-cells) and 100nM of unlabeled human recombinant Interleukin-2 (hr-IL2) to saturate specific sites failed to result in a significant specific binding (figure 4.18). The mean± SD % specific binding was 0±24.8%.

Figure 4.18: Characteristic $^{125}$-IL2 binding (a) Total and (b) Non-specific. The signal of non-specific binding is almost identical to the total. This signifies a failure to block specific sites with the Hr-IL2 molecule, even at concentrations of 1000x $K_d$.
4.4 DISCUSSION

4.4.1 SUMMARY OF FINDINGS

Key results of this study were the following:

- There is no difference in TSPO expression between normal gut tissue and CD segments.
- There is no difference in PK11195 and PBR28 binding in diseased versus normal gut segments from Crohn’s patients.
- Clinical scanning with TSPO radioligands to delineate or monitor diseased segments in patients with Crohn’s disease is therefore not justified.
- Binding affinity of PBR28 in gut is similar to previously published data on brain sections.
- Severely inflamed gut sections have higher expression of the high affinity α subunit of the IL2-R compared to moderately or mildly inflamed sections. This could be utilised in IL-2 based scanning in the future.

4.4.2 DISCUSSION OF FINDINGS

TSPO is a molecule that has been increasingly explored for its potential to act as a target in molecular imaging of inflammatory disorders. This data suggest that the background expression in normal intestine will likely prevent its use as a marker of inflammation in Crohn’s disease.

The expression of the receptor was first assessed semi-quantitatively with immunohistochemistry, using a previously employed composite score which factors in both the frequency and intensity of expression. There was no appreciable difference either in the mucosa or across the full thickness of the bowel wall in specimens with
histological features of moderate or severe inflammation compared to normal controls or mildly inflamed specimens.

Several studies have shown the potential for TSPO probes to demonstrate and quantify inflammatory processes. A high expression of PBR on peripheral macrophages was first described three decades ago. Since that time, several groups have demonstrated high TSPO expression in correlation with peripheral macrophage infiltrates. Bird et al. showed an abundance of PK11195–avid CD68+ve macrophages in *in vitro* specimens of carotid atherosclerotic plaques, which was 20 times higher than that in surrounding vascular smooth myocytes. A group from Amsterdam performed TSPO and CD68 immunohistochemistry in arthroscopically derived synovial specimens from patients with rheumatoid arthritis, as well as controls who underwent arthroscopy for mechanical knee injury. Their results demonstrate a relevant paucity of TSPO expression in control synovium, with increased expression in RA joints that closely co-localises with CD68. More recently, a Japanese group demonstrated high TSPO expression in a murine model of non-alcoholic fatty liver disease (NAFLD). This correlated with C11b expression, a marker of macrophage and lymphocyte activation.

TSPO in IBD has been investigated by Ostuni et al. The group used an immunohistochemical probe to confirm diffuse TSPO expression in normal human intestinal epithelium. In epithelial biopsies from patients with small bowel CD, the TSPO distribution extended throughout the length of the villus. The authors did not disclose the exact numbers of control or IBD human biopsies analysed. These observations of isolated epithelial TSPO expression in healthy tissue, which extends deeper into the villi in the inflamed state, were also replicated using a DSS murine model of colitis. In a recent study, a French group performed microPET imaging using a TSPO radioligand (*18F-DPA-714*) on mice treated with DSS or 2,4,6 trinitrobenzenesulfonic acid (TNBS) to
produce colonic inflammation. They report a significantly higher $^{18}$F-DPA-714 signal in both DSS and TNBS treated animals compared to controls. They also qualitatively demonstrate an increase on transmural colonic TSPO expression using an immunofluorescent probe \(^{227}\).

This work confirms the observation that the background expression of TSPO in normal epithelium is significant. I attempted, for the first time, to compare semi-quantitatively TSPO expression in normal specimens (mucosal and transmural) with that within sections containing active inflammation. The immunohistochemical data show that both in the mucosa as well as across the full thickness of the bowel wall, there is no appreciable increase in the TSPO expression.

The main goal of this project was to examine the applicability of TSPO-specific radioligands in clinical PET imaging of CD lesions. Current PET technology does not offer a high enough resolution to permit the differentiation between signal originating in the mucosa from that in deeper layers of the bowel. The transmural expression of TSPO (or any other target receptor) in inflamed versus normal samples has therefore more translational relevance than that in individual layers. While there may have been a trend towards a higher expression in grossly inflamed mucosal samples, the overall transmural expression was similar between sections of low and high GIS.

The reason behind this over-abundance of TSPO in normal gut has not been elucidated. Evidence suggests that the intestinal epithelium has an active role in maintaining immune homeostasis or a state of ‘controlled inflammation’. Firstly, epithelial cells are involved in MHC-mediated antigen processing and presentation \(^{228}\). Secondly, they have been implicated in the production of chemokines in response to Toll-like receptor ligation, which in turn leads to dendritic cell conditioning and migration \(^{229}\). In addition,
they are capable of apoptosis following bacterial invasion \(^{230}\). All of the above are unique characteristics of the gut, in response to a constant and diverse antigenic load of resident microbes. These have not been observed in other relatively sterile tissues such as the vascular endothelium or the synovium. All these functions are in direct relevance with some of the key effector pathways linked to TSPO. Several studies have suggested an immune-modulatory role for TSPO, which is modifiable by specific ligands such as PK11195 \(^{231,232}\). It is therefore possible that the abundance of TSPO on intestinal epithelial cells reflects their status as key players in the paradigm of controlled inflammation.

In addition, the double stains show that the majority of TSPO is not found in inflammatory cells. This was in contrast to synovium\(^{150}\), carotid atheroma\(^{148}\), steatohepatitis\(^{152}\), or neuro-inflammatory disorders\(^{153}\), where TSPO expression in background, non-inflammatory cells is very low. In order for molecular imaging in the gut to move forward from FDG, which is an inherently non-specific ligand, the need is to identify radio-tracers that selectively tag and quantify the inflammatory cells rather than rely on a relative increase in a receptor that is already abundant in normal state.

Subsequently, I examined the binding potential of a first generation (PK11195) and a second-generation ligand (PBR28). I was able to assess quantitatively the different receptor densities in ex-vivo intestinal resection specimens from patients with Crohn's disease, by performing saturation binding autoradiography in sections from the diseased core as well as the un-inflamed margins of these specimens. A non-significant trend towards a higher specific binding in inflamed sections was found, which corroborates the observations of immunohistochemistry.
Each of the two techniques above has major benefits and drawbacks. Immunohistochemistry on the one hand is widely applicable, at low cost, and can be preformed on larger numbers of stored formalin-fixed specimens. However, it can only offer, at best, a semi-quantitative measure of the abundance of the TSPO receptor, and provide very limited information on its behaviour as a target for radioligands. Autoradiography, on the other hand, was performed with tritiated versions of the exact radioligands currently used for in-vivo TSPO imaging, and as such, can provide a much more accurate representation not only of the location and density of receptors but of other parameters such as the signal-to-noise ratio in the binding. It too however has limitations: It has been shown that tissue fixation in (para)formaldehyde negatively impacts on the affinity of the majority of receptors\textsuperscript{233}. Previous attempts to perform PBR28 autoradiography, in particular on FFPE brain tissue were unsuccessful (Owen DR, personal communication). Therefore prospective recruitment with immediate processing of resected specimens was the only possible strategy of obtaining tissue for this project. This, in combination with the significant cost of re-agents and equipment required, only allowed us to examine a limited number of specimens. The observed differences between the specific binding in un-inflamed and diseased sections however were small, and hence larger numbers of tissue pairs would probably not have resulted in meaningful disparities.

Another major limitation was the technical aspect of handling unfixed tissue. The maintenance of the tissue orientation during snap-freezing was particularly challenging. It frequently resulted in significant distortion and malposition of the various layers (mucosa, submucosa, muscularis, serosa). In addition, the resulting tissue block was usually too large to be inserted whole onto the microtome for sectioning, and smaller fragments had to be produced. Commonly, these did not contain all layers of tissue, and, when they did, there was a variable degree of folding and superimposition. This was the
main reason for failure to obtain paired autoradiograms for all 12 pairs of tissue. With experience, the integrity and orientation of tissue blocks during snap-freezing and sectioning improved in sequential specimens. While this limits the statistical power of the observations, it is unlikely that it would have introduced any other type of bias in the measurements.

It has been shown that PBR28 and other second-generation TSPO radioligands have a significantly improved signal-to-noise profile, which was also evident on my samples. They do, however, exhibit significant between-subject variability, which has been accounted for by a genetic polymorphism (rs6971) in the gene encoding TSPO. Homozygote high-affinity and low-affinity binders, as well as heterozygote mixed-affinity binders have been described, and the dissociation constant (Kd) has been estimated for each of the three phenotypes, at least for TSPO extracted from brain tissue.

I was only able to genotype a subset of issue donors as the discovery of the rs6971 polymorphism was published while the recruitment was already in progress. Saturation binding was performed on the cell membrane fractions from homogenised specimens from 1 or 2 tissue pairs from each genotype (1 LAB, 2 MAB, 2 HAB). To date, the affinity of PBR28 on TSPO has only been described in receptors extracted from brain homogenates. Through this experiment I aimed to compare this to TSPO originating from peripheral tissue for the first time.

The measured Kd's from normal and inflamed gut tissue were statistically comparable. This suggests that while TSPO in inflamed tissue is relatively more abundant, its stereotactic conformation and binding properties remain stable in a diseased milieu. Moreover, the Kd's for the high and low affinity states proposed the experiment on
cellular membrane homogenates from intestinal tissue were similar to these described in the brain. However, methodological limitations conferred a degree of error to the measurements of $K_d$ in samples from the three phenotypes: Firstly, the proposed thickness of 5mm for each of the two rings of gut tissue obtained from each resection specimen often was too limited to allow good quality sections as well as enough tissue for a triplicate of saturation curves per specimen (which would be standard practice). The tissue stores only allowed a single saturation curve for each specimen, significantly increasing error. Secondly, protein concentration in membrane fractions was limited and therefore, even though ligand depletion was not observed, a fixed membrane dilution had to be used, and applied to saturation curves retrospectively, which can also act as a source of error.

Observation of the binding curves suggests that the $B_{\text{MAX}}$ in 4 of the 5 sets are higher in the diseased versus the normal specimens, which was not reflected in the autoradiograms. There are two possible explanations for this. Firstly, the tissue that was homogenised contained a significant proportion of mesentery, which was not included in the sections. A group from Berlin recently presented data suggesting that the mesenteric fat in CD is a rich source of macrophages. It is possible therefore that this observed trend for higher receptor density in diseased homogenates results in higher densities of TSPO receptors obtained from that compartment. Secondly, the sequential centrifugation protocol ensures that the receptor concentration in homogenates is by definition higher than that of whole tissue sections used on autoradiograms. Homogenate binding studies are therefore more likely to reveal significant differences in available receptors between the healthy and inflamed compartments. The drawback of the technique is that the spatial localisation of these receptors is not possible.
CD25 (IL2R-α) immunohistochemistry suggests that there are significant increases in CD25 expression across the full thickness of the bowel wall, in moderate and severe inflammation compared to normal or mildly inflamed sections. By far the greatest increase in CD25 expression was observed in specimens at the most severe end of the inflammation spectrum (GIS 5-6). These differences were not appreciable when mucosa biopsies were examined in isolation, but as discussed above, differences in transmural expression are more important in the context of the exploration of potential imaging probes.

These findings are in agreement with a small number of early papers from several decades ago, on the expression of IL-2, and its receptor, in CD: To assess the mucosal concentration of IL-2 amongst other cytokines, Brynskov et al. performed Enzyme-Linked ImmunoSorbent Assays (ELISA) on supernatants following homogenisation of 27 colonic and 4 ileal biopsies from patients with IBD and 19 controls. He reports IL-2 detection in 35% of the patient’s supernatants compared to 0% from controls. A more detailed analysis of positive ELISAs revealed that IL-2 is more likely detected in lesions of higher histology score, and that none of 4 ileal CD biopsies revealed any IL-2 irrespective of the histology score\(^2\).\(^{36}\)

Niessner et al. reports significantly higher IL-2 m-RNA levels in biopsies with active CD compared with controls, as assessed by reverse transcriptase polymerase chain reaction (RT-PCR). A comparison between active an inactive sites in the same patients did not reach significance\(^2\).\(^{37}\). Mullin GE et al. concurred with the above difference of IL-2 m-RNA concentrations in CD colonic biopsies versus controls in a similarly designed experiment. In addition, he reported a significant difference when active and inactive sites were compared in 5 CD patients \(^2\).\(^{38}\). Desreumaux et al. went a step further and examined the difference of IL-2 expression in chronic ileal lesions versus early ileal
recurrences and found a significantly higher expression in the former. This lead to suggestion of a shift in inflammatory type from Type 2 (IL-4 and IL-5 predominant) in early lesions to Type 1 later on in the disease’s natural history 239.

Two main studies focus on the expression of IL-2 receptors in active CD segments. Choy et al. examined the expression of CD25 in resection specimens from patients with IBD and observed that in normal small and large intestinal mucosa CD25+ cells are rarely seen. In ileal and colonic mucosa from CD patients, on the other hand, there were aggregated and scattered CD25+ cells. The lymphoid follicles and submucosa/muscle layers also had abundant CD25+ cells. Overall CD25+ cells made up 15-64% of lamina propria nucleated cells in Crohn’s disease, the majority of which were CD3+CD4+CD8-T-cells. Most CD25+ cells in sub-epithelial aggregates, on the other hand, were identified as macrophages 219. Mahida et al. performed comparative immunohistochemical staining for IL-2 R in tissue sections from CD patients and normal controls and confirmed the contrast between normal and diseased ileal mucosa, as well as CD25 abundance in the Peyer’s patches. He also noted that macrophages from diseased tissue expressing IL-2R had higher potential to undergo respiratory burst 240.

In addition to the aforementioned basic science papers on the abundance and distribution of IL-2 and its receptor in the context of Crohn’s disease, there is a significant body of literature from the field of SPECT imaging, which is suggests potential clinical applicability of an IL-2 based radio-pharmaceutical to quantify as well as monitor inflammatory activity in CD (reviewed in the Introduction).

The aim of the subsequent experiment was to confirm that these differences in transmural expression of the IL-2R between inflamed and normal intestinal segments would also translate into preferential binding of IL-2 radioligands in these segments.
Unfortunately, despite maximal efforts to optimise the binding parameters, the non-specific component of $^{125}$I-IL2 on tissue specimens was too high to permit any conclusions on the differential specific binding on un-inflamed versus diseased sections.

This study has many strengths. This is the first time quantification of TSPO expression in full-thickness sections of CD tissue was attempted. Moreover, this is the first study to investigate the potential of first and second generation TSPO-specific radioligands to delineate CD lesions and quantify the inflammatory activity within these. Finally, I attempt to quantify the affinity ($K_d$) of all three phenotypes of the binding status to 2nd generation TSPO radio-ligands in a peripheral tissue, demonstrating affinities comparable to those described in brain. With regards to the Interleukin-2 receptor, evidence was obtained that it is significantly over-expressed in inflamed bowel segments, in proportion to the degree of the global inflammatory score. This renders it an attractive potential target for molecular imaging. This study however failed to elucidate useful characteristics of the radioligand-IL2 receptor interaction such as the anticipated signal-to-noise ratio and the density of available IL-2 receptors in normal versus diseased bowel.
4.5 CONCLUSION

TSPO has a high background expression in normal gut epithelium and there was no obvious increase in transmural sections with significant inflammatory activity. In addition, the high expression in normal epithelial cells renders TSPO radioligands unsuitable as specific inflammatory tracers in CD. Full thickness autoradiograms and saturation binding studies from tissue homogenates suggested a non-significant trend towards higher specific binding in diseased versus healthy sections from the same patients.

Even though the IL-2R is potentially a more promising target, due to its higher selectivity for activated T-cells and its over-expression in diseased versus normal gut tissue, there are still many unanswered questions on the specificity and binding potential of an IL2-based radio-pharmaceutical for the investigation of Crohn’s disease activity.

The significance of these results and future studies that can originate from this work are further discussed in Chapter 5.
5. DISCUSSION AND FUTURE DIRECTIONS

Crohn’s disease is a chronic disorder, with a highly variable impact. Some patients exhibit a benign phenotype, with localised disease that remains quiescent over long intervals, while others have extensive disease which evolves more rapidly to cause complications such as fistulae and fibrostenosis\(^1\). Large population-based studies have shown that the latter phenotype with its accumulating complications is the commonest form \(^{1,15}\). Moreover, studies have shown a significant lack of association between the intestinal pathophysiology and symptom reporting by patients\(^ {8,34}\). This suggests that exclusive reliance on symptomatology is not an ideal strategy for characterising disease activity in clinical practice. The need for objective markers of disease activity has therefore been highlighted.

There are several instances in the natural history of CD when objective characterisation of disease burden and its change over time is needed. Firstly, at index presentation it is important to fully ascertain the extent and severity of the lesions. This will require a combination of several of the modalities currently available, which include imaging and endoscopic-histological assessment, as these measures are complimentary. Such an approach is advocated by current international guidelines such as those from the European Crohn’s and Colitis Organisation (ECCO)\(^ {241}\). Secondly, an assessment of inflammatory burden is important, particularly at times when escalation in medical therapy is contemplated. Finally, the evaluation of success of medical treatments also requires an objective parameter of disease activity. In view of the chronic and constantly evolving natural history of Crohn’s disease, it is therefore probable that each patient will require disease load monitoring at several time-points in their illness.

In addition to all these circumstances in which disease monitoring is clinically indicated, another major area of need for objective, reproducible outcome measures is that of drug
development. Currently, clinical trials rely heavily on clinical scores, such as the CDAI, and, more recently, patient-reported outcomes such as quality of life questionnaires and disability indices to assess the response of various therapies\textsuperscript{242}. These ignore the suboptimal correlation between mucosal inflammation and symptoms, and thus lead to an over or underestimation of the therapeutic effect. This leads to high variance and the need for larger trials for longer periods, complicating particularly early-stage development. While new approaches have been proposed, e.g. mucosal healing as assessed by endoscopy, (CDEIS and SES-CD are the two most frequently used evaluative indices), more evidence is needed to assess its impact\textsuperscript{243}.

Moreover, over the last few years, there have been a large number of new therapies that have entered the therapeutic pipeline\textsuperscript{25} and the need for new outcome measures has been increasingly vital. The roles that these new measures will be required to fulfill are to improve the definition of appropriate patient cohorts, and to reduce the rates of placebo response, which are currently high in studies relying on clinical scores and patient-reported measures. There is an opinion that the practice of using of placebo arms is gradually becoming impractical and unethical, in this era of ever increasing availability of advanced effective therapies in CD. Finally, the use of robust endpoints can act to reduce the sample sizes required to measure the studied effects.

The variability in disease phenotype, the disconnect between reported symptoms and ongoing bowel damage and the increasing throughput of costly and potentially toxic drugs currently in clinical practice or at various phases of development pose a currently unmet need for appropriate biomarkers that can assess disease burden in a variety of circumstances.
A biomarker is defined as ‘a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention’ \(^{244}\). Biomarkers can be subcategorised into prognostic, predictive, pharmacodynamic biomarkers and surrogate endpoints \(^{245}\). Prognostic biomarkers constitute of single or clusters of specific traits that separate the population, with respect to an outcome of interest, regardless of the type of therapy used. An example in CD would be an ileo-colonic distribution compared to isolated ileal or colonic disease, and/or the presence of perianal lesions, traits which carry poorer long-term outcomes \(^{246}\). Predictive biomarkers on the other hand constitute of baseline characteristics that can categorise patients by their degree of response to specified treatment \(^{247}\). To date, there is a distinct lack of predictive biomarkers in CD \(^{248}\). Pharmacodynamic biomarkers are those that are used to optimise drug-dosing schedules. A classical example in CD is the assessment of thiopurine methyl-transferase (TPMT) genotype to identify heterozygote patients with higher chances of myelotoxicity\(^{249}\). Finally, surrogate endpoints are intended to be substitutes of true clinical endpoints expected to predict clinical benefit (or harm) on the basis of epidemiologic therapeutic, pathophysiologic or other scientific correlations (FDA website). In the setting of clinical trials, surrogate endpoints are used as measures of effect on the basis of their correlation with true clinical endpoints\(^{250}\). For a biomarker to be deemed a surrogate endpoint, the corresponding clinical endpoint, population of interest and class of intervention must all be thoroughly defined.

**5.1 STATISTICAL ASSESSMENTS IN THE DEVELOPMENT OF BIOMARKERS**

The first step in the development of a biomarker is the determination of a normal cut-off level. This is done by comparison of inactive and active cohorts as determined by an accepted gold-standard investigation e.g. ileocolonoscopy. Biomarker values are then
plotted for the two groups and ROC analysis is used to determine a normal cut-off level as well as the sensitivity and specificity of the measure to determine disease status.

From that point on, appropriate statistical assessments for awarding biomarker status to a measure depend on the exact roles a biomarker is intended to fulfill. Prognostic biomarkers are usually assessed through prospective randomised controlled trials. Biomarker-positive patients are compared with biomarker-negative controls matched for disease activity and demographics, and are followed-up over a pre-determined time interval. At the end of the study period, the two groups are compared for an event in question e.g. maintenance of remission or flare and compared by \( \chi^2 \) test. P-value between the two groups assesses the power of the biomarker to predict the specific outcome.

Validation of an imaging biomarker in predicting therapeutic response involves a different methodology. A single cohort is commenced on a specified therapy and early re-imaging visit is carried out. An ROC analysis can then be done to determine the degree of change in the biomarker that is associated with subsequent clinical response. Combination of biomarkers can often enhance the accuracy of early prediction of a clinical outcome.

Finally, in order for a measure to be awarded “surrogate endpoint” status, several criteria have to be met. Firstly, proof of biological plausibility in the correlation of the biomarker with the corresponding clinical endpoint is essential. Plausibility building involves the accumulation and analysis of biochemical, tissue or animal data in order to describe a mechanism of action or to demonstrate the desired biological effect. Moreover, correlation statistics are used to show the degree of association between the surrogate and the true endpoint. Finally, additional important attributes of potential surrogate endpoints are reliability, validity and responsiveness, collectively termed clinimetrics\(^{251}\).
A reliable measure is one that produces results that are consistent, accurate, stable over time and reproducible. The two most important components of reliability in the measures examined in this study are test-retest, in other words the stability of an endpoint when assessed in the context of stable disease, and rater reliability i.e. the agreement of the results when a measure is assessed by independent observers.

Validity, in general terms, refers to the degree to which an instrument actually measures the parameter it is intended to measure, in other words, whether it samples the relevant areas that are expected to be influenced by a certain intervention. Validity also examines the correlation it exhibits with an accepted gold standard, or other accepted measures of the disease process. In the case of an inflammatory condition such as CD, evidence must be provided that a measure is actually examining a component of the inflammatory burden, and, in the absence of an acceptable gold-standard, that it correlates with other measures which have been validated to reflect disease activity e.g. clinical scores, blood and faecal biomarkers of inflammation, or endoscopic scores of activity.

Finally, responsiveness is defined as the ability of an outcome measure to either detect a concurrent clinically meaningful change or predict one in the future. Gauging an appropriate time interval for taking the repeat measurement is a crucial step in the design of both these approaches. Ultimately, however, both short-term as well as longer-term endpoints have to be appropriate and clinically important. In CD, examples of the former usually include a measure of clinical response of remission, whereas endpoints of a longer horizon include hospitalisations, surgeries, steroid avoidance and quality of life. A further complication in CD, however, is the fact that clinical remission as an outcome does not guarantee a favourable medium term prognosis. Therefore biomarkers that predict progression and complications in the longer term would be very helpful.
The lack of suitable gold-standard monitoring instruments for any of the situations outlined above has been discussed extensively earlier in this thesis. MRI has been increasingly evaluated for its potential to quantify disease activity, and several quantitative measures of inflammation have been proposed in recent years.\textsuperscript{74,97,108,192,199} PET, on the other hand, has shown some promise through its static correlation with endoscopic\textsuperscript{130,141} and histological markers of activity\textsuperscript{168}. When this research was planned, evidence of the value of MRI as a monitoring tool was scarce in the case of MRI and was completely lacking for PET.

This thesis makes a contribution to current knowledge through the simultaneous examination of a large number of outcome measures in a well-characterised patient cohort. The future roles of some or all of these measures in disease monitoring and the proposition of further work required for their validation depend on drawing appropriate conclusions from the data. In order for this to be achieved, the aforementioned criteria on biomarker selection and assessment should be used as a benchmark against which each measure can be objectively scrutinised.
5.2 $^{18}$F-FDG PET

The hypothesis that a reduction in inflammatory load corresponds to a decrease of glycolytic activity measured by $^{18}$F-FDG PET signal was tested in Chapter 2.

5.2.1 WHAT WAS ALREADY KNOWN

A number of studies have already contributed towards our knowledge basis on the potential of $^{18}$F-FDG–PET to act as a biomarker in the assessment of CD. Bowel SUV$_{\text{MAX}}$ greater that that in the liver has been shown to be an accurate cut-off to identify diseased segments $^{135}$. Correlation of SUV$_{\text{MAX}}$ and SUV$_{\text{MEAN}}$ with several clinico-pathological and endoscopic endpoints have also been demonstrated$^{138,141}$. Evidence on the plausibility of the hypothesis that FDG signal represents inflammatory cell infiltrates in tissue can be found in animal studies $^{142-144}$.

5.2.2 CONTRIBUTION OF THIS PROJECT AND FUTURE DIRECTIONS

Perhaps the most important finding of the PET study is the significant amounts of radioactivity within free faecal matter (10-30% of the recto-sigmoid activity) in the substudy of patients with distal colonic disease. This has a significant negative impact in the validity of the $^{18}$F-FDG PET as a method of monitoring lesional and global inflammatory change. This is because this luminal activity probably represents unbound FDG, which leaks or is secreted through extracellular space. Factoring it in as part of the total signal undermines the radioligand as a specific molecular marker of metabolic or inflammatory activity. This is because the free portion of the signal is more of a non-specific noise, rather than a probe being traced through its physiological pathway. Dynamic PET sequences$^{252}$, where a time series of images are obtained without any lag between injection and scanning
could help demonstrate the path of FDG on its way to the lumen and allow the process to be modeled. My hypothesis is that this is likely a radial inward migration originating in the mesenteric vasculature upon delivery of the ligand. Other, less plausible explanations are that the signal follows a ‘downstream’ migration pathway after being secreted at a remote site e.g. the biliary tree, or even that this is generated by epithelial cellular shedding into the lumen in the 100-minute interval between intravenous administration of FDG and bowel evacuation. This is not consistent however with the physiological transit time of small and large bowel.

With regards to the individual endpoints, my study highlights the measures of signal intensity such as segmental or global $S_{\text{UV}_{\text{MAX}}}$ and $S_{\text{UV}_{\text{MEAN}}}$, appeared much more reliable, had greater content validity reflected by their correlations with HBI, CRP and FC both statically and over time, and also significant responsiveness ratios. These should be explored further as possible biomarkers of disease activity.

In contrast to (G)$S_{\text{UV}_{\text{MAX}}}$ and (G)$S_{\text{UV}_{\text{MEAN}}}$, measures that incorporate signal volume, SLG and TLG are far less likely candidates to act as biomarkers for inflammatory activity in CD. Test-retest variability in these was of the order of 67%, which is exclusively attributable to the variance of the collective volume of voxels in each segment exhibiting signal stronger than Liv$S_{\text{UV}_{\text{MEAN}}}$. The positive correlations of SLG and TLG with clinico-pathological markers confer some validity, and the responsiveness ratios were significant, but in the context of such poor reliability, these have little value in supporting the use of these two measures. The large test-retest variability in the volume of lesional signal is an important observation, which should be corroborated with an independent study. This large variation cannot be justified by methodological shortfalls and it is conceivable that it reflects a genuine variability in the metabolic activity of a lesion even within an interval of 7 days. However, if it holds true, it is sufficient to render SLG and TLG unusable instruments to track change of
disease activity. In addition, it would suggest that inflammatory activity in a lesion is a much more dynamic process than it is currently believed. This hypothesis cannot be readily tested in humans with current techniques, as it will require serial full-thickness sampling of Crohn’s lesions, which would be prohibitively invasive.

Three characteristics of the modality render it an unlikely candidate as a serial monitoring tool in clinical practice: Limited availability, high cost and radiation burden. However, the potential role of FDG-PET in drug development is worthy of further study. Assuming that a large luminal component of the signal is not corroborated in larger studies, future work on FDG-PET in CD should explore its potential as a surrogate endpoint of response in Phase III clinical trials. I would first propose a dual time-point study on a larger cohort of CD patients before and after anti-TNF therapy, with concurrent assessment of endoscopic activity by CDEIS at the time of the baseline and follow-up scans. Subsequently, a study on the role of FDG-PET as a predictive biomarker of response to biologics, similar in design to work published in rheumatoid arthritis can also be useful. The follow-up scan can take place as early as two weeks after treatment initiation, and the patients subsequently followed up for 6 months for clinical response and endoscopic mucosal healing.

Finally, further studies to establish the specific cellular origins of the signal in humans are of scientific interest. It is possible that the increase in signal in inflamed segments is attributable both to the increased numbers of densely packed cells in the gut wall, as well as the increased activation of various immune cell lineages. Even if a focal increase in FDG uptake purely reflects increased cell numbers in the relevant gut regions compared to adjacent uninvolved segments, it is still valuable as it reflects a relevant measure of disease activity in a quantifiable, reproducible manner that also responds to change.
5.3 ALTERNATIVE PET RADIOLOGANDS

In parallel to the FDG-PET clinical imaging study, a separate line of my research involved evaluating the expression of molecular targets for alternative radioligands, which are, ostensibly, more specific to the inflammatory pathway. Immunohistochemical assessment was performed on stored tissue to assess the mucosal as well as transmural expression of TSPO and CD25, the principal subunit of the IL-2 receptor. The findings showed that TSPO expression in normal gut is considerable but the increased expression in diseased tissue proposed by previous studies\textsuperscript{206} was not confirmed. On the other hand, transmural expression of IL-2R, showed an appreciable increase with increasing inflammatory scores, in keeping with previous studies\textsuperscript{219,240}.

Subsequent attempts were made to extend these results using radioligand autoradiography in inflamed and normal sections from prospectively recruited resection specimens. Only non-significant increases in TSPO radioligand binding to the inflamed tissue were demonstrated. Unfortunately, due to experimental limitations (in combination with the high costs limiting consumables) satisfactory autoradiographic quantification of CD25 over-expression in diseased versus normal sections was not achieved.

Several different approaches can be taken to achieve a higher degree of specific \textsuperscript{125}I-IL2 binding. A higher concentration of hr-IL2 can possibly achieve a better block. Alternatively, an antibody against CD25 may prove more successful in saturating specific sites. If this also fails to provide a specific bind, then saturation binding studies on tissue homogenates, similar to that performed with PBR28 can also be attempted. This would inform on $B_{\text{MAX}}$ and $K_d$ but would not provide details on the spatial localisation of the signal.
The results on TSPO concentrations in normal versus inflamed specimens raise interesting research questions. Firstly, the reason for this significant expression of TSPO in enterocytes, and any associations in the local immune response are certainly worth exploring. Saturation-binding studies on cultured cell populations can help specify the precise cellular origins of TSPO in intestinal tissue\textsuperscript{148,255}. From there, hypotheses on its functionality can be tested.

In contrast to TSPO, IL-2R holds more promise as a target of molecular PET imaging. The over-expression of the receptor in inflamed bowel and good co-localisation with clusters of differentiation specific to leucocytes (CD45) and T-cells in particular (CD3) suggest that the receptor can lend itself as a more accurate marker of inflammatory activity. Further work will be required to specify the binding potential of the interaction in normal versus diseased specimens and, if a difference is confirmed, static and dynamic imaging studies can be justified.

5.4 QUANTITATIVE OUTCOME MEASURES OF MAGNETIC RESONANCE IMAGING

The hypothesis that quantitative MRI measures of disease activity such anatomical activity scores, diffusion weighted imaging parameters, small bowel motility and DCE MRI measures can act as surrogate endpoints of disease response following 3 months of anti-TNF therapy were tested

5.4.1 WHAT WAS ALREADY KNOWN

Studies have shown that bowel wall thickness and signal intensity in T\textsubscript{2} sequences correlate with histologic markers of disease activity\textsuperscript{71-73}. Moreover composite scores such as MaRIA and MEGS show good correlation with endoscopic scores \textsuperscript{74,75}, and faecal calprotectin\textsuperscript{192}
respectively. Functional endpoints such as motility and DCE endpoints have shown significant differences in normal versus diseased segments. Finally, ADC, a measure of water diffusivity across tissue, has shown high sensitivity and specificity figures in identifying segments with inflammation.

To date, studies on the role of MRI as a tool to monitor disease activity in Crohn's disease are very limited. Nevertheless, at least in the UK IBD practice, there is extensive empirical use of serial MRI enterography to monitor disease progress. The only evidence that can support this practice stems from the single timepoint studies which suggest good correlation between MRI measures such as bowel wall thickness, contrast hyper-enhancement and mesenteric vascular changes and histologic measures of activity, as well as MaRIA score and endoscopic scores. The latter is still a research tool without an established role in clinical practice. Current clinical guidelines, however, highlight the lack of evidence in support of the use of MRI in this context.

One major advantage of MRI is that it allows us to serially assess disease progress not only in the mucosa but also in the full thickness of the bowel wall and adjacent mesentery. This is a major step forward in our understanding of the natural history of the disease. Our access to the mucosa through endoscopic visualisation and biopsying has been possible for over 40 years. The potential of the mucosa to 'heal' or normalise both endoscopically and histologically has been repeatedly demonstrated, and the importance of this in predicting long term disease outcomes has been the subject of intense study in recent years. In contrast to this, before the advent of cross-sectional imaging there had been no opportunity to serially assess the transmural component of the disease process. This could only be studied in resection or post-mortem specimens, which give no information on the potential of lesions to reversibility.
Before existing imaging modalities are ‘put to the test’ on their abilities to demonstrate transmural healing in these patients it is important to consider firstly whether this outcome is at all possible, and secondly determine the time-course that such an endpoint can be achieved in.

So far it has been shown that lesional bowel wall thickness remains significantly higher than that in normal bowel in patients who are in clinical remission at 8 months\textsuperscript{256}, Van Assche et al. showed that MRI appearances had completely normalised only in a small minority (2/15) of a cohort with terminal ileal involvement, following treatment with IFX for 26 weeks\textsuperscript{199}. Importantly, these two subjects were the ones with the mildest changes at index scanning. Ordas et al. showed that some improvement in most markers of transmural disease can be observed 12 weeks into therapy, and these were seen in parallel to resolution of mucosal ulcers\textsuperscript{110}. This was however a secondary endpoint of the study looking qualitatively the persistence or absence of morphological markers rather than an in-depth scrutiny of transmural scores.

5.4.2 CONTRIBUTION OF THIS PROJECT AND FUTURE DIRECTIONS

This project produces a simultaneous examination of a large number of MRI outcome measures. An assessment of morphological scores such as MaRIA and MEGS, alongside newly-proposed functional endpoints such as the ADC, DCE MR and motility measures for the characterisation of tissue responses was performed for the first time in the same patient group.

The test-retest reliability appears to be very high in most MRI measures, with the exception of DCE, which also demonstrate the poorest responsiveness scores. The validity of DCE as a marker of inflammatory activity is also questionable as evidenced by poor static and
dynamic correlation with all clinico-pathological markers of this study, and histopathological markers of inflammation reported by other studies. It appears therefore that DCE MRI is not a suitable technique to monitor disease activity over time.

The performance diffusion-weighted imaging, ADC, was somewhat more ambiguous. It showed an acceptable variability of 21% on test-retest, but the responsiveness was significantly more modest than other measures. An interesting finding that merits further investigation is the fact that almost half of diseased segments showed a drop in diffusivity within the 12-week interval. This, as well as suggestions in the literature that the measure may also reflect other aspects of the pathophysiology e.g. fibrosis, cast doubts on the validity of the endpoint for actually measuring inflammatory load. One possible way of quantifying the relative contribution of fibrosis and inflammation in the reduction of ADC in diseased segments would be a prospective study in CD patients undergoing resection. Preoperative DWI imaging can be correlated with location-matched histopathological scores of fibrosis and inflammation, as was performed for other radiographic signs that correlate with inflammatory activity and fibrostenosis.

Assessment of small intestinal motility in this small cohort shows a significant improvement in responders at 12 weeks, as well as a favourable responsiveness for the marker. This probably suggests a tendency for physiological function to restore before any of the measures of GI morphology. Another example of a physiological process that shown a rapid response to therapy is gut barrier function which has been shown to almost normalise within 4 weeks of a single anti-TNF infusion. This early signal on the longitudinal performance of motility as a marker is certainly encouraging and worthy of further studies alongside morphological measures (see below). There is probably limited applicability of the method to colonic disease, however, as contractions are much more infrequent and the free-breathing algorithms required to capture these still pose significant challenges.
Morphological endpoints such as MaRIA and MEGS, were more far more reliable than functional measures in this cohort with figures comparable to $SUV_{\text{mean}}$ in PET. Some work is still required to validate these as monitors of inflammatory load. MaRIA, has been thoroughly validated against a mucosal endoscopic marker (MH) both statically and longitudinally, and MEGS was validated against FC, which was not reproduced in this cohort. Additional studies are still required that directly correlate the change of the measures to either a concurrent or a future clinically meaningful endpoint. In this study, neither marker correlated with absolute HBI at baseline or its change over a 12-week interval. Moreover, the responsiveness of the measures was modest. Important insight was gained through a more in-depth dissection of the behaviour of the various components of these scores. More specifically, partial resolution of perimural and mural oedema was noted, while complete resolution was less frequent. Ulcers typically persisted during the three months, particularly in colonic mucosa. Mural enhancement, loss of hastrations and the length of diseased segments showed modest changes between the baseline and the follow-up scan.

Future work on morphological measures should focus on strengthening their validity by demonstrating associations with clinically robust outcomes. A sensible study in this context could be one of serial MRIs in a single pre and post-treatment cohort to decide the optimal time of correlation of MRI measures with clinico-pathological response. An extension arm where endpoints such as steroid avoidance, surgeries and hospitalisations are also considered would certainly be valuable.

In total, MRI, at least in isolation, is probably not a suitable tool to assess early response to treatment. As discussed elsewhere in this thesis, the prevailing notion in the natural history of CD is that of a constantly progressive, albeit often silent, disease process which ultimately leads to significant bowel damage.\textsuperscript{8,15} There is still therefore a need to monitor the full...
thickness of the bowel wall even at longer intervals, and hence further studies required to systematically characterise the evolution of all the morphological measures along with motility, over longer time-periods.

Finally, a significant volume of research has recently been performed in the examination of the potential benefits of combining PET and MRI modalities. There are two strategies in doing so. Firstly, PET and MRI can be performed sequentially in different scanners, and the images co-registered using appropriate software. Alternatively, the scanning can take place in new, hybrid PET-MRI machines with a novel configuration of detectors, which simultaneously acquire functional PET data and anatomical images. This technique is already finding clinical application in oncology, where more accurate TNM staging for a variety of cancers has been achieved. The newest generation of PET MRI scanners also offers a PET time of flight camera that has a significantly higher sensitivity that the PET camera used for my study.

It is highly likely that sequential or simultaneous acquisition of functional and anatomical data using these machines can produce outcome measures that combine the reliability and anatomic precision of MRI data with the responsiveness observed for several of the PET measures. Exploring this technology is an exciting prospect which could potentially lead to accurate characterisation of Crohn’s disease inflammatory activity and structural burden.
5.5 REVIEW OF EXPERIMENTAL METHODS AND LESSONS FOR FUTURE WORK

This project, in common with many others, changed direction during the study sometimes due to circumstances outside my control. In addition, as with all research projects, there are a number of aspects which in retrospect could have been performed in a different way, and which provide useful lessons in how to plan and execute similar studies in the future.

5.5.1 CLINICAL STUDY (CHAPTERS 2 & 3)

5.5.1.1 $^{18}$F-IL2

The original aim of the clinical study was to use an $^{18}$F-IL2 probe under development in Groningen University, to examine the hypothesis that this probe can quantify the differential expression of the IL-2R in Crohn’s lesions versus un-inflamed tissue using clinical PET scanning. The aim therefore was to demonstrate the differential expression of IL-2R and binding potential of radio-labelled IL-2 \textit{ex vivo} before designing a pilot, ‘proof-of-concept’ study to investigate this new radio-chemical in a cohort of Crohn’s patients.

Approximately a year into my fellowship the production at Groningen was completed. Upon attempting to replicate the production of $^{18}$F-IL2 locally, it became clear that the molecule was being split in two at some stage during its synthesis. This raised important safety issues as the pharmacodynamics of the split product would have been impossible to predict, and the clinical scanning project was abandoned pending a full review of the radio-chemical method.

Several discussions during research planning meetings concluded that this setback would have pushed the project well outside its original timeline and that a revision of the $^{18}$F-IL2
production process could not be awaited. Two decisions were made: Firstly, to persevere with the *ex vivo* component of the project, which would still be valuable once an appropriate radiotracer became available. Secondly, it was decided that the whole clinical scanning study should be redesigned, with the focus on exploring knowledge gaps in currently available techniques such as FDG-PET and MRI.

This process made me realise early in my Fellowship that major setbacks are frequent in research and that adaptability in these circumstances is crucial. I was pleased to maintain the laboratory component of the IL-2 work, even though it would not be of direct relevance to the clinical study. In retrospect, I believe that the resulting study on current FDG-PET and MRI endpoints was a more useful training experience firstly because I was exposed in the complexities of larger recruitment, and statistical and methodological considerations of longitudinal assessment, and secondly because it produced a range of important findings.

5.5.1.2 RECRUITMENT

Another important difficulty was in participant recruitment. At the planning stages of the study it was estimated that 20 of the 40 patients anticipated to start anti-TNF therapy for Crohn’s disease at Imperial College Healthcare Trust over an 18-month period of recruitment would agree to participate in our study (also deemed adequate using power calculations). In practice, early uptake by potential participants was significantly slower at about 30%. The decision was then made and ethical approval was obtained to incentivise potential participants by offering reimbursement of £170 for each of the two scanning visits. This had an immediate positive effect on recruitment rates. Moreover, during the last 5 months of recruitment, permission was obtained for an additional centre to contribute patients to the study.
A major learning point from this was that recruitment is a major factor of delay in projects with strict timelines, and strategies that can expedite it should be implemented as early as possible. Moreover, I appreciated that applications to the REC for amendments and external R&D departments to broaden the recruitment base are timely processes, which are best avoided by optimising the recruitment strategy in the planning stages of a study.

5.5.1.3 DETERMINATION OF THE GOLD-STANDARD

This was a major point of contention during the planning of the scanning study. This is an issue that is shared with all studies examining novel biomarkers in CD. One option was to use endoscopy and CDEIS scoring concurrently to the two scans. This is the best validated technique against which all the scanning endpoints could have been compared. Factors that weighed against its use were the invasiveness of the test, and as such its potential negative effect on recruitment, as well as the inability to inform on components of disease load assessed by the scanning methods such as small bowel lesions proximal to the terminal ileum or on tissue planes deeper than the mucosa.

In planning future studies, an alternative strategy to that of exclusive reliance on HBI to determine our responder and non-responder group could be a consensus panel including other markers such as CRP and FC to determine the response status of each individual patient.
5.5.1.4 VOLUME OF INTEREST PLACEMENT

The VOIs both in PET scans and MRIs were drawn by myself and then corroborated by a Consultant in Nuclear Medicine or an MRI radiologist respectively. This approach was chosen in an attempt to maximise my training in the techniques of scanning interpretation and VOI derivation. A more robust way of producing the VOIs may have been for the experts to derive theirs independently and subsequently compare these with my own in a blinded fashion. This would also have allowed a formal inter-observer variability estimation. A similar point can be made for the method of histological and immunohistochemical scoring of the biopsies by the junior and senior histopathologist.

5.5.1.5 ASSESSMENT OF LUMINAL COMPONENT OF FDG SIGNAL

The observation that the highest signal intensity is observed from the centre of an intestinal section, and the hypothesis of a luminal component of FDG signal was suggested at the later stages of the study. At that stage there was only a limited number of patients remaining who had distal distribution of CD (n=3) on which this hypothesis could be tested using the pre and post enema study. Moreover, the direct method of measuring radio-activity in eliminated bowel contents and expressing it as a fraction of the total FDG activity in that segment was only implemented in the latter two patients, while the first underwent repeat PET scanning following the enema administration which introduced significant sources of error in the interpretation.

This highlights the importance of a thorough interim analysis of the images or other data as this hypothesis could have been formulated earlier in the process, and tested on a larger number of participants, hence producing more conclusive results.
5.5.2. LABORATORY STUDIES (CHAPTER 4)

5.5.2.1 POOR DEFINITION OF THE IHC CONTROL GROUPS

In order to retrieve appropriate FFPE biopsies and sections for the immunohistochemical expression of TSPO and IL-2R, the electronic database of the Histopathology Department was interrogated for ‘findings’. GI specimens coded as ‘normal bowel’ were used as controls without assessment of the demographics or indication of the endoscopy/surgery. A potential source of error here could have been that un-inflamed samples from patients with either IBD or other inflammatory conditions may have been included in the control group masking any differences between it and the CD samples. A cross-interrogation of the endoscopic database would have ensured that normal specimens are not derived from CD patients. The strict definition of the control group is a crucial step in study design, and care must be exercised in its detailed designation.

5.5.2.2 SUBOPTIMAL OPTIMISATION OF THE TECHNIQUE OF SNAP-FREEZING

My training on tissue snap-freezing and subsequent sectioning on the cryotome was done on porcine tonsils, which were available at the lab at the time. When the first human gut specimens were collected, several difficulties arose. Firstly, the method of immersing the tissue in the isopentane/dry ice solution resulted in the folding and malrotation of the specimen. This step could not have been reversed and repeated. After several attempts, a variation of the process where the snap-freezing solution was poured over the fixed gut section was implemented, producing much better tissue orientation. Secondly, as the various gut layers are of very different consistencies, sectioning on the cryotome proved significantly more challenging than with the porcine tonsils. It would have been much
preferable if the training had taken place in animal gut specimens, as those challenges could have been resolved before working with the limited human tissue that was available.

5.5.2.3 LIMITED AMOUNT OF FRESH TISSUE

Another major limitation of the autoradiographic and homogenate binding studies was the amount of tissue obtained from each participant. My concern when applying for ethics approval was to avoid the criticism of compromising the clinical histo-pathological reporting of the specimens by removing too much tissue at the time of the operation. It subsequently transpired that the approved thickness of 5mm from each section impacted negatively on both the number of sections for autoradiography as well as the tissue that was subsequently available for homogenisation.

The lesson from this was that while it is important to demonstrate to the REC that the impact of a research project on clinical care is minimised, it is equally vital that the quality of experimental data resulting from the study is optimised. In retrospect, section thickness of 20.25mm instead of the proposed 5mm would have enabled me to produce much more robust data without impacting on the histo-pathological reporting of the specimens.

In conclusion, most of the above learning points are a testament to the importance of the planning stages of each study. The planning meetings are opportunities for weaknesses in the hypotheses, study design, methodology and proposed analysis to be identified and addressed early and effectively. If this is not achieved then their impact on the output of the project is often irreversible. An additional learning point is that adaptability during the execution of a research project is very important so that adjustments, when required, can be made early rather than late.
5.6 CONCLUSION

In conclusion, Crohn’s disease is a condition with a rapidly modifiable inflammatory component, and significant long-term structural sequelae. Through this project I attempted to demonstrate the need for new outcome measures that can improve monitoring of disease activity, assessment of therapeutic success, as well as the efficiency of the processes in new drug development. This study shows that both anatomical MRI scores as well as functional PET measures, either using FDG or alternative radioligands more specific to the inflammatory pathway, have the potential to fulfill some of these roles. These pilot results can be used as a guide to determine appropriate combinations of outcome measures and scanning intervals, in order to conduct larger studies that could further establish them as valid instruments in clinical practice or early clinical trials.
6. REFERENCES


17 November 2010

Dr Evangelos Russo
Clinical Research Fellow
Imperial College London
Clinical Imaging Centre,
Hammersmith Hospital, Du Cane Road
London
W12 0NN

Dear Dr Russo

<table>
<thead>
<tr>
<th>Study Title:</th>
<th>Investigation into the differences on the expression of Translocator Protein and Interleukin-2 in stored biopsy and tissue specimens of healthy bowel and bowel from Crohn’s Disease patients, as assessed by immunohistochemistry.</th>
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<tr>
<td>REC reference number:</td>
<td>10/H0801/59</td>
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The Research Ethics Committee reviewed the above application at the meeting held on 15 November 2010. Thank you for attending to discuss the study.

Ethical opinion

You were told that the Committee found no ethical issues with this project, you said that there was an excellent laboratory available with the pre-2006 tissue which could be used for the project, and that you did not believe that there would be any significant changes in that tissue compared to that collected post-2006.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.
be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at [http://www.rdforum.nhs.uk](http://www.rdforum.nhs.uk).

Where the only involvement of the NHS organisation is as a Participant Identification Centre (PIC), management permission for research is not required but the R&D office should be for PICs is available in IRAS. Further advice should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

**Approved documents**
The documents reviewed and approved at the meeting were:

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<th>Date</th>
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<td></td>
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<td>26 October 2010</td>
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<td>29 July 2010</td>
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**Membership of the Committee**
The members of the Ethics Committee who were present at the meeting are below.

**Statement of compliance**
The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

**After ethical review**
Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

- guidance for notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email...
s best wishes for the success of this project

Yours sincerely

pp. Dr Shelley Dolan
Chair
Email: Rosalind.cooke@imperial.nhs.uk

Enclosures: guidance for researchers

Copy to: Lucy Parker, Imperial College

South West London REC 1
Attendance at Committee meeting on 15 November 2010

Committee Members:

<table>
<thead>
<tr>
<th>Name</th>
<th>Profession</th>
<th>Present</th>
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</thead>
<tbody>
<tr>
<td>Mr Roger AHern</td>
<td>Medical Statistician</td>
<td>Yes</td>
</tr>
<tr>
<td>Dr. Sonya Babu-Narayan</td>
<td>Cardiologist Specialist Registrar</td>
<td>Yes</td>
</tr>
<tr>
<td>Mr Jeremy Butler</td>
<td>NHS Non Executive Director</td>
<td>Yes</td>
</tr>
<tr>
<td>Dr Robin Chung</td>
<td>Academic Medicine Trainee doctor and Engineer</td>
<td>Yes</td>
</tr>
<tr>
<td>Dr Shelley Dolan</td>
<td>Chief Nurse</td>
<td>Yes</td>
</tr>
<tr>
<td>Dr Adam Jacobs</td>
<td>Medical Statistician</td>
<td>No</td>
</tr>
<tr>
<td>Mr Simon Jordan</td>
<td>Consultant Thoracic Surgeon</td>
<td>No</td>
</tr>
<tr>
<td>Mr Philip Kimberley</td>
<td>Clinical Governance Information Manager</td>
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</tr>
<tr>
<td>Mrs Patricia Pank</td>
<td>Retired University Lecturer</td>
<td>Yes</td>
</tr>
<tr>
<td>Dr Nazima Pathan</td>
<td>Consultant PICU</td>
<td>No</td>
</tr>
<tr>
<td>Mrs Paula Rogers</td>
<td>Research Nurse Manager</td>
<td>No</td>
</tr>
<tr>
<td>Ms Cate Savidge</td>
<td>CT Scanning Superintendent</td>
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<tr>
<td>Dr Elliot Shinebourne</td>
<td>Consultant Paediatric Cardiologist</td>
<td>Yes</td>
</tr>
<tr>
<td>Dr Mary Taj</td>
<td>Consultant Paediatric in Oncologist</td>
<td>Yes</td>
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Also in attendance:

<table>
<thead>
<tr>
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<tr>
<td>Mrs Rosalind Cooke</td>
<td>Co-ordinator</td>
</tr>
<tr>
<td>Dr Michael Schachter</td>
<td>Clinical Pharmacologist</td>
</tr>
</tbody>
</table>
11 July 2011

Dr Evangelos Russo
Clinical Research Fellow
Imperial College London
CIC, Burlington Danes Building
Hammersmith Campus
Du Cane Road London
W12 0NN

Dear Dr Russo

Study title: Investigation into the expression of TSPO and Interleukin-2 radio-ligand binding sites in intestinal resection specimens from patients with Crohn's Disease.

REC reference: 11/LO/0380

Thank you for your letter of 29 June 2011, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a sub-committee of the REC. A list of the sub-committee members is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study:

Management permission or approval must be obtained from each host organisation prior to...
the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at [http://www.rdforum.nhs.uk](http://www.rdforum.nhs.uk).

Where a NHS organisation’s role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

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<td>03 March 2011</td>
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<td>03 March 2011</td>
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<td>Protocol</td>
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<td>RED application</td>
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<td>03 March 2011</td>
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<tr>
<td>Response to Request for Further Information</td>
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<td>03 March 2011</td>
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Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

11/LO/0380 Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

[Signature]

Dr Christine Heron
Chair

Email: kristy.randell@imperial.nhs.uk

"After ethical review - guidance for researchers"

Copy to: Ms Lucy Parker, Joint Research Office
20 August 2012

Dr Evangelos Russo
Imanova, Burlington Danes Building
Hammersmith Campus
Du Cane Road
W12 0NN

Dear Dr Russo

Study title: Longitudinal assessment of tissue responses to anti-TNFα therapy in Crohn's Disease

REC reference: 12/LO/1018

The Research Ethics Committee reviewed the above application at the meeting held on 10 July 2012. Thank you for attending to discuss the study.

Ethical opinion

The following issues were discussed with you.

i. The Committee enquired whether the PET scan and oncology would detect chemo and will not affect clinical practice. The Committee discussed the biological with you and you explained that the clinical team look at different pathways and may not stop the biological all together.

ii. The Committee queried whether the PET and CT are intended for the abdomen and chest; you explained that it will be looking at the abdomen and pelvis.

iii. The Committee queried the amount of radiation, you explained that the radiation satisfies clinical practice.

iv. The Committee queried whether the colonoscopy would be a higher risk in chromes, you reassured the Committee that it isn't and summarised the risk and procedures.

v. The Committee explained that the sharing of data with pharmaceutical companies should be in the consent form.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).
Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation’s role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

1. The PIS needs to include safety guidelines and risks including driving after having the procedure.
2. In the section ‘will my taking part in the study be kept confidential’ there is missing text which should read ‘on hospital computers’.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation.

Approved documents

The documents reviewed and approved at the meeting were:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
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<tr>
<td>Covering Letter</td>
<td></td>
<td>06 June 2012</td>
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<td>28 July 2011</td>
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<td>10 May 2012</td>
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<td>Evangelos Russo</td>
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<td>30 May 2012</td>
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<tr>
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<td>23 February 2012</td>
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<td>01 June 2012</td>
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Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/LO/1018 Please quote this number on all correspondence

With the Committee’s best wishes for the success of this project

Yours sincerely

pp

Prof David Russell-Jones
Chair

Email: nrescommittee.secoast-surrey@imperial.nhs.uk

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments
“After ethical review – guidance for researchers”

Copy to: Ms Becky Ward, AHSC Joint Research Compliance Office
NRES Committee South East Coast - Surrey

Attendance at Committee meeting on 10 July 2012

Committee Members:

<table>
<thead>
<tr>
<th>Name</th>
<th>Profession</th>
<th>Present</th>
<th>Notes</th>
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<tr>
<td>Dr Julia Boyle</td>
<td>Director Surrey CRC/Pharmacologist</td>
<td>Yes</td>
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<tr>
<td>Miss Elizabeth Cheshire</td>
<td>Consultant in Emergency Medicine</td>
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<tr>
<td>Dr Matthew Dickinson</td>
<td>Consultant Anaesthetist</td>
<td>No</td>
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<tr>
<td>Mrs Margaret Handyside</td>
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<tr>
<td>Dr Stephen Houston</td>
<td>Consultant Medical Oncologist</td>
<td>Yes</td>
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<tr>
<td>Ms Wendy Joy</td>
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<tr>
<td>Mrs Chrissie Lawson</td>
<td>Nurse Specialist</td>
<td>Yes</td>
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<tr>
<td>Dr Charles Li</td>
<td>Consultant Physician</td>
<td>Yes</td>
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<tr>
<td>Ms Georgina Marshall</td>
<td>Coordinator</td>
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<td></td>
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<tr>
<td>Mr Michael Morris</td>
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<td>Yes</td>
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<tr>
<td>Dr JHP Powell</td>
<td>Consultant Physician</td>
<td>Yes</td>
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<tr>
<td>Prof David Russell-Jones</td>
<td>Professor of Diabetes and Endocrinology</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Mrs Ann Sayer</td>
<td>Lay Member</td>
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<tr>
<td>Dr L Selby</td>
<td>General Practitioner</td>
<td>No</td>
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<tr>
<td>Dr Jane Stuart</td>
<td>Public Health Researcher</td>
<td>Yes</td>
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Volunteer Information Sheet

You will be given a copy of this Information Sheet to keep.

Radioligands for TSPO and IL2 in Crohn’s Disease

Chief Investigator: Dr Evangelos Russo

You are being invited to take part in a research study. Here is some information to help you decide whether or not to take part. Please read the following information carefully and discuss it with friends, relatives and your General Practitioner (GP) if you wish. Ask us if there is anything you do not understand or if you would like further information. Please take your time to decide whether or not you wish to take part in this study.

What is the purpose of the study?

Our aim is to investigate in the laboratory how gut cells from patients with Crohn’s Disease interact with chemical imaging agents used to demonstrate inflammation. These cells will be obtained from sections of bowel removed at operation. The agents we are investigating are already in use in the imaging of other inflammatory conditions. If our study shows that these agents attach themselves preferentially to inflamed gut cells, then we will investigate using them in scanning techniques to help detect and monitor Crohn’s Disease.

Why have I been chosen?

The reason we have approached you for this study is because you and your Gastroenterology physician have decided that the best course of action for your symptoms is to have an operation to remove a diseased bowel segment.

Do I have to take part?

No, it is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You will always be able to withdraw at any time and without giving a reason.

What will happen to me if I take part?

1. **Blood test:**
   You will be asked to donate a blood sample (maximum 50ml, 3 tablespoons) before surgery.

2. **Intestinal samples**
   Surgery to remove a diseased segment routinely involves removing all the diseased part as well as a rim of healthy bowel on each side, as surgeons always prefer joining together healthy bowel edges after resection. Parts, but not all of this diseased area and healthy rim of...
bowel are then examined under the microscope by histopathology specialists as a routine. If you agree to take part in our study, the histopathologists will provide us with one small part of the diseased section and one small part of the healthy edge which is not necessary for their analysis. We would like to emphasise that if you chose to participate in our study, this will NOT result in your surgeon removing any additional bowel during your surgery than he/she would normally remove. We would also like to reassure you that, if you decide to donate these parts of tissue for our research, this will not hinder the work of our histopathologists in any way.

What do I have to do?

To take part in the study you will need to provide us with a blood sample around the time of your operation (this can be obtained at the same time as blood tests requested by your clinical team). In addition, you have to give your permission for the pathology department of the hospital to provide us with the two specimens described above. This can be done by signing our consent form.

What is being tested?

We are testing to see if the inflamed and non-inflamed sections of your bowel wall respond differently to our imaging markers.

Where will the tests take place?

Your specimens and blood sample will be frozen and stored at the Burlington Dane Building, Imperial College, Hammersmith Hospital Campus, and analysed at Imperial College. Other onsite laboratories may also be used, such as the Biology laboratories at the GlaxoSmithKline Clinical Imaging Centre on the Hammersmith Hospital site.

What will happen to my samples after this project is completed?

Unused blood or tissue samples will be will remain anonymised (given a study code) and stored. These samples may be used in relevant future projects, provided appropriate ethical approval is granted. Your samples may be stored for a maximum of 15 years in authorized storage facilities of the university following which they will be destroyed.

What are the possible disadvantages and risks of taking part?

Blood tests can cause brief discomfort, and bruising which may persist several days.
What are the possible benefits of taking part?

You are not expected to gain any direct personal benefit in terms of improved health, fitness or sense of well-being from taking part in this study. Therefore we will not be informing our participants individually about the results of our experiments.

What if something goes wrong because of this study?

Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation. If you are harmed due to someone’s negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Evangelos Russo 0208 008 6178). The normal National Health Service complaint complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Office.

What will happen if I don't want to carry on with the study?

Since this research study is voluntary, you are free to withdraw at any time, without giving a reason. If you wish to withdraw from our study, we may still use your specimens and blood sample unless you specifically request that we destroy them.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept confidential to the extent permitted by law. If you join the study, some parts of the data collected for the study will be looked at by authorized persons at Imperial College London. They may also be looked at by representatives of regulatory authorities and by authorized people (from the Trust, other NHS bodies) to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

What will happen to the results of the research study?

We will aim to publish the results of this study in an appropriate medical journal. You will not be identified by name in any report or publication.
Who is organising and funding the research?

This research is organised by Imperial College London. The project is co-ordinated by the Chief Investigator, Dr Evangelos Russo, whose salary is paid by Imperial College London, The Wellcome Trust and GlaxoSmithKline. No member of the research team is being paid to individually recruit people.

Expenses and payments

There will be no payments for participating in this study.

Who has reviewed the study?

This research study has been reviewed by an ethics committee - a committee of people separate from your doctor, whose primary concerns are the safety, rights and welfare of patients on this study. The South East London 5 ethics committee has reviewed and approved all written materials about this study including this information sheet.

Contact for Further Information

Please ask any questions now that you wish to. A copy of this information and of the consent forms will be given to you to keep. If any questions occur to you later, or you have other concerns or would like to discuss any aspect of the study, please contact the following persons:

Evangelos Russo – 0208 008 6178

THIS INFORMATION SHEET IS VALID FOR USE UNTIL:

Signed (REC Chairman) Date
Title of project: Radioligands for TSPO and IL2 in Crohn’s Disease

Chief Investigator: Dr Evangelos Russo

CONSENT FORM

Study Number:
The participant should complete this whole sheet himself or herself.
(please initial each statement if it applies to you)

I have read the Information Sheet (version 2, 09 Feb 2011)

I have been given the opportunity to ask questions and discuss this study.

I have received satisfactory answers to all my questions.

I have received enough information about the study.

I agree that my medical information can be shared and that blood samples and tissue from my gut may be frozen and stored during the course of the study, as explained in the sheet for research participants.

I understand that sections of any of my notes may be looked at by responsible individuals from Imperial College London, the Trust or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to access my records that are relevant to this research.

I understand that I am free to withdraw from this research study at any time, without having to give a reason for withdrawing and without affecting my future medical care.

I agree to allow the research team to obtain a blood sample and two segments from the part of bowel removed during surgery, which I donate for research purposes.

The study has been explained to me by:
Prof/Dr/Mr/Mrs/Ms_________________________

I agree to take part in this study.

Signed.................................................................................Date.................................
(NAME IN BLOCK CAPITALS)..........................................

Investigator’s signature....................................................
(NAME IN BLOCK CAPITALS).......................................................... 

1 copy for participant
1 copy for investigator
1 copy to be filed in notes where relevant
Participant Information Sheet

Title: Longitudinal assessment of tissue responses to anti-TNF therapy in Crohn’s Disease

Protocol Number: CRO1973

Version: 9, 28 January 2014

Date Approved:

Invitation

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you.

A member of our team will go through the information sheet with you and answer any questions you may have. You may wish to talk to others about the study to help you make your decision. Remember that even if you agree to take part, you may change your mind at any time and leave the study without having to give a reason.

What is the purpose of the study?

This study is part of an Imperial College Research degree (PhD) on “The application of novel imaging modalities in the investigation of clinical outcomes in Crohn’s Disease”

We are trying to understand the effect the new “anti-TNF” treatment you are about to start on has on the inflammation of your bowel. We want to assess this effect by testing chemicals in your blood and your faeces, as well as doing special scans of your bowels that help us see inflammation.
Why have I been invited?

You and your gastroenterologist have decided that the best course of action to manage your Crohn’s Disease is to start one of the powerful “anti-TNF” medications. To help us understand how to better use this kind of medicine in the future, we are studying its effects on chemicals in the blood and on special kinds of images. As you are just about to start one of these medicines, we are inviting you to participate in our study so that we can watch how things change over the first weeks that the medicine is used.

Do I have to take part?

No. It is up to you to decide if you want to take part in this study. If you decide to take part you will be asked to sign a consent form. You will be given a copy of this for you to keep.

Your General Practitioner (GP) will also be informed that you have volunteered for this study and will be asked if he/she knows any reason why you should not take part.

You would still be free to withdraw from the study at any time.

The investigator or study doctor also could ask you not to participate in the study if he or she feels that your health might be affected by the study, if useful results will not be able to be obtained or for other administrative reasons.

What will happen to me if I take part?

This study involves your participation on 5 different occasions.

Screening Visit
Outpatient department of your treating hospital or Imanova Imaging Centre.

If you agree to take part in this study you will be asked to come to a screening visit, when we will discuss any medical problems you have had and give you a brief medical examination. We will ask you about your health, symptoms, information about any medications you are taking, smoking of cigarettes, use of alcohol and drugs, as well as the history of any similar disorders in your family. In addition, during screening, blood and urine samples (including a urine pregnancy test) will be taken for routine safety tests. No more than 30ml of blood (5 teaspoon) will be obtained at this visit. We will also ask you to bring a stool sample on the day, in a specialised contained which will be provided in clinic. Finally, we will give you a brief questionnaire and examine you to assess the severity of your Crohn’s. The whole of the screening visit should not last longer than 60 minutes in total.

If no problems are identified that would prevent you from safely contributing useful results for the study, you will be invited to come to the Imanova Imaging Centre at
the Hammersmith Hospital in London for two scanning visits. Wherever possible, for your convenience, we will try to combine the Screening Visit and the Scanning Visit 1 (see below), and they will both take place at the Imaging Centre.

Scanning Visit 1
Imanova Imaging Centre, Hammersmith Hospital campus

This visit will take place within 2 weeks of your screening visit. It will be at the Imanova Imaging Centre based at Hammersmith Hospital. It should last no longer than 5 hours and will involve the following:
1) Being registered as a patient at Hammersmith Hospital. The research team will organize this for you
2) A repeat of the same questionnaire and physical examination to re-assess the severity of your Crohn’s disease at the time of the visit
3) A blood test (no more than 20ml or 3.5 teaspoons)
4) A stool test (You will be asked to bring in a sample on the day)
5) A urine pregnancy test (if positive then no scanning will take place)
6) A positron emission tomography or “PET” scan
7) An Magnetic Resonance Imaging or “MRI” scan (optional)

Scanning Visit 2
Imanova Imaging Centre, Hammersmith Hospital campus

This will take place either 6 or 12 weeks after starting your new anti-TNF treatment. It will be identical to the first scanning visit.

Study Visit 3
Imanova Imaging Centre or Outpatient department of your treating hospital

This will take place approximately 4 months after starting the anti-TNF treatment and it can take place either at Imanova Imaging Centre or the outpatient clinic of the hospital where your Crohn’s Disease is looked after. No additional scanning will take place at this visit. It should last no longer than 1 hour and will involve the following

1) The same questionnaire and physical examination to assess the severity of your Crohn’s disease
2) A blood test (no more than 20ml or 3.5 teaspoons)
3) A stool test (You will be asked to bring a sample on the day)
4) A discussion around the medication you have taken for your Crohn’s

Final Study Visit
Endoscopy Department of your treating hospital
This visit will take place approximately 6 months after the anti-TNF treatment has started. It may happen sooner if your physician decides that the treatment is not helping you enough to continue. It will happen at the endoscopy department of the hospital where your Crohn’s Disease is looked after. It should last no longer than 4 hours, although the colonoscopy itself takes approximately 40 minutes. It will involve the following:

1) The same questionnaire and physical examination to re-assess the severity of your Crohn’s disease
2) A blood test (no more than 40ml or 7 teaspoons)
3) A stool test (You will be asked to bring a sample on the day)
4) A colonoscopy
5) A discussion around the medication you have taken for your Crohn’s

What does the PET Scan involve?

“PET” or Positron Emission Tomography relies on a sensitive machine that can “see” small amounts of radiation released from a sugar molecule (FDG or [18F]fluorodeoxyglucose), which we will inject into your body to be taken up in areas of inflammation in your bowel. When this sugar molecule is taken up by the cells, the small amounts of radiation released can be detected by the PET scanner to produce three-dimensional images showing us where they are in the body.

Before the beginning of the PET scan, you will have a “cannula” inserted in a vein. Cannulae are very small (about the thickness of a piece of string), flexible plastic tubes which are inserted through the skin into a blood vessel to make it easier to withdraw blood samples or inject a medicine.

After the cannula has been inserted, approximately 20ml of blood will be withdrawn for tests and then you will be injected with the sugar molecule known as FDG and then you will ask to rest in a quiet room for 60 minutes, while it is absorbed in the tissues. During that time you will be asked to drink approximately 800ml (1 and one half pints) of a contrast solution that helps to open up the small bowel so that it is easier to see in the scan. Once the 60 minutes have passed, and before you are moved into the scanner, you will be given a medicine (buscopan) through the cannula that stops the bowel from contracting during the scan.

Immediately afterwards, you will be placed on the scanning bed of the PET scanner. Soft foam pads will be placed around your head and upper body to make you more comfortable and to help you to remain still during the scan. Once you are comfortable, the bed will be moved into the scanner.

While you are lying still in the scanner, you may experience stiffness, discomfort from the pressure of the bed or mild backache. The foam pads provided should help to reduce this and the staff will help you to gently shift your position, if necessary. Staff will always be on hand to help you and to minimize any discomfort.
The PET scan will last up to 30 minutes, during which you should try to keep as still as possible. If necessary, the scan can be interrupted in order to have a break.

On some occasions, where radioactivity accumulates in the lower end of the bowel we may want to find out if this is coming from bowel tissue or if it’s mixed in with the stool. The study doctor may therefore ask for your an enema. This will bring about a bowel movement. Subsequently, when you’re ready, we will invite you to re-enter the scanner for another 10 minutes. This component of the PET scan is entirely optional.

For the rest of the day, it is advised that you drink plenty of fluids (about 1 and one half pints), and you may eat as normal. You should empty your bladder frequently. For the rest of that day only, the sugar molecule injected into your body will be giving off very tiny amounts of radiation, so you should not sit next to pregnant women or children for long periods.

What does the MRI Scan involve?

The MRI part of this study is optional and you can decide not to have an MRI scan and still take part in the study.

MRI is a special technique that uses powerful magnets, radio waves and computers to produce detailed images (or scans) of the inside of the body. If you agree to take part in the MRI part of the study after the PET scan you will be asked to drink an additional of up to 1600ml (approximately 3 pints) of the same contrast solution, or as close to that amount as you can tolerate. After approximately 90 minutes you will be asked to lie on your back on a table, which slides inside a cylinder-shaped machine. The radiographer may use pillows or straps to adjust your position and help you to stay still. You will then be placed in the middle of the scanner. The machine is open ended so you won’t be completely enclosed at any time. The tunnel in the scanner is just over one metre (about four feet) long.

Your radiographer will operate the scanner from behind a window and will be able to see and hear you during the scan. The MRI scanner makes a loud knocking or buzzing sounds throughout the scan. You will usually be given earplugs or headphones to wear, and you can listen to music during the scan. It can take several minutes for each image to be taken, so it's important to lie very still and breathe gently. Your radiographer will ask you to hold your breath at certain times during the scan.

After the first series of scans, you will be given another dose of buscopan through the cannula and undergo another short set of scans in the MRI machine. The final dose of buscopan will then be given, together with a special dye (contrast medium) called gadolinium, also injected through your cannula. This dye is used during the scan to produce more detailed images. You will then be asked to undergo another series of scans and again your radiographer will ask you to hold your breath at certain times during the scan.
After the end of the scan, the cannula will be removed from your arm. After completion of the MRI you will usually be able to go home when you feel ready. None of the procedures that take place during the scanning visit should interfere with your ability to drive home if you want to.

What does the colonoscopy involve?

A colonoscopy is a test where a doctor looks into your large bowel. It is very likely that you would have had one or more in the past as part of your Crohn’s diagnostic work-up.

Colonoscopy is usually done as an outpatient or day case. It is a routine test which is commonly done. You will usually be given a sedative to help you to relax. This is usually given by an injection into a vein in the back of your hand. The sedative can make you drowsy but it does not ‘put you to sleep’. It is not a general anaesthetic.

You lie on your side on a couch. The operator will gently push the end of the colonoscope into your anus and up into the colon. Modern colonoscopes transmit pictures through a camera attachment on to a TV monitor for the operator to look at.

The operator may take biopsies (small samples) of some parts of the inside lining of the colon - depending on why the test is done. This is painless. The biopsy samples are sent to the lab for testing, and to look at under the microscope. At the end of the procedure the colonoscope is gently pulled out.

You should get instructions from the hospital department before your test. The sort of instructions given include:

- The colon needs to be empty so that the operator can get a clear view. You will be instructed on how to take a special diet for a few days before the test. You will also be given some laxatives to take.
- You will need somebody to accompany you home, as you will be drowsy with the sedative.

As you may remember from previous colonoscopies, we do request that an adult friend or relative is available to accompany you home after the test, unless you select not to have any sedation during the procedure. We would advise that you refrain from driving on the day of your procedure, until the sedatives are cleared from your system.

What are the possible disadvantages and risks of taking part?

- The PET molecule
FDG is a PET molecule used extensively in clinical practice. It is used in very small amounts and you should not feel different after receiving it. It has an excellent safety profile.

• PET Scanning and Radiation

Each PET scan will involve exposure to radiation. It is a very small dose of radiation – about the same as you would get naturally from the environment in three years. The exact amount you will receive throughout both scans is estimated at 11.2 mSv. If you agree to be re-scanned following an enema, this will add 1.2mSv to the additional dose, bringing the total up to 12.4mSv. For comparison purposes, the UK legal limit set by the Ionising Radiations Regulations (1999) for a classified person who works with radiation to be exposed to in any given year is higher, at 20mSv.

However, this radiation does carry a very small, theoretical risk of increasing your chance of getting cancer. Cancer is common: 1 in 4 people – 25% - will die of cancer. Your exposure could increase your risk by 0.05% (i.e., five of one hundred parts of 1%) so that your theoretical risk could rise from 25% to 25.05%. Although this statistical risk exists, follow up studies of people who work with radiation and who are exposed to bigger doses have found no increased cases of cancer.

If you were to participate in further studies which involve the use of radiation you should inform the study team that you have been exposed to up to 12.4mSv of ionising radiation in this study.

**IF YOU PARTICIPATE IN OUR STUDY, WE ADVISE THAT YOU SHOULD NOT PARTICIPATE IN FURTHER RESEARCH STUDIES INVOLVING IONISING RADIATION FOR THE NEXT 3 YEARS**

• Administration of the enema

An enema is fluid that is placed in the rectum through the anus (back passage) to clear the bowel. The phosphate enema that you will be given is a single dose disposable enema that will clean the section of your bowel that will be re-scanned subsequently.

It is usually very well tolerated. Rare side-effects include rectal bleeding, blistering, burning and itching.

• MRI SCAN

An MRI is a very safe test and there are no known complications or side-effects from the magnetic field used during the scan. You may feel slightly claustrophobic and uncomfortable from being inside the scanner. In addition if you have any metal implants for example, heart pacemaker, an inner ear hearing aid (cochlear implant), an intra-uterine contraceptive device or coil, shrapnel or gunshot wounds, a body piercing or tattoos that have been done using a metallic
ink you may not be able to have a MRI scan. The contrast solution you will
drink prior to the MRI may give you short-lived diarrhoea. This isn't a complete
list - your radiographer will go through a safety checklist with you before the
scan.

• MRI dye (gadolinium)

It's possible to have an allergic reaction to the dye used, though this is very
unlikely. If you have any itching or shortness of breath during the scan, tell your
radiographer immediately. Medicines are available to treat the allergic reaction.

Patients with severe kidney disease and those who have had, or who are awaiting,
liver transplantation are at risk of a disease called nephrogenic systemic fibrosis
(NSF) after gadolinium administration, this can cause, skin pains and blisters
amongst other symptoms and possibly death. You will be screened before
scanning to make sure you do not have kidney disease. If you do not have kidney
disease, then you are at no increased risk of developing this condition.

Other common side effects of the dye may include; dizziness, headache, a change
in taster sensation, vomiting, nausea, pain, feeling hot, feeling cold and problems
at the injection site which could include; coldness, swelling, warmth, pain and
irritation. Ask your radiographer to explain how these risks apply to you. If
you're worried about this or anything to do with the MRI scan please talk to your
radiographer.

• Cannulation and blood sampling

Insertion of a cannula into a vein may cause brief discomfort as the cannulae
penetrates the skin (like a needle), but there should be no further discomfort.

Risks of any cannulation include minor local bleeding and bruising. Very rarely, a
blood clot could form around the cannula.

A maximum of 200 mL (approx 11 tablespoons) of blood will be taken during the
whole study, and this will be done through the iv cannula wherever possible

• Intravenous buscopan

Buscopan is a drug given intravenously to prevent the bowel from contracting
during the scan. Its effects are very short-lived, and therefore the dose will have
to be repeated during the scan. Just after you have a dose, you may experience
mouth dryness for a few minutes, or, more occasionally, your heart rate may go
up for a few minutes after each dose. We will be asking you if you have been
shown to suffer from raised eye pressure (glaucoma) in which case we will not be
giving you buscopan.
• Colonoscopy

As with every procedure, there are some risks associated with colonoscopy.

Side-effects: These are the unwanted, but mostly temporary effects you may get after having the procedure. After having a colonoscopy you may feel bloated and uncomfortable due to trapped wind. You may find that lying on your front can sometimes help. Trapped wind usually passes after a few hours. You may also bleed a little from your back passage if you have had a biopsy or polyp removed.

Complications: Complications are when problems occur during or after the procedure. A very small minority - far less than 1% - are affected by any of these complications listed below.

• You may have a reaction to the sedation, which can affect your breathing or your heart. You will be monitored throughout the procedure and treated quickly if these problems develop.
• The colonoscope and the other instruments used during the procedure can cause a tear in the bowel. This happens in less than 1 in 1000 people who do not have any polyps removed during colonoscopy. If this happens you may need an operation to repair it.
• You may have heavy bleeding if you have had biopsies or polyps removed.

Are there any lifestyle restrictions?

If you are a woman of childbearing potential, or a man whose female partner is of childbearing potential, you should follow the guidelines below to ensure that pregnancy is avoided during the course of this study. From the time of the first PET scan until 2 weeks after the second PET, you should either be sexually inactive, or use one of the contraceptive methods listed below.

• Oral contraceptive
• Injectable progestogen
• Implants of levonorgestrel
- Estrogenic vaginal ring
- Percutaneous contraceptive patches
- Intrauterine device (IUD) or intrauterine system (IUS)
- Double barrier method: condom and occlusive cap (diaphragm or cervical/vaultcaps) plus spermicidal agent (foam/gel/film/cream/suppository)

**What are the possible benefits of taking part?**

This study will not help you, but the information we get from this study may help improve the way that we care for people with Crohn’s disease in the future. The screening tests may be of benefit to you if we find an important medical problem, but they could reveal something you would prefer not to know about. Imperial College will be the owner of the study results and may use the results to get patents, or make a profit in other ways. You will not be paid any part of this.

**Will I receive payment for being part of this study?**

You will receive reimbursement for all your travel expenses, as well as meals / refreshments during each visit. In addition, you will receive compensation of £170 for each of the two scanning visits.

**What if something abnormal is picked up on the scans?**

The PET scan and MRI (if you undergo this) will be over and above the imaging you would normally have as part of your usual care. The images of your bowel obtained for this project will therefore not be formally reported by a radiologist. However, if these images pick up anything which could impact on your future health and well-being, the images will be sent for review by a radiologist. If the abnormality is felt to be potentially important, a member of the research team will contact your GP, so they may arrange further investigation if necessary. We cannot guarantee however that an abnormality (if present) will definitely be detected.
What if the blood tests show an unexpected abnormality?

Similarly, if the blood tests are abnormal we will inform you and inform your GP so that you can get appropriate advice or treatment.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions:

   Dr. Evangelos Russo: 07733 236009
   Prof Tim Orchard: 0776 9606039

If you feel unwell, you can contact the Principal Investigator, Dr. Evangelos Russo, to ask for advice. If the situation will require further assistance, you may be suggested to contact your GP, or, in some cases, to go to an A&E department. If the situation is serious or potentially life threatening, you will be suggested to dial 999 and call for an ambulance.

What if something goes wrong?

Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator Evangelos Russo. The normal
National Health Service complaint complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

**Will my taking part in the study be kept confidential?**

Yes. All information which is collected about you during the course of the research will be kept strictly confidential. This information is covered by the Data Protection Act and will be stored on hospital and university as well as research centre computers protected by passwords and data encryption. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. A sheet of paper with your name and date of birth linking you to your study ID will be kept in a locked drawer in a room accessible by swipe card only within Imperial College and will be accessible only to the research team doing the study.

The radiographers at Imanova Imaging Centre will have your name entered into the Radiology Information System so that they can keep a record of who is scanned but this information will be restricted to authorised individuals. Participants in the study will need to be registered as patients with the Hammersmith Hospital as part of the agreement for resuscitation cover. We will also contact your GP by letter to inform them of your participation. We will inform your GP for you if we find anything clinically abnormal in the blood tests or on the scans. Research studies are monitored to make sure that the research is conducted ethically and properly. People involved in monitoring the research study may have access to your personal information.

**How long is the study data kept?**

Imperial College London will keep the data in anonymised form for 10 years and it may be used in other studies. Imanova will keep the data in anonymised form for 15 years.

**What will happen to the images obtained and any samples I give?**

The PET and MRI images and associated information will be anonymised and studied by researchers at the Imanova and Imperial College. To ensure the greatest benefit from your contributions, we also may share anonymised images and associated information with scientists at other universities and companies including
GlaxoSmithKline, which has contributed partial support for this study and which carry out related research in other centres.

As mentioned earlier, if you choose to take part in this study, you will be asked to give blood, urine and stool samples. Blood samples will be collected by trained staff. Urine and stool will be collected by you, as instructed by study nurses.

Some tests will be performed by the study team at Imperial College or within the Imanova Imaging Unit, which is on the Hammersmith Hospital site. We will also ask other organisations (such as a university, hospital or company) to perform other parts of the tests. However, when your samples are transferred, any labelling that might allow you to be identified will be carefully removed. These samples will then be frozen and stored ready for analysis. Your samples will be kept in locked storage and may be stored for up to 10 years from the end of the study. Any sample remaining at that time will be destroyed.

If in the future you decide to withdraw from the study, your samples will be stored and analysed unless you ask to destroy them.

**What will happen to the results of this study?**

A brief summary of the results will be made available to the treatment service you are attending. The data and results of this study also will be shared with other scientists, although your name will not be used and you will not be otherwise identified in doing this or on any report or publication related to the study. The results of this study will be presented at scientific conferences and published in a scientific journal (we expect this to happen about one to two years after the end of the study). Data obtained from your blood tests, and scanning data may be used in an anonymous form for future research. You will not be contacted by anyone carrying out such research and they will not be given access to your medical records.

**What will happen if I don’t want to carry on with the study?**

You can withdraw from the study at any point without the need to give a reason. If you choose to withdraw from the study at any point there will be no penalty or loss of benefits. Any medical care you receive outside of the study will not be affected. Information collected may still be used. Your stored blood or tissue samples will still be analysed unless you ask us to destroy them.

**Who has reviewed the study?**
All research is looked at by an independent group of people called a Research Ethics committee, to protect your interests. This study has been reviewed and given approval to continue by the South East Coast- Surrey Research Ethics Committee.

Further information and contact details

If you have any questions or concerns about this study you can contact the Principal Investigator Evangelos Russo on 07733236009. If you do experience an adverse event which you or your family consider serious or potentially life threatening dial 999 and call for an ambulance. Do not delay treatment by trying to contact the doctor in charge of the study.

If you would like further information about research or advice as to whether you should take part you can contact the NHS Patient Advice and Liaison Services (PALS). Or alternatively you can discuss with your GP or consultant if you should enter the study.
# Consent Form

Patient identification number for this trial:

Study number/Title of project: CRO1973/. Longitudinal assessment of tissue responses to anti-TNF therapy in Crohn’s Disease

Name of Researcher: Evangelos Russo

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<td>1.</td>
<td>I confirm that I have read and understand the information sheet dated 28 January 2014 for the above study. I have had the study sufficiently explained to me and had the opportunity to consider the information, ask questions and have had these answered satisfactorily.</td>
<td>Please initial box</td>
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<td>2.</td>
<td>I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason without my medical care or legal rights being affected. In the event of my withdrawal, I understand the research team still analyse any data already obtained during my participation, unless I instruct them to destroy it.</td>
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<td>3.</td>
<td>I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from Imperial College London, Imanova, regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.</td>
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<td>4.</td>
<td>I understand that my anonymised images/anonymised data may be shared with Scientists at other universities and companies (including GlaxoSmithKline), here and in other countries.</td>
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<td>5.</td>
<td>I agree to my GP being informed of my participation in the study and I authorise my GP to disclose details of any relevant medical or drug history in confidence.</td>
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<td>6.</td>
<td>I agree that the Unit/Study Physicians may notify my General Practitioner of any abnormalities detected during the study after discussing these with me and that the Physician may consult with other Imanova and non-Imanova staff as appropriate for my care should I suffer a serious adverse event during the study.</td>
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<td>7.</td>
<td>I have read and understand the compensation arrangements for this study as</td>
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8. I agree to be registered at the Hammersmith Hospital as a patient.

9. I have read, understand and agree to the study restrictions as specified in the information sheet.

10. I have read, understand and agree to the contraception requirements for the duration of the study.

11. I agree that my blood samples may be collected, frozen, tested and stored as explained in the sheet for research participants. I understand that my blood samples could be transferred outside the European Union. I agree that my samples may be stored for use in ethically approved projects in the future.

12. It has been explained to me that the procedures and/or compounds being tested in this study may involve risks to me which are currently unforeseeable.

13. I am not participating in any other studies and understand that other drug trial units may be contacted about my participation in/registration for studies elsewhere. I understand that I will be registered on The Over-volunteering Prevention System (TOPS) at screening, to record my intention to participate in this study.

14. I agree to take part in the above study.

15. I agree to take part in the optional MRI part of the study.

16. I agree to take part to the optional repeat PET scan following an enema if requested.

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