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ABSTRACT

Crohn's disease (CD) is a chronic and relapsing inflammatory condition of the gut. Diagnostics of small bowel CD is difficult, as conventional endoscopy can only reach the proximal and distal ends of the small bowel. Cross sectional imaging lacks sensitivity, whereas small bowel capsule endoscopy (SBCE) has limited specificity. Positron emission tomography (PET)-imaging using 18-fluorodeoxyglucose ([^{18}F]-FDG) can be used to evaluate the glucose metabolism of different tissues. Combining PET-imaging with magnetic resonance enterography (MRE) yields exact anatomical data that localizes increased glucose metabolism that is caused by inflammation.

The aim of this thesis was to improve both the diagnostics and follow-up of small bowel CD. [^{18}F]-FDG PET-MRE was performed on patients with suspected small bowel CD and patients diagnosed with CD had a follow-up [^{18}F]-FDG PET-MRE to assess their response to medical therapy. The accuracy of [^{18}F]-FDG PET-MRE was compared to MRE, endoscopic and biochemical findings. In addition, predictive clinical and biochemical markers of CD were investigated in a retrospective study conducted using patients who had previously undergone SBCE for suspected small bowel CD.

Higher standardized uptake values (SUVs) were measured in patients with CD. A higher SUV predicted the need for more advanced therapies. In this unique setting with a follow-up [^{18}F]-FDG PET-MRE, SUV decreased significantly thus depicting a clinical response to treatment. Patients with low fecal calprotectin, negative cross-sectional imaging results, and no endoscopic ileitis had a very low probability of CD. Thus they may be monitored without SBCE.

No single gold standard method exists for the diagnostics of small bowel CD, but the use of [^{18}F]-FDG PET-MRE is potent in diagnostics, and even in predicting disease severity. The findings presented in this thesis aid in the diagnostics of small bowel CD.

KEYWORDS: Crohn's disease, fusion imaging, IBD, PET-imaging, PET-MRI, SBCE, small bowel capsule endoscopy

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Sisätaudit
JUHO MATTILA: Fuusio-PET-MRI ja ohutsuolen kapseliendoskopia ohutsuolen Crohnin taudin diagnostiikassa ja seurannassa
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TIIVISTELMÄ

Crohnin tauti (CD) on pitkäaikainen ja uusiutuva tulehduksellinen suolistosairaus. Ohutsuolen CD:n diagnostiikka on vaikeaa, sillä tavanomaisilla tähystystoimenpiteillä pystytään tutkimaan ohutsuoli vain osin. Leikekuvantamisen herkkyys on rajallinen ja ohutsuolen kapselikamerakuvauksessa (SBCE) tarkkuus on rajallinen. Positroniemissiotomografiakuvantamisella (PET) 18-fluorodeoksiglukoosia ([^{18}F]-FDG) merkkiaineena käyttäen voidaan arvioida kudosten glukoosiaineenvaihduntaa. Yhdistämällä PET-kuvantaminen magneettienterografiaan (MRE) saadaan tarkkaa anatomista dataa tulehduksen aiheuttaman kiihtyneen glukoosiaineenvaihdunnan paikallistamiseksi.

Väitöskirjatyön tarkoituksena oli parantaa sekä ohutsuolen CD:n diagnostiikkaa, että seurantaa. Teimme [^{18}F]-FDG PET-MRE:n potilaille, joilla epäiltiin ohutsuolen CD:a ja potilaille, joilla CD todettiin, tehtiin uusi [^{18}F]-FDG PET-MRE lääkehoitovasteen arvioimiseksi. [^{18}F]-FDG PET-MRE:n tarkkuutta verrattiin MRE:n, endoskooppisiin ja biokemiallisiin löydöksiin. Lisäksi tutkimme retrospektiivisesti kliinisiä ja biokemiallisia ennusmerkkejä ohutsuolen CD:n potilailta, joille oli tehty SBCE epäillyn ohutsuolen CD:n vuoksi.

Havaitsimme korkeampia merkkiainekertymiä (SUV) potilailla, joilla todettiin CD. Lisäksi korkeampi SUV näytti ennustavan tarvetta edistyneille lääkehoidoille. Tässä ainutlaatuisessa tutkimusasetelmassa, jossa hoitovastetta seurattiin [^{18}F]-FDG PET-MRE:llä, SUV laski merkittävästi kuvastaen kliinistä vastetta lääkehoidolle. Potilaita, joilla oli matala ulosteen kalprotektiini, negatiiviset kuvantamis- ja endoskopialöydökset, oli hyvin matala todennäköisyys ohutsuolen CD:n ja heitä voidaan näin ollen turvallisesti seurata ilman SBCE:ta.

Ohutsuolen CD:n diagnostiikka on edelleen vaikeaa, sillä mitään yksittäistä herkkää ja tarkkaa tutkimusmenetelmää ei ole. [^{18}F]-FDG PET-MRE osoittautui lupaavaksi menetelmäksi sekä diagnostiikkaan, että tautiaktiivisuden seurantaan ja voi jopa ennustaa taudin vaikeusastetta. Tämän väitöskirjan tutkimustulokset tarkentavat ohutsuolen CD:n diagnostiikkaa.

AVAINSANAT: Crohnin tauti, fuusiokuvantaminen, IBD, PET-kuvantaminen, PET-MRI, SBCE, kapselikamerakuvaus

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Abbreviations

[^{18}F]-FDG	18-fluoride-fluorodeoxyglucose
^{11}C	carbon-11-isotope
^{13}N	nitrogen-13-isotope
^{15}O	oxygen-15-isotope
2D	2-dimensional
3D	3-dimensional
3T	3 tesla
ACG	American College of Gastroenterology
ADA	anti-drug-antibodies
AGA	American Gastroenterological Association
Alb	Albumin
anti-CBir1	anti-flagellin antibody
anti-OmpC	anti-outer membrane porin C
anti-TNF	anti-tumor necrosis factor alpha
APC	antigen presenting cell
ASCA	anti-Saccharomyces cerevisiae-antibody
ATG16LI	autophagy related 16 like 1 gene
BH	breath hold
CCR9	C-C chemokine receptor type 9
CD	Crohn's disease
CD127	cluster of differentiation 127
CD4	cluster of differentiation 4
CD56	cluster of differentiation 56
CDED	Crohn's disease exclusion diet
CD-group	patients diagnosed with Crohn's disease
CECDAI	capsule endoscopy Crohn's disease activity index
CI	confidence interval
CRP	C-reactive protein
CT	computed tomography
DNA	deoxyribonucleic acid
DWI	diffusion weighted imaging

EANM	European Association of Nuclear Medicine
ECCO	European Crohn's and Colitis Organisation
ECM	extracellular matrix
EEN	exclusive enteral nutrition
EIM	extraintestinal manifestation
ESR	eryhtrocyte sedimentation rate
FC	fecal calprotectin
FIESTA	fast imaging employing steady-state acquisition
FMT	fecal microbiota transplantation
FODMAP	fermentable oligosaccharides, disaccharides, monosaccharides and polyols
GI	gastrointestinal
GLUT	glucose transporter
GWAS	genome-wide association study
HR	hazard ratio
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IFN	interferon
IgG1	immunoglobulin-G1
IL	interleukin
ILC	innate lymphoid cell
IQR	interquartile range
IRGM	immune related GTPase-M
IUS	intestinal ultrasound
JAK	Janus kinase
keV	kiloelectron volt
LIFT	ligation of intersphincteric tract
LMR	lactulose-mannitol ratio
LOR	line of response
MAdCAM	mucosal vascular adressin cell adhesion molecule
MaRIA	magnetic resonance index of activity
MBq	megabecquerel
MHC	major histocompatibility complex
MRAC	magnetic resonance attenuation correction
MRE	magnetic resonance enterography
MRI	magnetic resonance imaging
MUC1	mucin-1
MUC2	mucin-2
NF-κB	nuclear factor kappa B
NK	natural killer

NLR	nucleotide binding domain like receptors
NOD	nucleotide binding domain
Non-CD-group	patients not diagnosed with Crohn's disease
NPV	negative predictive value
NSAID	nonsteroidal anti-inflammatory drugs
OR	odds ratio
OmP	outer membrane porin
PAMP	pathogen-associated molecular pattern
PD	percutaneous drainage
PEG	polyethylene glycol
PET	positron emission tomography
PPV	positive predictive value
PRR	pattern recognition receptors
PSC	primary sclerosing cholangitis
PVE	partial volume effect
Q1	quartile 1
Q3	quartile 3
RCT	randomized controlled trial
RF	radiofrequency
RNA	ribonucleic acid
ROC	receiver operator characteristic
SBCE	small bowel capsule endoscopy
SD	standard deviation
SES-CD	simple endoscopic score for Crohn's disease
SGLT-1	sodium glucose cotransporter-1
sMARIA	simplified magnetic resonance index of activity
SUV	standardized uptake value
TAC	time activity curve
TE	echo time
TGF	tissue growth factor
Th	T-helper
TLR	toll-like receptor
TNF-alpha	tumor necrosis factor alpha
TR	repetition time
UC	ulcerative colitis
WBC	white blood cell

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Mattila J,. Stenholm T,. Löyttyniemi E,. Koffert J. Predictive Markers of Crohn's Disease in Small Bowel Capsule Endoscopy: A Retrospective Study of Small Bowel Capsule Endoscopy. *Journal of Clinical Medicine*, 2022; 15: 4635.

II Mattila J,. Kallio J,. Löyttyniemi E,. Nuutila P,. Koffert J. Combined [^{18}F]-Fluorodeoxyglucose Positron Emission Tomography-MR Imaging: A Promising Tool for Diagnostics of Small Bowel Crohn's Disease. *Digestive Diseases,* 2024; Nov 13:1-11.

III Mattila J,. Kallio J,. Löyttyniemi E,. Nuutila P,. Koffert J. Combined [^{18}F]-FDG PET-MR Imaging can predict clinical remission in small bowel Crohn's disease. *Manuscript*.

1 Introduction

Crohn's disease (CD) is a chronic inflammatory condition of the gastrointestinal tract characterized by progression and flares. The incidence of inflammatory bowel diseases (IBD) in Finland is rapidly rising. The number of patients diagnosed with IBD has increased by 2.4% per annum from 2000-2020. On a population basis, 1% of people in Finland already suffer from IBD.[1] The disease is typically progressive; chronic inflammation leads to complications such as fibrosis. Regular medical treatment and follow-up is needed for most patients. Early medical intervention is linked to better long-term outcomes and fewer complications.[2]

CD can affect the whole gastrointestinal (GI) tract from the mouth to the anal canal, but it can also be isolated in the small intestine. This makes the diagnostics challenging, as only the proximal and distal regions of the small intestine can be reached by conventional endoscopy. Cross-sectional imaging such as computed tomography (CT) or magnetic resonance enterography (MRE) can be used in diagnostics of severe CD[3–5], but their sensitivity is limited in luminal disease; in 43-60% of the patients the diagnosis can not be established.[6] With small bowel capsule endoscopy (SBCE), the whole small intestine can be visualized, but this method lacks specificity, as healthy individuals also have mucosal erosions in their small intestine.[7] SBCE is also a time-consuming and costly procedure, and it comprises the risk of capsule retention. Choosing the right patients for SBCE is demanding, as symptoms of CD often resemble those of e.g. functional disorders of the gut and a quarter of patients with IBD in remission suffer from IBS (irritable bowel syndrome) -symptoms.[8] Due to the progressive nature of CD, the diagnosis should be established as soon as possible to start medical intervention.[2]

Positron emission tomography (PET)-imaging using 18-fluorodeoxyglucose ([^{18}F]-FDG) can be used to evaluate glucose metabolism of different tissues in the human body. Increased glucose metabolism is caused by inflammation, infection or tumors. PET-imaging can be combined with MRE to add exact anatomical data to enable measurement of glucose metabolism (standard uptake value, SUV) from even small locations such as the mucosa of the small intestine.[9,10] However, there is very limited data on the use of fusion PET-MRE in diagnostics and follow-up of small bowel CD and no diagnostic thresholds exist to date. Up till now, no single gold

standard method exists for diagnostics of small bowel CD, so there is a true demand for better diagnostic measures.

2 Review of the Literature

2.1 Crohn's disease

2.1.1 Epidemiology

CD and ulcerative colitis (UC) are both IBDs, which are idiopathic inflammatory bowel disorders.[11] The incidence and prevalence of IBD have increased worldwide in the past 50 years.[12] Both incidence and prevalence rates are high especially in the Western Countries[13] and these diseases are becoming a global burden to healthcare.[14] Although some reports indicate a plateau phase in the incidence in the Western countries in the 21st century[15], the incidence is still rapidly rising in developing countries and Asia[16].

The highest prevalence values reported for CD are in Europe and North America (322 per 1000 in Germany and 319 in Canada).[11] Finland has one of the highest incidence rates and prevalence of IBD in the whole world, as approximately 1% of the population suffer from IBD. IBD incidence has been increasing in Finland in all age groups between 2000 and 2020. The incidence of CD has increased by 2.2% annually. The prevalence of IBD increased from 376 to 972 per 100000, with an average increase of 4.7% per year.[1] In 2023, according to Social Insurance Institution of Finland, there were 60,910 patients in Finland who had claimed for medication reimbursement for IBD, which would make the prevalence of IBD 1.1%.

2.1.1.1 Impact of Crohn's disease

Due to the remitting and relapsing disease course, CD often needs lifelong treatment and surveillance.[17] The nature of the disease seems to be progressive, but this course can usually be altered by medical therapy and surgery at least temporarily.[2] A recent nationwide study in Finland including 21,964 IBD patients with 236,129 person years showed a slightly increased overall mortality in patients with CD and with UC, compared with the general population.[18] The overall malignancy mortality rate was slightly increased among males in CD. CD-patients have been reported to have a substantially increased risk of colorectal cancer, small intestine cancer, lymphoma, melanoma, and hepatobiliary cancer.[19] Lifelong regular monitoring and physicians'

appointments seem important to detect these diseases in time.[17] Indeed, patients with IBD participating in dysplasia screening colonoscopies have a reduced mortality compared to those without surveillance (OR 0.34).[20]

As the diagnosis is often made at a young age, the disease has a huge impact on the patient as well as the healthcare system. The increasing numbers of patients and evolving new medical therapies may mean increasing costs for society. On the other hand, some reports show that the total costs have stabilized due to novel, effective therapies, which have decreased the need for hospitalization and surgery.[21,22] In a recent study in Southwest Finland, CD was shown to have higher mean drug costs annually than UC (2369€ vs 902€). Higher disease activity also correlated with increasing costs.[23]

Although rarely fatal, the young age of onset and the morbidity associated with the disease and treatment have the potential to profoundly affect patients; not only physically, but also through limitations in social, educational, professional and emotional activities. Multiple studies have shown CD to have a remarkable influence on quality of life of the patients. Indeed patients suffering from CD have a lower quality of life compared to patients with UC or healthy controls.[24] In the current global situation with increasing demand and costs of public healthcare, it is important, that clinicians are aware of the costs of the medication used to treat CD. However, the treatment decisions should not be based solely on costs, but instead cost-effective decisions should be made in co-operation with the patients to avoid unnecessary suffering from ineffective treatment or side effects.

To avoid increasing costs, the initial diagnosis of CD should be made rapidly. As the disease course is progressive in nature, a delayed diagnosis and initiation of effective treatment can lead to sometimes irreversible complications. Early diagnosis and medical intervention have been shown to improve the prognosis of the disease and lead to fewer complications.[2] As the symptoms can be confounding and be confused with many other disorders of the gut such as IBS or infectious diseases, there is an urgent need to develop the diagnostics to identify patients suffering from CD and on the other hand, to avoid unnecessary procedures.

2.2 Pathogenesis

2.2.1 Introduction to pathogenesis

CD was first reported in 1623 by a German surgeon, Wilhelm Fabry.[25] The disease was later described in detail as a regional ileitis in a series of 14 patients published 1932 by U.S. physician, Burril B. Crohn and it was later named after him.[26] CD is still considered to be idiopathic, although several mechanisms behind this are currently known. Genetics has been known to play a role in CD since the 1930's.[27]

The role of the microbiome is widely under research as there is an interplay between strains of beneficial and harmful bacteria, which can induce inflammation in weakened epithelial barrier of a genetically suspect person. This change in the permeability activates the immune cells which congregate in the lamina propria. The innate immune system becomes less tolerant to antigens and autophagy of harmful intestinal bacteria is impaired. Imbalance of regulatory and effective T-cells and cytokines and increased number of leukocytes causes a disturbance in the adaptive immune response. Several environmental factors may act as triggers to start the disease process (Figure 1).[28]

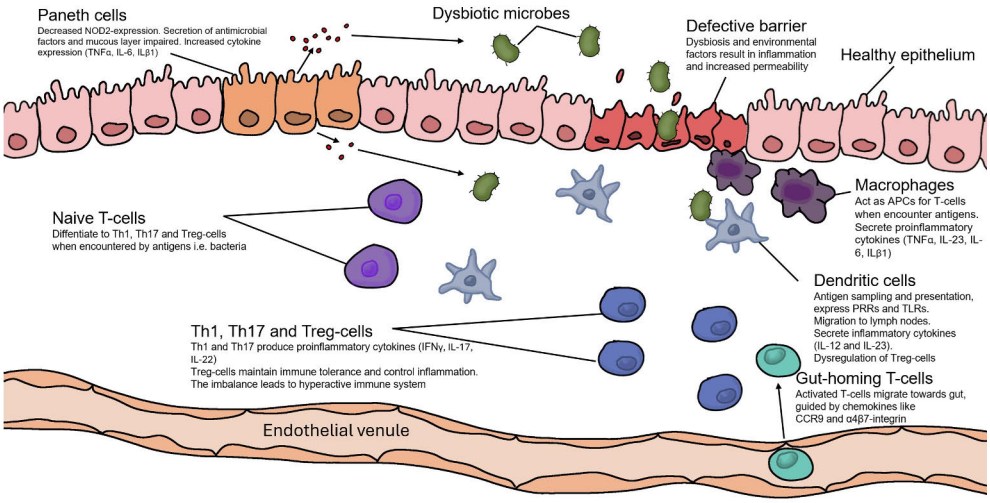

Figure 1. Schematic presentation of pathogenesis of CD. Dysbiotic bacteria are abundant in the gut resulting in inflammation and damage to the endothelium. The gut barrier becomes defective allowing bacteria to pass through the epithelium. Macrophages act as antigen presenting cells for T-cells directing the maturation. Proinflammatory cytokines are secreted by macrophages, dendritic, T-helper and Paneth cells. Dendritic cells migrate to the lymph nodes increasing migration of T-cells homing to gut. These are also attracted by chemokines such as CCR9 (C-C chemokine receptor type 9) and α4β7-integrin causing more leukocytes to enter the inflamed area. Regulatory T-cells are dysregulated by dendritic cells resulting in reduced immune tolerance and uncontrolled inflammation.

2.2.2 Genetics

There is a well-known risk for IBD among relatives of patients with CD or UC. This has been proven in both family and twin studies. Orholm et. al compared the risk of 637 patients with CD or UC to general age- and sex-adjusted population and identified a 10-fold risk for first-degree relatives to have the same disease as the patients.[29] In a ten-year follow-up study by the IBSEN-group[30], the age-adjusted risk for development of concordant disease was 25.9 for the siblings, and 8.6 for the

parents of CD-patients. Concordance rate is significantly higher among monozygotic twin pairs compared to dizygotic twin pairs, which strongly points to genetic influence on occurrence of CD.[31] Woodrow et al.[32] found a good agreement for disease location and disease behaviour and a moderate agreement for age of onset in concordant monozygotic twins in a population of 260 IBD twin pairs. A German study on 189 twin pairs found that 11 out of 31 (35%) monozygotic pairs were concordant for CD.[33] Concordance rate is lower for UC in both studies by Spehlmann[33] and Orholm[31] compared to CD, therefore the genetic contribution to the development of IBD seems to be more important in CD than in UC. The relatively low concordance rates also highlight the role of environmental factors as triggers rather than CD being solely a genetic disorder.

Genome wide association studies (GWAS) have revealed more than 240 risk loci for IBD.[34] Monogenetic disease forms are rare, and only encountered in the paediatric population[35], but even in most early-onset cases the disease is polygenetic instead.[36] Several genes associated with immune and cytokine pathways have been linked to the development of CD.[37] Genes controlling extracellular matrix and inflammatory processes have been associated with formation of strictures.[38] Specific genes have also been associated with response to anti-tumor necrosis factor (anti-TNF) therapy.[39]

The complex nature of multiple interactions between genome, proteome, transcriptome, epigenome, metabolome and microbiome (multi-omics) requires machine-based learning that can combine demographic and multi-omic data. In future, multi-omic data can be combined with clinical data to reveal risk profiles for both disease onset and to create a risk profile for CD-patients, which can aid the clinician in planning the medical interventions. Identifying risk genes may also improve our understanding for disease pathogenesis and enable us to choose an effective treatment for each patient (precision medicine).[40–42]

2.2.3 Pathogenesis

The mucosa of the small bowel comprises epithelial cells, Goblet cells, Paneth cells and immune system cells. These cells help in maintaining homeostasis and structural integrity and play an important role in defence against pathogens. The epithelium interacts and signals bidirectionally with the intestinal microbiota and the immune system.[43] Damage in the epithelium may result in an unwanted response to microbiota by the immune system, which can lead to mucosal inflammation and excessive recruitment of natural killer (NK) cells and monocytes (Figure 1).[44]

Gut barrier dysfunction seems to be one of the key mechanisms behind IBD inflammation and has been suggested to be a risk factor for both CD and UC.[45–47] An intact intestinal barrier offers protection against toxins and invasion of micro-

organisms. In contrast, the barrier must be permeable to allow the absorption of essential fluids and nutrients. The exact role of gut barrier dysfunction has been under debate for decades, but over that same time period new research methods have been developed. For example, the use of urinary fractional excretion of lactulose-to-mannitol ratio (LMR) provides more accurate data on the subject; an increased absorption of lactulose (and hence increased LMR) is observed under conditions of increased intestinal permeability.[48]

The first line of defence in the gut is a single layer of epithelial cells covered by mucus biofilm secreted from goblet cells. An inflamed terminal ileum in CD patients have a decreased expression of the mucin gene MUC1, which results in an insufficient mucin cover.[49] The fluxes between epithelial cells are normally blocked by tight junctions. In CD, the tight junctions become leaky.[50] Increased permeability allows antigens from the gut to reach the lamina propria more easily. Immune cells in the lamina propria are densely concentrated. Tight junction proteins have been linked to permeability changes, T-cells and cytokines typical of CD inflammation.[28] Paneth cells found in the epithelium defend the mucosal barrier by excreting antimicrobial peptide granules, alpha defensins, mediating autophagy (of unwanted cytoplasmic contents that are targeted by lysosomes for degradation) and prevent cell death (apoptosis). Impaired function of the Paneth cells in CD patients results in inflammation.[51] The epithelium is covered with a mucous layer that protects it from harmful substances. Bacterial translocation might be promoted by emulsifiers commonly present in the Western diet[52] or by mutations in the MUC2-gene responsible for coding the mucous layer.[53] Decreased expression of MUC1 is seen in the terminal ileum of CD-patients which further suggests that mucin cover becomes insufficient.[54] Defects in genes related to autophagy such as ATG16L1 and IRGM have been identified as risk factors for CD.[55] Bacterial products may cross the defective gut barrier more easily and induce an immune response even before disease onset.[47] Changing intestinal permeability may offer a new target in both disease prevention and therapeutics.[56]

The gut microbiota seems to have a vital role in the development and activity of both CD and UC. The change in composition of microbiota is commonly referred to as dysbiosis, but the term as such is poorly defined. A healthy microbiota enables a balanced functional capacity for nutrient metabolism, immune regulation and energy homeostasis. Dysbiosis could be defined as a shift in this balance causing negative effects on the human host.[57] Several studies have described reduced diversity of micro-organisms, decline in beneficial anaerobic species, and increased presence of potentially harmful pathogens in patients with IBD.[58,59]

The gut microbiota consists of four major bacterial phyla (*Bacterioidetes, Firmicutes, Actinobacteria* and *Proteobacteria*). These include thousands of mostly anaerobic species that colonize the human gut.[60] In IBD-patients, there is a decrease of

bacteria with anti-inflammatory capacities and an increase in bacteria with pro-inflammatory capacities compared to healthy individuals.[61,62] Almost a third of patients with ileal CD have an abundance of adherent-invasive *eschericia coli* which can cross the mucosal barrier, invade intestinal epithelial cells, replicate and provoke abundance of TNF-alpha.[63,64] However, there is no difference in the abundance of *E. coli* or *lactobacillus* between IBD-patients in remission or with an active disease. A Danish systematic review showed that patients with active CD had a lower abundance of *C. coccoides, C. leptum, F. prausnitzii* and *Bifidobacterium.*[65] Strains of *bifidobacterium* and *C. coccoides, C. leptum* and *F. prausnitzii* are widely known to have various anti-inflammatory properties.[66,67] Another study of 70 IBD-patients revealed that both the disease genotype and phenotype are associated with shifts in intestinal microbiota. Patients with IBD had *inter alia* a decreased abundance of *Faecalibacterium* and increased levels of *E. coli* in their stools.[68] The adaptive immune system seems to be dysregulated because of unbalanced microbiota, decreased number of beneficial bacteria and an abundance of harmful bacteria. Genetic variance, genetic susceptibility and environmental factors result in changes in the immune system and microbiome composition, which is a major mechanism in IBD pathophysiology.[69]

The field of host-microbial interactions requires a lot more research as this is a potential target for both treatment and prevention of IBD. The exact functional pathways that are disrupted in dysbiosis are not well understood. Altering the whole gut microbiome without knowing the precise effects seems to be unpredictable and too robust a strategy, but more focus is needed on the functional interplay between microbes and the immune system.[57]

Diet is one way of altering the composition of gut microbiota, and several studies have been conducted to learn more about this. The theory is, that diet can change the composition of the microbiome indirectly which affects the intestinal immune function. Diet can have a direct effect on the mucosal barrier and induce disorders in intestinal mucosal innate immunity.[70] FODMAP (low fermentable oligosaccharides, disaccharides, monosaccharides and polyols) is known to reduce symptoms in patients with IBS, but has not shown any benefit in inflammatory markers in patients with IBD.[71] In this randomized controlled trial (RCT) the researchers reported that patients had symptomatic relief and higher health related quality of life scores. However, a FODMAP-diet of four weeks resulted in decreased numbers of beneficial bacteria such as *b. adolescentis*, *b. longus* and *f.prausnitzii* and thus the researchers suggest limiting the duration of low FODMAP diet to four weeks. Although lacking a placebo-arm, another RCT that compared specific carbohydrate diet and mediterranean diet, showed that symptomatic remission was common, but only a few patients achieved combined symptomatic remission and resolution of inflammation.[70] Exclusive enteral nutrition (EEN) may be beneficial in inducing remission in CD in adults[72], and in pediatric populations EEN has shown to be as

effective as corticosteroid induction therapy in inducing mucosal healing, and is linked to improved nutritional outcomes.[73] EEN may alter the microbiota by promoting the presence of beneficial bacteria and reducing the number of pathogens.

Some strains of probiotics have shown efficacy in UC[74,75], but there is no clear data for CD and future research is needed. Another powerful way of altering the gut microbiota is fecal microbiota transplantation (FMT), which is currently being actively investigated. This procedure involves transfer of fecal content from a healthy individual to the gastrointestinal tract of a patient with the aim of restoring healthy gut microbiota. Theoretically, this is intriguing, as there is a huge potential to restore the dysbiosis and profoundly affect the adaptive immune system behind IBD inflammation. So far, several studies have demonstrated the efficacy of FMT for UC[76,77], but the data for CD showing possible efficacy are more scarce.[78,79] There is a need for more research on this topic, as there are several questions unanswered: the optimal route of delivery of FMT, the frequency of delivery, the dose of FMT and the heterogeneity of donor samples. A lot of research to optimize and standardize this potential form of treatment is ongoing.[80]

Population of innate immune cells recognize luminal nutritional particles and molecular patterns (pathogen associated molecular patterns (PAMPs)) such as lipopolysaccharides, peptidoglycan-derived muramyl dipeptide, lipoteichoic acid, single and double-stranded RNA and methylated DNA. This can be thought of as the body's initial line of defence forming a part of innate immunity. The innate immune cells have a major role in both maintaining the intestinal homeostasis and recognizing pathogens. Recognition of PAMPs leads to the activation of dendritic cells, which triggers a series of signalling cascades that result in the maturation of dendritic cells thus enabling them to present antigens to T-cells. Pattern recognitions receptors (PRRs), such as Toll-like receptors (TLR) and nucleotide binding domain (NOD) like receptors (NLR), act as mediators in this interaction. Dendritic cells express the widest range of these recognition receptors and interpret microbial patterns to guide other immune cells towards either tolerance, or to provoking an immune response.[27] These form transepithelial dendrites which scan luminal antigens[81] by acting as antigen presenting cells (APC) priming T-cell response.[82] The distribution and phenotype of dendritic cells correlates with CD activity.[83] TLRs have an ambiguous role, as their signalling may either inhibit or provoke inflammation. The TLR patterns in patients with IBD are very different to those of healthy controls. In patients with IBD, TLR 2, 4, 8, and 9 are upregulated compared to healthy individuals[84], whereas TLR3 are downregulated in IBD patients.[85] The ability of dendritic cells to induce regulatory T-cells that inhibit inflammation may be decreased. NOD2 is a PRR strongly associated with CD, weakened inflammatory cytokine response, ineffective autophagy and IL10-transcription. NOD2 activates the nuclear kappa-light-chain (NF-κB) enhancer of activated B-cells in signalling

pathways.[86] Loss of function mutations are seen in NOD2 and this is currently thought to be the most widely implicated gene in the etiology of CD.[87] NOD2-variants modify the recognition of bacterial wall components, which increases NF-κB -activation in monocytes.[86] This cascade reduces the individuals' ability to eliminate invasive and pathogenic micro-organisms thus leading to chronic inflammation. Activated dendritic cells also upregulate major histocompatibility molecules (MHCs), which are essential for effective antigen presentation and for T-cells to initiate the adaptive immune response.[88]

PRR activation on dendritic cells also leads to the production of cytokines and chemokines that contribute to the inflammatory response and recruitment of immune cells to the site of inflammation. Intestinal macrophages have a role in the degradation of apoptotic or defective cells and in tissue remodelling. Neutrophils in turn are responsible for early response to microbial stimuli in inflammation and mediate adaptive immune system by cytokines. Interleukins (ILs) are cytokines generated by cells in the innate immune system. APCs produce IL-12, IL-23 and IL-27 during antigen presentation to naïve T-cells. IL-35 instead is a product of regulatory T- and B- cells. ILs are critical to regulating cellular pathways, that maintain intestinal homeostasis and activate the inflammatory response to antigens and they also play a role in preventing uncontrolled, over-exaggerated immune response that is behind autoimmune diseases.[89]

Innate lymphoid cells (ILCs) found in the epithelium and lamina propria are partly responsible for the integrity of the epithelium. ILCs also respond to microbial and dietary content *inter alia* by secreting TNF-alpha, IL-22 and interferon (IFN) gamma. In particular, ILC1 and ILC3 are found in abundance in patients with CD and have been shown to have a role in pathogenesis of CD.[90,91] IL23-responsive ILCs have been shown to express increasingly Th17-associated cytokine genes. A significant increase in CD127+CD56 ILCs have been seen in patients with CD but not in UC. This indicates that IL-23-responsive ILCs are associated with selective accumulation of a phenotypically distinct ILC population, which drives inflammatory cytokine expression.[90] Experimental and genetic studies have highlighted the role of IL-23/IL-17 pathway in the pathogenesis of IBD.[92] The adaptive immune system is thought to mediate inflammation instead of initiating it.

T-cells are white blood cells that originate from stem cells in bone marrow. T-cells migrate to the thymus, where they are differentiated, hence the 'T' for thymus in the name. In IBD, there seems to be an imbalance between the CD4-positive T-effector cells and the regulatory T-cells.[93] The effector T-cells (Th1 and Th17) which defend the mucosa against pathogens, are found in large numbers in the inflamed-CD-patient's intestine.[94] Th1 and Th17-cells defend the mucosa by secreting interferon gamma, TNF-alpha, IL-17 and IL-22. Regulatory T-cells, in turn, maintain the balance in the healthy gut by secreting IL-10, IL-35 and tissue growth-factor

(TGF). IBD-patients have decreased levels of IL-10, which regulates IL-23, and an excess IL-23 allow the stimulation of Th17-cells.[95]

Cytokines in the intestinal lumen promote chemotaxis of Th1-cells to the mucosa. Neutrophils and macrophages migration to the site of inflammation is guided by chemokines synthesized by lymphocytes (such as TNF-alpha and IFN-gamma). Other cytokines (IL-1, IL-6, IL-23) and TGF-beta guide lymphocytes to differentiate to Th17-effector cells that are responsible for synthesizing IL-17. This IL-17 stimulation attracts more neutrophils on site, which provokes more inflammation in patients with CD. Macrophages and APCs also contribute to IL-12, IL-6, TGF-beta and IL-23 synthesis. This inflammatory cascade also creates a feedback loop that stimulates the production of even more Th1 and Th 17 cells.[96,97] TNF-alpha has a pivotal role in the pathogenesis of CD. In addition to acting as a messenger in chemotaxis, TNF-alpha directly promotes the apoptosis of epithelial cells. TNF-alpha also promotes angiogenesis in the inflamed tissue thus supplying the oxygen and nutrients to cells that have migrated to the inflamed areas. TNF-alpha acts synergistically with ILs to amplify the inflammatory cascade.[98]

B-cells, also known as B-lymphocytes, also develop from hematopoietic stem cells in bone marrow. B-cells produce antibodies, differentiate into plasma cells and act as APCs. In IBD, the B-cells are known to produce several types of antibodies. Antimicrobial antibodies such as anti-*sachharomyces cerevisiae*, anti–*Escherichia coli,* OmpC, and anti-flagellins (anti-CBir1, anti-flagellin 2, and anti-flagellin X) antibodies are often detected in patients with CD. These antibodies can also be detected in patients years before disease onset.[45,99] In a more recent study, 51 antibodies were shown to identify patients who were to develop IBD up to 5 years before diagnosis.[100]

Autoantibodies are present especially in complicated CD, which further supports the role of B-cells in disease pathogenesis.[100] The exact role of the serological antibodies present in IBD-patients remains unclear. It is likely that they are markers of aberrant immune response, rather than acting as drivers for the pathogenesis of the disease. Increased number of plasma cells are found in the lamina propria in patients with CD and skewing of the antibody production has been observed.[101] The role of B-cells in the pathogenesis of CD is far less known, than other areas of adaptive immunity.

NK-cells are found in both the epithelium and the stroma of the intestine. They encounter antigens of the gut lumen, contribute to both maintenance of immune homeostasis and the development of an efficient immune response. They interact with epithelial cells, fibroblasts, macrophages, dendritic cells and T lymphocytes primarily by the production of cytokines such as IFN-gamma, which can further stimulate the recruitment of additional NK-cells from the peripheral blood, which amplifyies the antimicrobial response.[102]

Ongoing chronic inflammation eventually leads to fibrosis in the intestine. This is thought to be due to the upregulation and excessive deposition of extracellular matrix (ECM), which is caused by complex interactions between cellular and inflammatory mediators. Mesenchymal cells regulate profibrotic factors. Fibrogenic cells are thought to emerge from multiple sources. This process is driven by inflammatory cytokines such as IL-13, IL-17 and TGF-beta. The pathway is associated with TGF-beta and is an especially fibrogenic factor that regulates cells differentiation into profibrotic mucosal cells.[103] Fibrosis is a dynamic process with continuous balancing between ECM deposition and degradation. This balance is regulated by matrix metalloproteinases that break down the ECM and are regulated by tissue inhibitors. Smooth muscle hyperplasia has also been observed in intestines of IBD patients. Induced hyperplasia and hypertrophy may result in fibrotic strictures and stenosis in CD.[104]

Inflammatory cells migrate from the vessels to the intestinal mucosa, which is driven by chemoattractants and antigen recognition by the APCs. This migration is mediated by adhesion molecules and chemokines that are expressed on the surface of endothelial cells of the blood vessels, such as IL-8, macrophage inflammatory proteins and monocyte chemoattractant proteins. This induces changes in adhesion molecules and ligands on lymphocytes such as α4β7-integrin and mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1). Simultaneously proinflammatory cytokines such as IL-1 and TNF-alpha are secreted by macrophages, which upregulate the expression of adhesion molecule ligands, thus allowing leucocyte adhesion and extravasation into the intestinal tissue. Leukocyte trafficking perpetuates chronic inflammation in IBD by continuously recruiting and activating the immune cells in a response to ongoing stimulation.[27,105]

Although environmental issues related to the development of IBD have been investigated for decades, no clear pattern of their association to disease onset has been established. Multiple longitudinal cohort studies have identified a number of factors that contribute to the disease risk. As the potential role of gut microbiota has been well established in the disease process, there has been a lot of discussion on the role of hygiene. The hypothesis is that improved hygiene changes the variability of microbes in the gut. This hypothesis is supported by the fact that the prevalence of IBD is lower in rural areas compared to urban areas.[106] This can be also seen in studies that compared childhood living area with the risk of IBD onset.[107] Furthermore, the incidence and prevalence of both CD and UC are higher in western countries than in developing countries. In newly industrialized countries the incidence of IBD is rapidly rising.[13] A higher socioeconomic status has been linked to an increased risk of IBD especially in developing countries. This may be due to differences in lifestyle in addition to better hygiene and sanitation. However, studies on this are heterogeneous and the concept remains controversial.[108]

The onset of CD often occurs after infectious gastroenteritis (hazard ratio, HR 2.4)[109] and has a distinct microbe flora (dysbiosis)[110] often containing intramucosal bacteria with adhesive properties.[111] Several bacteria may associate with a risk of IBD as discussed earlier. Breastfeeding is confirmed to have a protective role against IBD in several studies, which may be due to changes in gut microbiota.[112] Both prenatal and postnatal courses of antibiotics also increase the risk of IBD onset.[108] The use of antibiotics also increases IBD risk at an adult age, and according to a study by Faye et al., is highest at the age of 40 and older. The risk seems to be highest at 1-2 years after exposure to antibiotics.[113] The use of combined oral contraceptive pills are associated with the development of CD as reported in multiple studies; most recently Pasvol et al. reported an increase in CD, odds ratio (OR) of 1.60, in a recent large case-control-study.[114] The use of nonsteroidal anti-inflammatory drugs may also increase the risk of CD, (HR 1.59.[115] The role of appendectomy and the risk of IBD has been studied a lot; overall the risk for CD seems to be higher after appendectomy, relative risk (RR) of 1.61, as demonstrated in a large umbrella study. However, this may be superimposed due to unnecessary appendectomies for patients with incipient CD and symptoms suggesting acute appendicitis. In contrast, appendectomy seems to protect from UC.[116,117] Inflammation in the appendix did not show an association with disease severity or prognosis in CD patients with ileocecal resection in a recent Dutch study.[118]

Smoking has been recognized to influence IBD as early as in the 1980s. Active smoking had nearly a twofold risk of CD (OR 1.76) and decreased risk of UC (OR 0.58) in an umbrella study by Piovani et al. High levels of physical activity have a protective role against CD as well as manual-work occupations that make physical demands on a person are linked to a lower risk of IBD compared to office workers.[116,119]

The interplay between diet and IBD has been investigated for decades. Piovani et al.[116] found that the consumption of soft drinks increased the risk of IBD, but there was no clear evidence of excessive consumption of carbohydrates and sugars, which had previously been linked to an increased risk of IBD onset. Moreover, those authors found a significant risk in diets containing high amounts of polyunsaturated fats. A more recent review study did not confirm this but showed that the results for diet as a risk factor were rather controversial and conclusions could not be drawn from the material from 24 studies included in the review.[120] A recent Nordic study found out that a high-quality diet rich in fish and vegetables was protective regarding IBD, whereas high intakes of sugar sweetened beverages were associated with an increased risk of IBD onset.[121] Narula et al. reported a higher hazard for developing CD (HR 1.82) for patients with ultra processed food intake for five or more servings per day.[122] There is a huge gap in the knowledge of this field, as manipulating the diet could be a potential treatment approach for disease prevention in IBD.[123]

2.3 Symptoms and disease prognosis

CD typically presents with loose stools (with or without gross bleeding), abdominal pain (typically in the right lower abdomen), fatigue and weight loss, although sometimes the disease is asymptomatic. The symptom pattern is often unique and there are not truly pathognomic features. Loose stools lasting for more than six weeks often helps to distinguish IBD from a gastrointestinal infection.[17] Crohn's disease presents with ileal, ileocolonic, or colonic disease in about one-third of patients each. Upper-GI-involvement is less common and is almost always accompanied by ileal or colonic involvement. Only a minority of the patients experience a change in disease location over time.[124]

Although diagnosis can not be based on symptoms only, Danese et al. have developed a “Red Flag Index” for referral of patients for symptoms suggestive for CD. They found eight features that were associated with CD rather than IBS as follows: non healing fistulae or perianal abscess, a first degree relative with diagnosed IBD, weight loss of more than 5% over 3 months, chronic abdominal pain for more than 3 months, nocturnal diarrhea, mild fever for more than 3 months, no abdominal pain 30-45 min after meals and no rectal urgency. Each symptom has a rounded coefficient with maximum total score of 24, and a total score of 8 or more has a sensitivity for CD 97% according to their receiver operator characteristics analysis (ROC).[125]

Patients with CD limited to the colon often present with loose mucous stools and rectal bleeding. For small bowel disease, the typical symptoms are lower right-sided abdominal pain, diarrhea, weight loss due to malabsorption and increased secretion from inflamed areas, low-grade fever, fatigue and malnourishment. In the presence of strictures, postprandial pain, bloating, nausea and vomiting are common. Sometimes the first manifestation of CD is acute occlusion. Enterourinary fistulae can cause fecaluria or pneumaturia, rectovaginal fistulae stool discharge through vagina and enteroenteric fistulae abdominal abscesses or malnutrition via bypassing segments of the GI-tract. Perianal fistulae may cause perianal pain and drainage or fever and purulent discharge when abscesses are present.[126] Oral involvement may present with aphthous ulcers or pain in the mouth and gums. Esophageal involvement may present with dysphagia. Gastroduodenal involvement can cause upper abdominal pain, nausea and postprandial vomiting.[127]

Most patients have a non-penetrating, non-stricturing disease behavior (luminal CD), but up to half of the patients will develop intestinal complications such as: stricture, fistulae or abscess within 20 years after diagnosis (Figure 2). Patients with upper-GI-involvement, ileal or ileocolonic disease are more likely to develop complications. Deep ulcerations and extensive anatomic involvement are also risk factors for the more complicated disease. Over a long time, 20-30% of the patients with CD have a nonprogressive disease course. It is well known that symptoms do

not correlate well with CD inflammation and therefore diagnosis or decision making about therapy should not be based on symptoms only. Up to 25% of patients with CD present with perianal fistulae, which indicates a more severe clinical course of CD.[124] Flares are typical for CD, and they can be followed by long asymptomatic periods. Diagnostic delay is often long, and patients often have suffered from GI-symptoms for years before diagnosis. Missing a diagnosis often leads to a more complicated disease, as treatment is then started after formation of strictures, fistulae or other complications.[128,129]

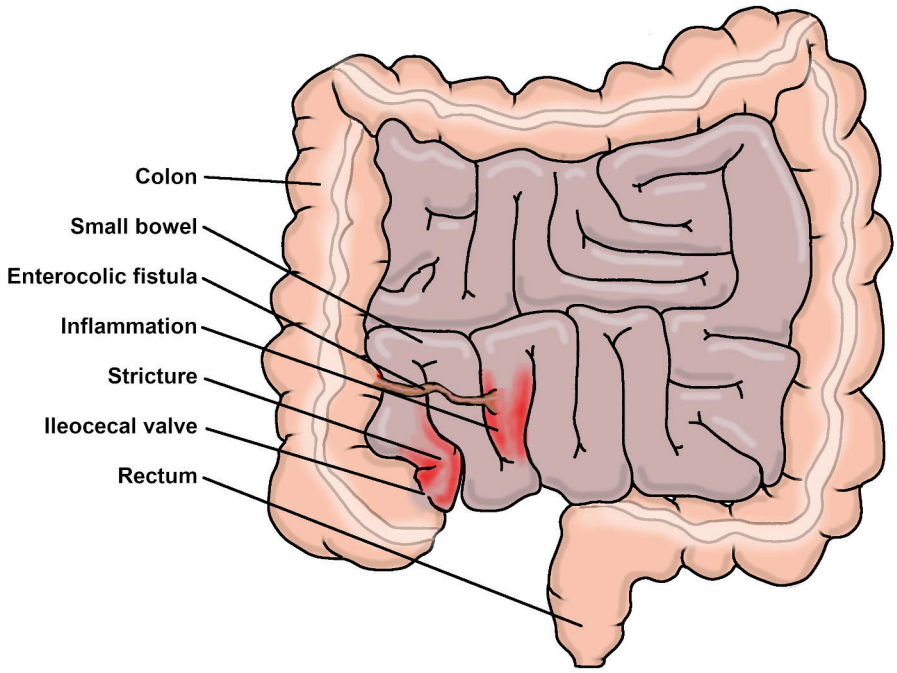

Figure 2. Manifestations of small bowel CD showing the colon and the small intestine. Areas with inflammation shown with a stricture and a fistula from the small intestine to the ascending colon.

CD is often linked to extraintestinal manifestations (EIMs); up to 25-40% of IBD patients suffer from EIMs. Patients with CD are more likely to suffer from more EIMs than patients with UC. Typically, EIMs presented are musculoskeletal (peripheral and axial inflammatory arthritis), ocular (uveitis, episcleritis), cutaneous (erythema nodosum and pyoderma gangrenosum) or hepatobiliary (primary sclerosing cholangitis, PSC). Intestinal activity of CD and activity of EIMs are sometimes discordant especially in uveitis, PSC, and ankylosing spondylitis. These diseases are also progressive in nature and need medical attention to prevent complications and to improve the wellbeing of patients.[130]

As CD can present in many different forms, a structured classification was created by a panel of experts in the 2005 Montreal World Congress of gastroenterology. The Montreal classification was developed to aid clinicians in patient counselling, assessing disease prognosis and choosing the most appropriate therapy for each disease phenotype. The Montreal classification considers age at disease onset, the location of the disease and the behavior of the disease in terms of penetrance. The age is classified as A1: 16 years or younger, A2: 17-40 years, A3: >40 years. Disease location is categorized by L1: ileal, L2: colonic, L3: ileocolonic, L4: isolated upper-GI. The disease behavior is classified as B1: non-stricturing, non-penetrating, B2: stricturing, B3: penetrating. The letter 'p' is added to B1-B3 when perianal disease is present (Table 1).[131]

Table 1. Montreal classification of CD by age, disease location and behavior in terms of penetrance. (A) L4 can be added to L1-L3 when upper-GI involvement is present. (B) p is added to B1-B3 when perianal disease is present.[131]

Variable	Montreal classification
Age at onset (yr)	A1: ≤16
	A2: 17-40
	A3: >40
Disease location	L1: ileal
	L2: colonic
	L3: ileocolonic
	L4: isolated upper-GI (a)
Disease behavior	B1: nonstricturing, non-penetrating
	B2: stricturing
	B3: penetrating
	p: perianal disease (b)

Numerous studies have shown different patterns of disease behaviour in patients with younger age of onset. The age classification is thought to be important due to different phenotypes of the disease; patients diagnosed at an early age have a higher risk of penetrating disease with stricture formation and fistulae. The disease is more likely to have more extensive involvement of the GI-tract including a higher risk of upper- GI involvement. Older age, on the contrary, seems to increase the odds of having CD limited to the colon with less penetrative presentation. The Montreal classification is currently in global use in both everyday clinical and scientific work.[132]

2.4 Diagnostics of Crohn's disease

2.4.1 Laboratory findings

As described earlier, CD can not be diagnosed based on symptoms alone. Therefore the diagnosis of CD should be established based on symptoms, laboratory findings, imaging and endoscopic findings including histology. According to guidelines on CD diagnostics of both the European Crohn's and Colitis Organisation (ECCO) and the American College of Gastroenterology (ACG), a routine whole blood count, C-reactive protein (CRP) and fecal calprotectin (FC) should be assessed. Microbial analysis of the stool should be included to rule out C. difficile enteritis and other common microbial pathogens. Whole blood count often reveals anaemia, microcytosis due to iron deficiency or thrombo- and leukocytosis due to active inflammation. CRP and erythrocyte sedimentation rate (ESR) are often elevated in CD and correlate with the severity of CD. Both CRP and ESR may be normal in luminal disease as well. CRP is more sensitive for CD than UC; as in UC CRP is usually elevated only during acute severe colitis. This difference in sensitivity is probably due to the intramural inflammation caused by deeper ulcerations in CD increasing CRP more frequently. Serum albumin (Alb) may be decreased in CD due to malnutrition. Coeliac disease should also be serologically ruled out. Genetic or serological testing is currently not recommended for routine diagnostics of CD.[17,124]

All the biomarkers are nonspecific, thus no pathognomous laboratory marker exists for CD to date. In addition, there are no cutoff values suggestive for CD in the blood samples. FC is commonly used in the differential diagnostics between IBD, IBS and other functional disorders of the gut. FC shows an estimate of the number of neutrophils migrated to the gastrointestinal tract due to inflammation. FC correlates well with endoscopic disease activity indexes. However, FC is not specific for IBD, but can also be elevated in the presence of infections, polyps and neoplasias.[133] In addition, no clear diagnostic cutoff value exists for FC, although values ranging from 100 µg/g to 250 µg/g have been suggested.[134–138] Both ECCO and AGA recommend the use of CRP and FC as markers in follow-up of patients diagnosed with CD. When both of these markers are low indicate a clinical response. On the other hand, when CRP and FC are elevated indicate a loss of response or flare. A cutoff for clinical remission CRP <5mg/l is suggested and <250 µg/g for FC.[17,139–142]

2.4.2 Endoscopy

Patients with symptoms and laboratory findings suggesting CD should undergo endoscopy as the first line of diagnostic measurement. Differential diagnostics can

be made in ileocolonoscopy to rule out infectious colitis, UC, infectious diverticulitis, ischemic colitis and colorectal cancer. In addition to evaluating the extent and degree of macroscopic inflammation, biopsies should also be taken from both inflamed and normal appearing sections of the bowel. Over 80% of IBD patients have mucosal involvement within the reach of a colonoscope. The terminal ileum can be reached in more than 90% of patients via flexible colonoscopy[143], although CD strictures may cause the valvula to be impassable for the scope.[17,124] Patients suspected of CD who have nausea, dyspepsia and vomiting will also benefit from gastroscopy. However, upper endoscopy is not routinely suggested for asymptomatic patients with known CD.[17]

Endoscopic findings suggestive for CD include mucosal nodularity (cobblestone appearance), erythema, loss of visible vasculature, ulcerations, friability, and stenosis. Compared to UC, the inflammation is often segmental with inflamed and healthy looking segments following each other.[124] The severity of the endoscopic appearance and disease extent should be carefully documented. Simple Endoscopic Score for Crohn's Disease (SES-CD)[144] is widely used to assess disease activity (Table 2). In addition to diagnostics, ileocolonoscopy is often performed as a follow-up measure to assess disease activity, possible strictures and neoplasms; patients with colonic CD have an increased risk for colorectal neoplasias. During ileocolonoscopy, short strictures can be dilated with an inflatable balloon device to avoid bowel obstruction. Additionally, endoscopic polypectomy can be done. Ileocolonoscopy, however, cannot give information about extraluminal complications or nature of the strictures as there is no transmural visualization. Ileocolonoscopy also requires bowel preparation and often sedation, which is time consuming for both the patient and endoscopist and unpleasant for the patient. There is also a small risk of complications such as perforation or bleeding.[145]

Table 2. Simple Endoscopic Score for Crohn's disease (SES-CD)-score for assessing endoscopic activity of CD inflammation. Scores presented in the table are calculated for each segment (ileum, right side of colon, transverse colon, left side of colon and rectum) and added together.[144]

Points	**0**	**1**	**2**	**3**
Size of ulcers	None	Aphtous ulcers (1-5mm)	Large ulcers (5-20mm)	Very large ulcers (>20mm)
Ulcerated surface	None	<10%	10-30%	>30%
Affected surface	Uneffected	<50%	50-70%	>75%
Presence of narrowing	None	Single, can be passed	Multiple, can be passed	Cannot be passed

In addition to getting a macroscopic assessment of the mucosa, in the ileocolonoscopy, mucosal biopsies can be collected for histological analysis. In diagnostic endoscopy, biopsies should be collected from each segment including nonaffected sites. The main difference between infectious colitis and IBD is acute inflammation and preserved crypt architecture. In CD, granulomas are rarely present but usually confirm the diagnosis. Focal crypt architectural abnormalities with focal or patchy chronic inflammation, defined by the presence of lymphocytes and plasma cells in the lamina propria or mucin preservation at inflamed sites, are histological features typical for CD. Neutrophil granulocyte infiltration is seen in active disease. Metaplastic changes can be seen in the form of pyloric gland metaplasia or Paneth cell metaplasia. There is no hallmark score for histopathological diagnosis or severity of CD.[17,146,147]

2.4.3 Small bowel capsule endoscopy

The terminal ileum is often affected by CD inflammation, however, the terminal ileum can not be reached via colonoscopy in every patient, or the disease may be limited to the proximal parts of the small intestine (in up to 30% of patients with CD).[124,131,148,149] The small bowel can be examined with a wireless capsule camera device called the SBCE. In this procedure, the patient's bowel is prepared with polyethylene glycol (PEG) solution and the patient then swallows the small camera device. The device transmits wirelessly still images of the patient's intestine to a recording device. The images can later be analyzed with computer software. Although it may lack specificity, SBCE is a non-invasive and sensitive procedure for finding even smaller superficial lesions in the small bowel. The lack of specificity is an issue though, as nearly 10% of healthy subjects demonstrate mucosal breaks and erosions in their small bowel.[7] The diagnosis of small bowel CD should not be established by SBCE findings alone, but a combination of clinical symptoms, laboratory, endoscopic, histologic and imaging findings together should be used for the assessment.[124,150,151] Figure 3 shows typical findings for CD in SBCE.

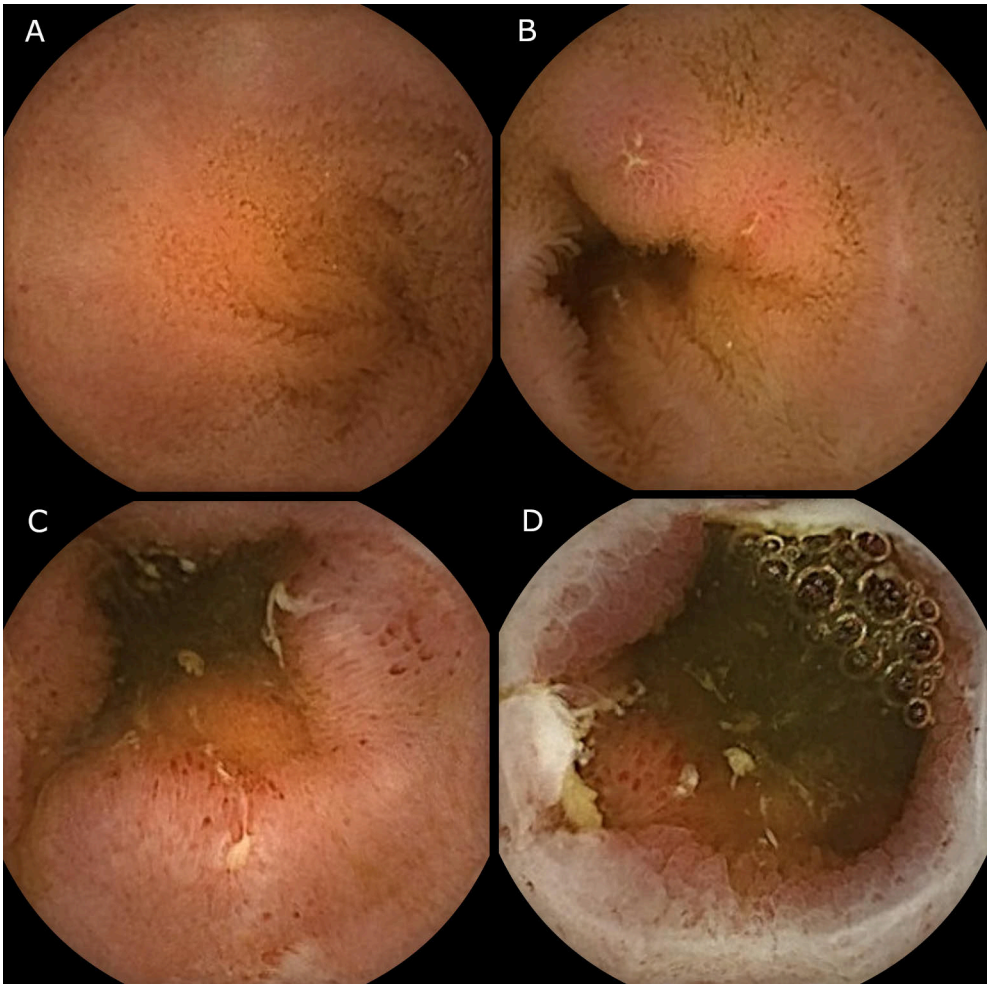

Figure 3. SBCE findings in the small bowel of a 62-year-old female patient who participated in Study II. A shows the normal mucosa. B has two small aphtae. C shows larger ulcerations, and D shows a stricture barely passable for the capsule camera.

According to both ECCO and ACG guidelines, patients who suffer from symptoms suggestive for small bowel CD, with no explicatory findings in ileocolonoscopy, should undergo SBCE to directly visualize the mucosa of the small intestine.[17,124] Before SBCE, the risk for capsule retention should be assessed and MRE or small bowel CT performed to rule out strictures. The rate for capsule retention in general is 2.1%, but in patients with suspected CD the rate is 1.2%.[152] Despite being stuck, the capsule may pass through the stricture spontaneously, or with the help of endoscopic dilatation, but often needs to be surgically removed. Even acute occlusion may occur.

The findings for SBCE should be documented systematically therefore two grading systems have been validated: the Lewis-score and Capsule Endoscopy Crohn's Disease Activity Index (CECDAI) (Table 3). CECDAI is a scoring system of mucosal injury in CD of the small bowel, which is easily reproduceable and independent between interpreters and the latest ECCO-guideline on CD also encourages its use in follow-up.[17,138,153–155]

Table 3. CECDAI for classifying inflammatory lesions SBCE. Small bowel is divided into two segments, proximal and distal. The score is calculated as proximal (AxB+C) + distal (AxB+C).[154]

Score	A. Inflammation	B. Extent of disease	C. Stricture
0	None	No disease	None
1	Mild to moderate edema, hyperemia/denudation	Focal disease (single segment)	Single-passed
2	Severe edema, hyperemia/denudation	Patchy disease (multiple segments)	Multiple-passed
3	Bleeding, exudate, aphtae, erosion, ulcer <5mm	Diffuse disease (>3 segments)	Obstruction (non-passage)
4	Ulcer 5-20mm, pseudopolyp	-	-
5	Ulcer >20mm	-	-

2.4.4 Imaging in small bowel Crohn's disease

2.4.4.1 Magnetic resonance enterography

MRE is a powerful tool for assessing intestinal inflammation and has been increasingly used in the last decade for diagnostics and disease monitoring in CD.[156] MRE can be used to detect severe CD lesions such as transmural inflammation, stenosis, fistulae or extraintestinal abscesses, thus the diagnosis of even small bowel CD is relatively easy to achieve.[157,158] (Figure 4.) MRE, however is not sensitive enough to detect luminal lesions in the small bowel[3–5,159], in which case a SBCE is usually recommended. Samuel et al. showed that more than half of patients with known CD and negative results for ileocolonoscopy had active inflammation in the small bowel.[149] The disease location in the proximal ileum or disease located in the intramural layers or the mesentery may not be visible in endoscopy. MRE gives useful information on the disease extent, extraluminal inflammation and possible complications based on bowel wall thickness and intravenous contrast accumulation. MRE has high sensitivity of bowel wall enhancement, mucosal lesions and T2 hypersensitivity as suggestive of intestinal inflammation. Cross sectional imaging of the abdomen, intestinal ultrasound (IUS) or SBCE is recommended for all patients

with CD diagnosed by endoscopies.[17,124] In more than 50% of cases, the treatment is altered based on MRE findings.[160]

The recent ECCO-ESGAR (European Society for Gastrointestinal and Abdominal Radiology) guidelines[161] recommend evaluation by MRE and to report the number and anatomical location of inflammatory areas. In addition, bowel wall thickness, edema, ulcerations, vascularization, and adipocyte proliferation should be reported. When assessing MRE for penetrating disease, the number and anatomic location of transmural inflammation, strictures and fistulae must be evaluated.[161]

Several grading systems have been developed to quantify the inflammation in CD. The Magnetic Resonance Index of Activity (MaRIA) is one of the most used, but heterogenous gradings have been used in research. MaRIA correlates with SES-CD and CRP and can predict mucosal healing in CD patients.[162] MaRIA-score was further developed to a simplified version, the sMARIA-score[163], which accurately identifies active lesions in patients with known CD[164] (Table 4). Due to no radiation being used, MRE is preferred over CT for young patients.[17,124] Compared to endoscopies, no bowel preparation is needed but intraluminal contrast such as mannitol is often used. Compared to CT, MRE is more expensive, and imaging is slower, the latter of which may be an issue for patients suffering from claustrophobia.[165] In the METRIC-trial, participants were asked if they were willing to go through a repeat colonoscopy or MRE and 91% were willing to undergo another MRE compared to 75% for colonoscopy.[166]

Table 4. sMARIA-score for evaluating CD inflammation by MRE. Score for a single segment is calculated according to MRE findings by adding the points together. All variables are categorized as present or absent.[163]

Variable	Points
Bowel wall thickening >3 mm	1
Edema	1
Fat stranding	1
Ulcers	2

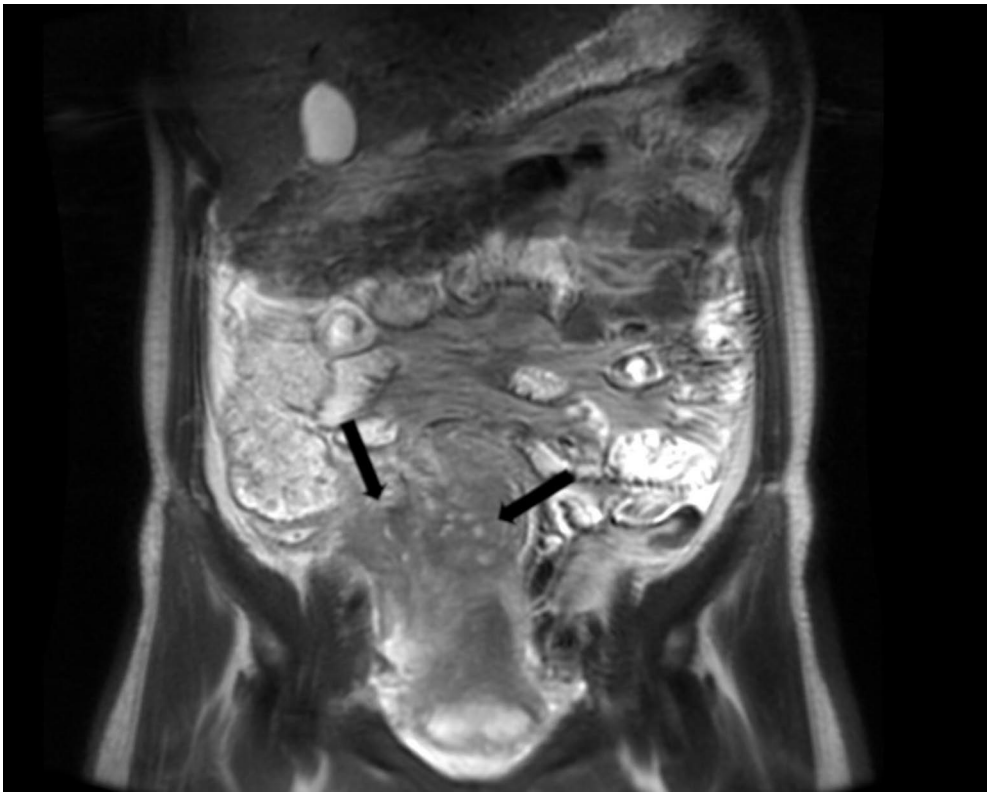

Figure 4. T2-weighted MRE image of a 22-year-old male presenting with active CD in small bowel with massive inflammatory phlegmon (black arrows). Modified from Study II with permission from Karger publishers.

2.4.4.2 Computed tomography

An alternative option to MRE is CT, which is widely available in both diagnostics and follow-up of CD. Compared to MRE, CT has similar sensitivity and specificity for detecting active CD inflammation, strictures, fistulae and abscesses.[167,168] In CT bowel wall thickening, increased intravenous contrast enhancement and mesenteric fat can be seen as markers of active and chronic inflammation similar to that found for MRE. However, the soft tissue contrast of CT is inferior to MRE.[169] Due to harmful ionizing radiation, repeated CT imaging should be avoided and instead MRE used especially in young individuals. In an ideal situation, CT should be reserved for emergency use only and to patients with contraindications to MRE.[17,124]

2.4.4.3 Intestinal ultrasound

For the patient IUS is a comfortable and non-invasive procedure for examining the intestine that has been emerging during the last few years. In ideal conditions, good

visibility can be reached especially in the terminal ileum, but visibility is often poor in the proximal small bowel. IUS is even less invasive than MRE, as no contrast is necessarily needed. IUS can be performed while the patient is seeing the gastroenterologist on a point-of-care basis and the treatment options discussed simultaneously. IUS is widely available and less expensive than cross-sectional imaging.[170,171] IUS is rather simple to learn and can detect ileocolonic CD with performance close to MRE.[172] However, there are concerns about the sensitivity and specificity of IUS compared to CT or MRE at the diagnostic phase.[173–175] Although strictures, fistulae or abscesses may be seen with IUS, the sensitivity is lower than that of CT or MRE.[175] Bowel wall thickness is the most widely validated measure of inflammation. Additionally, mural changes such as ulcerations and edema can be seen. Perienteric inflammation, fat accumulation and enlarged lymph nodes represent inflammation. Blood flow of the vessel wall can also be assessed by Doppler-ultrasound; increased blood flow is related to inflammation. The different layers of the bowel wall become blurrier with inflammation. Accurate thresholds are so far unclear, but on an individual basis, bowel wall thickness in IUS could be seen as a potential method for assessing response to treatment.[170,176] Scores such as Simple Ultrasound Activity Score for CD (SUS-CD)[177] and International Bowel Ultrasound Segmental Activity Score (IBUS-SAS) have been validated and are reliable options for evaluating activity in CD.[178]

2.4.4.4 Positron emission tomography

PET scans using 18-fluoride-fluorodeoxyglucose ([^{18}F]-FDG) tracer can be used to assess glucose metabolism in the human body. Increased glucose metabolism *inter alia* may be due to inflammation, infections, and malignancies.[179,180] This high metabolic activity is due to higher energy demand and thus increased nutrient transport, glycolysis, mitochondrial activation and expression of cytokines.[181] [^{18}F]-FDG PET imaging has also proven to be sensitive and specific for active lesions in CD.[182–188] PET imaging does not yield very clear anatomical data, but this drawback can be compensated by using fusion imaging whereby a CT or MRE scan is done simultaneously. PET-CT scanners are more widely available than PET-MRI scanners. As discussed earlier, CT has limited soft tissue contrast compared to MRI. MRI techniques have improved significantly with motion correction techniques thus enabling a more detailed anatomic map in combination with PET scan.[189,190] Figure 5 shows a sample PET image.

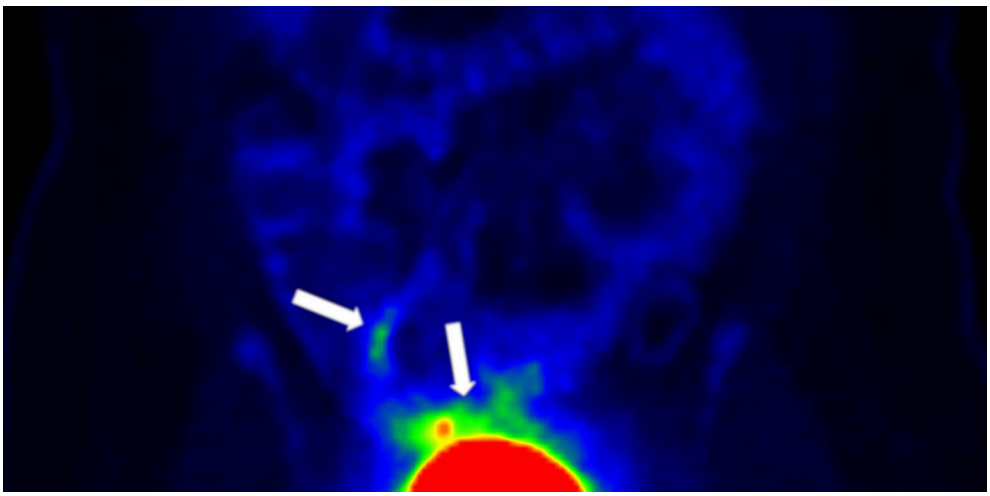

Figure 5. A PET-image of a 27-year-old female with active small bowel CD in the terminal ileum and the proximal ileum (black arrows). Anatomical structures and the exact location of any inflammation is difficult to visualize in PET sequences.

Fusion [^{18}F]-FDG PET-MRI has been documented to be a reliable tool for evaluating intestinal metabolism[9,10,180], but its use has been limited due to cost and accessibility. There is also a lack of standardized methods to quantify inflammation. Bettenworth. et al. showed a significantly increased [^{18}F]-FDG uptake in 87% of deep mucosal lesions in patients with IBD.[184] In a study by Tenhami et al., fusion PET-MRI using [^{18}F]-FDG tracer has been shown to correlate with endoscopic inflammation activity in patients with known or suspected IBD in the colon or the terminal ileum.[191] There are a few studies of [^{18}F]-FDG PET-CT use for the detection of active CD inflammation, but no diagnostic thresholds have previously been established.[192] In a study by Catalano et al., sensitivity of [^{18}F]-FDG PET-MRI was higher than MRI alone (88% vs 80%) and the specificity of [^{18}F]-FDG PET-MRI was superior to that of MRI (91% vs 83%) in detecting active CD inflammation in patients with known CD.[193] Preoperative [^{18}F]-FDG PET-MRI is accurate in detecting active lesions and extraluminal disease in the small bowel CD.[194] It has been suggested that [^{18}F]-FDG PET-MRI can be used to assess inflammatory activity of strictures in CD, which could aid the clinician's decision making in whether the patient needs a step-up with medication or surgical resection of the inflamed area.[195] Whether [^{18}F]-FDG PET imaging can be used to evaluate the response to treatment remains unclear, as studies on this are relatively few and those that do have small series.[196,197] Due to this paucity of information, PET imaging is not yet suggested to be used in standard CD diagnostics or follow-up in the most recent guidelines issued by the ECCO.[17] The recent guideline of European Association of Nuclear Medicine (EANM) lists the indications for fusion PET imaging for IBD as follows: 1)

evaluation of the disease extent, 2) early assessment of therapy and 3) differential diagnostics between fibrotic and inflammatory stricture.[198]

[^{18}F]-FDG PET-MRI can be considered a safe procedure in diagnostics, the main risk being the radiation dose. Due to improved imaging techniques, the doses have significantly decreased though.[199] In PET-MRE performed using the protocol of Study II and III, the patients received 5.3 mSv of radiation, which is equivalent to that of 1 year and 7 months of background radiation. For comparison, the effective dose for a chest X-ray is 0.07 mSv and 6.7 for an abdominal CT.[200]

2.4.5 Treatment of Crohn's disease

2.4.5.1 Treatment targets

CD can be a debilitating condition especially when left untreated and often leads to irreversible complications. Chronic inflammation tends to be destructive. The goal for treatment is complete disease control, the prevention of disease progression and the restoration and maintenance of normal quality of life. In the short term, the treatment should aim to gain deep (endoscopic and clinical) remission as these reduce the risk of hospitalization and surgery due to complications. The long-term goals focus on preventing the progression of bowel damage, including strictures, fistulae and abscesses, which in turn can lead to surgery, loss of bowel function and disability.[2]

Historically, the treatment of CD was based on clinical symptoms only. Recently, theories such as treat-to-target have emerged, as the symptoms and disease activity do not often correlate. The treatment goals have been shifted towards deep remission meaning normalization of biomarkers and endoscopic findings including histology.[201] In the CALM-study, monitoring of CRP and FC combined with clinical symptoms data resulted in better endoscopic and clinical outcomes compared to treatment guided by symptoms only.[202] Due to discrepancies between symptoms and the degree of inflammation, it is important to measure the disease activity at regular intervals with laboratory tests, imaging and endoscopy. This enables the clinician to react and adjust the treatment to avoid disease progression.[141]

The STRIDE II-consensus paper has so far suggested the most concrete targets for treatment of CD. Endoscopic healing is the most documented parameter linked to better long-term outcomes possibly reducing bowel damage. Response was defined as SES-CD <3. Although patients often think of clinical symptoms as the most valuable variable in treatment goals, the remission of symptoms must be ensured with objective measures. The consensus suggested that a >50% decrease in abdominal pain and stool frequency and Harvey-Bradshaw Index score of <5 should be pursued. Normalization of quality of life and absence of disability and restoration

of growth of children were also defined as goals. Normalization of serum CRP and decrease of FC are reasonable targets as they correlate well with degree of inflammation, however the clear cutoff target is somewhat unclear. The consensus agreed to suggest a target FC of 100-250 µg/g in both CD and UC. Transmural healing assessed by imaging lacks data on long term outcomes and was suggested to be an adjuvant assessment rather than a solid target. Lack of data also applies for normalization of histology and indeed, only a small percentage of CD-patients achieve complete histological remission.[141]

2.4.5.2 Medical treatment

The medical treatment for CD can be divided into three categories. First, induction of remission, second, maintenance therapy and third, fistulizing perianal disease.[203] Despite the growing number of advanced therapies (biologics and small molecules), the treatment should always be individually tailored to CD-patients balancing between the benefit of treatment and possible adverse effects and costs. Disease location, activity and severity should always be considered, but also the duration of CD, other comorbidities, individual risk factors (i.e. patient age, family planning immunodeficiencies, history of severe infections or malignancies) must always be considered and thoroughly discussed with the patient.[204]

Although no gain in terms of clinical remission, early combination therapy of immunosuppressive and anti-TNF-therapy was noted to decrease the risk of hospitalization or major surgery in the REACT-study[205] which has led to discussion between top-down (early introduction of advanced therapies) and step-up strategies (immunomodulator therapy in the beginning and step-up to biologics when necessary). In a systematic review and individual-patient meta-analysis of all placebo-controlled trials of biologics approved for CD; the study showed significantly higher ratios for remission in CD with disease duration <18 months in both the treatment and placebo-arms, which suggests the importance of early intervention.[206] This may be due to plasticity of the underlying disease process, which allows for more change in the early disease course. The PROFILE-study recently revealed that top-down strategy increases the odds for steroid and surgery free remission without significantly increasing the risk of therapy related adverse effects.[207] This strategy also decreases the risk of postoperative recurrence of CD.[208]

Aminosalicylates are no longer suggested for treatment of CD by ECCO. They have been shown to have similar properties to placebo and have no effect on induction or maintenance of remission of CD, and only a minor effect on postoperative disease prevention.[203,209]

Antibiotics can only be used for short term treatment of complications of CD such as perianal or intra-abdominal abscesses, septic infections or fistulae with

drainage combined to anti-TNF. They have no effect on achieving endoscopic remission or on maintaining remission even in luminal CD.[203,209]

Corticosteroids are often used in the induction of remission. Corticosteroids act widely on cytokines and increase phagocytosis and apoptosis of WBCs.[210] Systemic corticosteroids such as prednisolone are effective on mild-to severe CD in achieving remission in all disease locations but lack efficacy in the long term maintenance of remission. Local acting corticosteroids such as budesonide can be used in the induction of remission in patients with ileal or ileocecal mild-to-moderate CD and have fewer side effects. Side effects of corticosteroids are many and well-known including osteoporosis, metabolic issues (diabetes, hypertension, cataract, hypothalamus-pituitary-axis), mental issues. Corticosteroids also increase the risk of abdominal and pelvic abscesses in CD. ECCO does not suggest using any type of corticosteroids for sustaining the remission of CD and corticosteroid-free remission is often a key endpoint in clinical studies of novel CD treatments.[203,209]

Thiopurines (azathioprine and mercaptopurine) are antimetabolites that inhibit nucleic acid synthesis in T-cells by driving them to apoptosis. Methotrexate is another immunomodulator that acts as an antagonist of folic acid. At high doses, it inhibits DNA and RNA synthesis by cytotoxic and antiproliferative mechanisms disrupting the proliferation of WBCs such as T-cells.[211] Thiopurines and methotrexate are immunomodulators, which can be used in the maintenance of remission in CD in corticosteroid-dependent disease or for the induction of remission when combined with glucocorticoids. They also have an important role in immunogenicity as they prevent the formation of anti-drug-antibodies (ADAs) when used with anti-TNFs. Efficacy of thiopurines in achieving clinical remission or endoscopic healing is significantly lower than anti-TNFs.[212] Immunomodulators are somewhat poorly tolerated, they commonly cause myelosuppression, increased liver enzymes or GI-symptoms. Thiopurines can also cause pancreatitis and increase the sensitivity of skin for sunlight. Thiopurines are also linked to an overall increased risk of malignancies, especially lymphomas in young males. These immunomodulators also increase the risk of infections.[203,209]

Anti-TNFs are one of the most efficient current therapies for CD. In Finland, infliximab and adalimumab are available. Anti-TNFs are humanized monoclonal anti-IgG1 antibodies that are produced in cell cultures and which neutralize the cytokine TNF-alpha thereby leading to a decreased inflammatory response.[213] ECCO suggests that anti-TNFs should be used for the induction of remission in patients that do not respond to corticosteroids or patients with a complex disease (deep ulcerations, fistulae, strictures, abscesses). A large systematic review and meta-analysis that compared current advanced therapies, found that infliximab had the best efficacy in both induction and maintenance of remission in luminal CD.[214] The REACT-study showed that early introduction of adalimumab decreases the risk of

hospitalization or surgery.[215] Infliximab is the only advanced therapy with reported efficacy on perianal fistulizing CD in a randomized placebo-controlled trial, where fistula healing is the primary enpoint.[216,217] The CHARM-study has reported that adalimumab showed efficacy compared to placebo for perianal fistula healing as a secondary endpoint.[216] Anti-TNFs also successfully treat strictures and several extra-intestinal manifestations such as pyoderma, uveitis, psoriasis, and axial spondyloarthropathy. They are also effective for preventing postoperative recurrence and are relatively safe to use during pregnancy. Anti-TNFs are often used in combination with immunomodulators for the first year to reduce the risk of ADAs, as up to 30% of patients lose response over time.[218] The SONIC-trial reported that the combination of azathioprine and infliximab was more effective in achieving clinical remission and also mucosal healing compared to monotherapy with infliximab.[212] Anti-TNFs were introduced as early as in the 1990s and currently there are widely available biosimilars of infliximab and adalimumab, which cut costs of the treatment without compromising safety or efficacy. The side effects of anti-TNFs include serious infections and psoriasiform eczematous reactions of the skin. Importantly, combination therapy with immunomodulators should be used carefully in elderly patients due to the higher associated risks in this population.[203,209]

The second line of biologics available is the anti-integrins, currently vedolizumab is a widely available molecule approved for CD. Vedolizumab is an anti-α4β7 integrin monoclonal IgG1 antibody that is gut selective and it allows for a favourable safety profile. Gut selectivity though limits its use in treating EIMs. α4β7 is expressed on a subset of T-cells; vedolizumab blocks the interaction between α4β7 integrin and MAdCAM-1 that is mostly expressed in the endothelial cells of the gut. The mechanism of blockage is principally by reducing the migration of T-lymphocytes into the gut. ECCO recommend the use of vedolizumab for both the induction and maintenance of remission in moderate-severe CD. Vedolizumab can also be used in patients who have failed anti-TNFs (both primary failure and secondary loss of response due to ADAs).[203,209] Data on treating complex CD with vedolizumab are lacking, but vedolizumab may be useful even in perianal disease.[219] Other molecules targeted against leukocyte trafficking such as etrolizumab and ontamalimab have not shown significant efficacy in CD, however.[209]

Another option for anti-TNFs are molecules targeted against interleukins. Ustekinumab was first introduced to treat CD. It is an anti-IL-12 and IL-23 p40 IgG1 antibody. ECCO recommends the use of ustekinumab for treating moderate to severe CD in both the induction of remission and as a maintenance therapy in patients who have failed conventional therapy or anti-TNFs or who have contraindications for either of these. Ustekinumab and vedolizumab are both recommended as valid options for a second line of advanced therapy. The SEAVUE head-to-head trial showed similar rates of clinical and endoscopic remission in induction therapy with

ustekinumab and adalimumab in biological naïve patients with moderate-to-severe CD (65% vs 61% at one year, respectively).[220] Several studies have shown that the rate of adverse effects is similar between ustekinumab and vedolizumab.[203,209] These findings emphasize, that both ustekinumab and vedolizumab can also be used in elderly and possibly immunocompromised patients. This hypothesis was confirmed in a retrospective study by Holvoet et al.[221]

Several new molecules that affect the IL-12/23 pathway have been developed. Currently, risankizumab is available in Finland. Risankizumab is targeted against the p19-subunit. Risankizumab is expected to provide better efficacy over ustekinumab and it can also be effective in patients with previous failure with ustekinumab. For example, in both ADVANCE and MOTIVATE trials the response rates to risankizumab were higher than placebo (37.2% vs 15.8% and 36.1% vs 10%, respectively).[222] Risankizumab seemed to be the most powerful in achieving and maintaining clinical remission in luminal CD with patients previously exposed to advanced therapies.[214] Risankizumab is also recommended in the latest ECCO-guidelines as both induction and maintenance therapy of moderate-to-severe CD.[223] Mirikizumab and guselkumab are also promising molecules targeting the same pathway, but not yet available for CD treatment in Finland.[209]

The first janus kinase (JAK) inhibitor approved for CD is upadasitinib. The JAK-inhibitors are small molecules that can be administered perorally, compared to other advanced therapies that must be administered intravenously or subcutaneously. The JAK-inhibitors are fast acting, lack immunogenicity, and have a short half-life compared to other advanced therapies. Upadasitinib is effective for both induction and maintenance of clinical remission and endoscopic response was shown in U-EXCEL, U-EXCEED and U-ENDURE studies.[224] JAK-inhibitors have advantages of treating several extraintestinal manifestations, but an increased risk for thromboembolic events is linked to JAK-inhibitors, as well as increased cholesterol levels, risk for major cardiovascular events, and cancer. Thus JAK-inhibitors should be cautiously used in patients over 65 years or older.[209] JAK-inhibitors have been reported to be teratogenic in animal studies and are therefore contraindicated during pregnancy, however, small studies on tofacitinib (a JAK-inhibitor approved for UC) suggest that pregnancy and neonatal outcomes in UC studies of tofacitinib are close to those in the general population.[225] Upadacitinib is so far the only JAK-inhibitor recommended in the latest ECCO-guideline as both induction and maintenance therapy of moderate to severe CD.[223]

In addition to medical therapies that are targeted towards inflammatory process in CD, nutritional deficiencies in CD patients should also be corrected. The role of dietary therapy has been under investigation for a long time. Although no single specific diet has been shown to be effective for long term efficacy in the remaining remission, due to its overall health benefits, a mediterranean diet should be suggested

to all IBD patients according to recent guidelines of American Gastroenterology Association (AGA).[226]

Recently, food-based therapies have been proposed to reduce inflammation and may be considered as a potential adjunct to medical therapy. Crohn's Disease Exclusion Diet (CDED) has been shown to be effective for induction and remission of mild-to-moderate CD with or without EEN. CDED is a whole-food diet for which pro-inflammatory foods are avoided.[227,228] The most recent ECCO guideline on the treatment of CD recognizes these results and recommends that partial enteral nutrition may be considered as a strategy for maintaining remission and the role of dietary counselling is highlighted especially during CD flares. However, neither ECCO nor The European Society for Clinical nutrition and Metabolism (ESPEN) recommend a specific diet for all CD patients.[223,229]

The patients should also be monitored for therapy-related side effects such as infections and cancer. The patients should receive guidance concerning vaccinations, sun protection and osteoporosis. All CD-patients should be advised to cease smoking.[203]

2.4.5.3 Surgical treatment

Despite evolving medical therapy and the growing number of molecules targeted at CD, surgery remains an important part of treatment in CD. In fact, nearly half of the patients with CD need surgery at some point of their lives.[230,231] Rates of surgery have not declined in Europe compared to cohorts from 25 years ago. The rates are similar across Europe even though advanced therapies are more commonly used in Western Europe.[232] Care for CD often requires a multidisciplinary team to optimize and tailor medical treatment and consult for surgery. Nutritional optimization, steroid-tapering, control of infection (with antibiotics) and prevention of thromboembolic complications are important. Preoperative corticosteroid treatment (prednisolone 20 mg/day) is associated with an increased risk of postoperative complications and corticosteroid tapering may reduce the risk of complications, but the risk of a worsening of inflammation should be considered.[233] Perioperative anti-TNF, vedolizumab or ustekinumab therapy do not increase the risk of complications.[234,235] Postoperative treatment should also be planned to reduce the risk of disease relapse.[236]

CD surgery can be divided into two parts: acute emergency surgery, due to complications such as acute obstruction of the bowel, perforation or fulminant colitis, and elective surgery, due to medically refractory disease and complications progressed beyond the reach of medical therapy. The indications for perianal CD surgery are complex: fistulae, perianal abscesses, and also septic pelvic inflammation which is also an emergency surgery indication.[236]

Pelvic sepsis due to complex fistulae or fistulae refractory to medical or surgical interventions can be controlled by a diverting stoma.[237] Fistulae can be treated by advancement flaps or ligations of the intersphincteric fistula tract (LIFT). Controlling perianal sepsis by proper examination under anaesthesia and appropriate Seton drainage are key measures. In the case of perianal disease, anti-TNF therapy should be initiated. Fibrin glue or stem cell therapies may be effective and safe treatment options for complex perianal fistulae.[236]

Treating intra-abdominal abscesses is difficult, as antibiotics may not be sufficient in the case of a large lesion and immunosuppression can also be hazardous. Ultrasound or CT guided percutaneous drainage (PD) of well-defined, accessible intra-abdominal abscess is recommended as the primary approach by ECCO. After resolution of acute abscess, elective surgery should be considered, as the recurrence rate of abscesses is high in PD only compared to PD followed by surgical resection. PD in the acute setting may be considered as a bridge therapy to elective surgery after optimization of nutritional and medical treatment.[236,238]

Treatment of strictures in the acute setting is often conservative including fluid resuscitation, bowel rest and gastric decompression. In the case of a short stricture (<5cm), endoscopic balloon dilatation is a suitable option for surgery in the terminal ileum, although 76% of the patients will undergo surgery within 5 years.[239] For multiple strictures and strictures in the more proximal small bowel, a stricturoplasty is a feasible option as more bowel can be spared compared to resection of long segments of bowel. Regarding colonic CD, segmental resection can be appropriate for patients with only a single involved segment, whereas subtotal or total colectomy is preferred with multiple involved segments. A diverting stoma may postpone or even avoid the need for colectomy in an acute setting or in extensive perianal disease refractory to advanced therapies.[236]

2.4.6 Small intestine

2.4.6.1 Anatomic structure and function

The small intestine is approximately 5-6m long in adult humans. The diameter is normally less than 25mm. It is divided anatomically into three segments starting from the duodenum, which connects the small intestine to the ventricle in the pylorus. The duodenum secretes a mucus rich alkaline secretion containing bicarbonate, which is mixed with digestive lipolytic and proteinolytic enzymes secreted by the pancreas, and also gastric chyme which neutralize the acidic chyme. Following the duodenum is the jejunum, which is lined by enterocytes that are specialized at the absorption of sugars, amino acids, and fatty acids. The distal part of the small intestine is the ileum, which is connected to the proximal colon via the

ileocecal valve. The ileum absorbs mainly vitamin-B12 and bile acids. The duodenum is located retroperitoneally, and the more proximal parts are attached to the mesentery in the abdominal cavity.[240]

The small intestine is folded, which increases the surface area and thereby increases its absorptive capacity. The lining of the small intestine consists of permanent circular folds (*plicae circularis*), which further amplifies the surface area and, hence enhances efficient nutrient absorption. The plicae circulares are covered by villi, which are about 1mm in length, and between the villi are crypts. At the base of crypts, new epithelial cells are constantly formed which renew the epithelial layer. The wall of the small intestine consists of the following layers: mucosa, submucosa, muscular layer, and the serosa. The mucosa can further be divided into three sections, intestinal epithelium, lamina propria and muscularis mucosa (longitudinal and circular). The epithelium has a crucial role as the first line of defence against luminal pathogens in the small intestine as discussed earlier. The submucosa is fibrous connective tissue that supports the muscular layer and is abundant in the blood and lymph vessels. The muscular layer is responsible for peristalsis, which moves and mixes the luminal contents to aid digestion and absorption. The serosa acts as connective tissue that covers the whole intestine.[240] (Figure 6.)

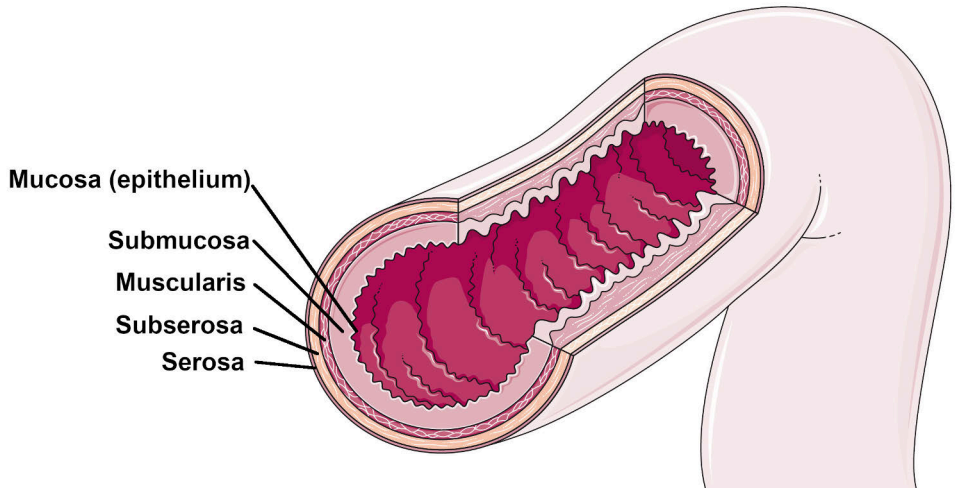

Figure 6. The anatomic layers of the small intestine. Illustrated partly using Servier Medical Art; https://smart.servier.com/, provided by Servier, licensed under a Creative Commons Attribution 3.0 Unported license.

The blood circulation of the small intestine mainly comprises branches of superior mesenteric artery (distal part of duodenum, jejunum and ileum). The

duodenum also receives blood from the celiac trunk via the superior pancreaticoduodenal artery. Arterioles in the villi are divided into multiple segments that form capillary granules. The veins follow the arteries and return to the portal vein.[240]

2.4.6.2 Glucose metabolism

In addition to the network of capillaries, the villi contain fine lymphatic vessels (lacteals) close to their surface. Amino acids and carbohydrates are absorbed by and translocated into the capillaries, whereas lipids are absorbed by the lacteals. Glucose is absorbed by the epithelial cells via the sodium-glucose cotransporter (SGLT-1) and by facilitated diffusion glucose-transporter 2 (GLUT2) located at the apical side of epithelial cells. The sodium-potassium pump, pumps out sodium from the epithelial cells to create a gradient for glucose cotransporter (GLUT2) thereby allowing glucose to diffuse into the capillaries at the basal surface of the epithelial cells.[241] Metformin has been shown to increase glucose uptake in the small intestine by up to two-fold.[9]

2.5 Fusion PET-MR imaging

2.5.1 Principles of magnetic resonance imaging

Magnetic resonance imaging (MRI) creates images of the body by strong magnetic fields, magnetic field gradients and radio waves. In contrast to CT and PET, MRI does not generate ionizing radiation, that is harmful to humans. Hydrogen nuclei are present all over the human body, particularly in water and fat tissues. In MRI, the hydrogen nuclei at an area of interest are exposed to a strong oscillating magnetic field at the appropriate resonance frequency. The hydrogen atoms are excited by a strong radiofrequency pulse (RF) that spins them out of their equilibrium state due to the pull of the magnetic field. Normally, the atoms are randomly oriented, but when excited become aligned along the magnetic field. After the RF-pulse, the hydrogen atoms return to their steady state (relaxation) and thereby release energy that can be detected as a radiofrequency signal by a sensor that spins rapidly around the patient in the MRI device. Essentially, MRI images map the location of water and fat in the body.[200]

T1 is the process of tissue returning to its equilibrium state. In T1-images, the magnetization is in the same direction as the static magnetic field. Magnetization is then allowed to recover before measuring the RF signal. The repetition time (TR) can be changed to create a T1 weighted image. T1-weighted images enhance the signal of fatty tissues and suppress the signal of water. In a T2-image the

magnetization is transverse to the static magnetic field. To create a T2-image, magnetization is allowed to decay before measuring the RF signal emitted. This can be adjusted by changing the echo time (TE). In contrast to T1, T2-weighted images increase the signal of water and decrease the signal of fat. T2-weighted images are better for detecting oedema and inflammation in the intestine, whereas T1-weighted images can reveal intestinal fat, which is a sign of chronic inflammation. Intravenous contrast can be administered to increase the speed at which the protons realign with the magnetic field. Faster realignment of protons results in a brighter image.[200,242]

2.5.2 Principles of positron emission tomography

PET imaging is a functional imaging technique that uses radioactive tracers or radioligands to measure quantitatively metabolic processes or receptors in the human body *in vivo*. These processes include blood flow, absorption, and chemical composition of the area in the target. Various tracers have been developed to measure different parameters in different parts of the human body. A tracer is simply a radioactive molecule that is attached to a molecule that is injected intravenously, and blood flow then distributes the tracer around the body. Its decay is then measured by a camera. In clinical practice, PET imaging is most widely used in oncology for the imaging of tumors and to detect and locate metastases. Inflammatory conditions can also be assessed with PET imaging such as fever of unknown origin.[200]

In PET imaging, the concentration of a radioactive tracer in the body is measured. The radionuclei of the tracer undergoes beta decay, in which a positron i.e. a positively charged electron is emitted and transfers through tissues constantly losing its kinetic energy, until it collides with an ordinary electron. In the collision, annihilation takes place thus releasing two photons and 511keV energy. As the photons are released, they move in exactly opposite directions at the speed of light. A PET device consists of gamma-counter detectors placed around the patient, which register these photons released in the annihilations. Only the photons received in the exact opposite direction are measured (coincidence detection). The trajectory of the photons heading in the opposite directions is called line of response (LOR). The counts measured by the detectors are proportional to the radioactivity along the LOR. Despite the exact place of annihilation being unknown, 2D images can be reconstructed using a coordinate system. The angle and distance from detector of each annihilation can be calculated and then plotted to a single point on a graph. Composite grouping of all angles is called a sinogram, which is presented as a function of time. These can then be converted to a matrix, where each row represents the number of counts at a single angle. As there are multiple rows of detectors in different angles placed 360 degrees around the patient in modern PET imaging devices, from 2D data a 3D image can later be constructed. In this image, the voxels

represent the number of annihilations of the radioisotopes in the tracer, which is proportional to the concentration of tracer at the location.[243,244] (Figure 7.)

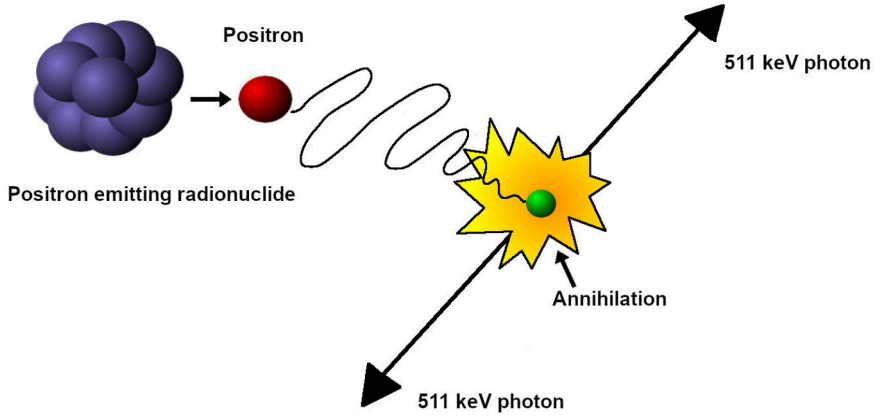

Figure 7. The radioactive positron emitted collides with an electron while travelling through tissue. Collision results in an annihilation, where two photons each with 511keV of energy are released. The two photons travel in exactly opposite directions. The gamma counter devices in the PET scanner register the photons received at exactly opposite directions. The exact place of annihilation is unknown, but a coordinate system can be used to reconstruct a 2D image. In modern PET scanners, there are multiple rows of detectors placed 360 degrees around the patient and data from these detectors can be combined in a matrix and further converted to 3D images.

There are several factors that degrade the image quality in PET imaging. First, the half-life of the tracers is rather short, approximately 110 min for ^{18}F, 20 min for ^{11}C, 10 min for ^{13}N, and 2 min for ^{15}O. Thus, the time from creating the radiotracer to the moment when it is injected into the patient should be minimized to avoid excess decay in order to maximize signal intensity. As the decay of each tracer is known exactly, decay correction can be calculated and applied to the image data. Different tracers also take different times to accumulate into the tissue of interest. For example, it takes roughly 20-60 min for the blood flow to spread tracers based on small molecules and peptides evenly across the body.[245] Larger proteins and antibodies, on the other hand, have slower kinetics and may require days for even distribution and clearance. Second, there are random events; the photons emitted in the annihilation do not always arrive at the detector at exactly the same time. Therefore, a time window is set for the detectors to count the photons. The longer the time window, the more counts, but this in turn increases the number of false counts due to scattering increasing noise in the final image. Noise is essentially random variations in pixel intensity, which is decreased with more counts. Noise can thus be decreased by increasing the scan time, by using higher dose of tracer or increasing the sensitivity of the detectors. Third, there is attenuation caused by part

of the true counts scattering, absorbed and not detected at the LOR. Attenuation depends on the density of the material (i.e. the type of tissue). Attenuation causes noise because of lost data. Attenuation also depends on the site of annihilation; when annihilation happens at the center of the subject, the photons must travel longer and through more tissue than photons emitted near the surface of the subject. With increased distance, there is higher risk of scattering. Additionally, the tissue near the surface gathers more scattered photons from annihilation taken place in more central locations, which causes higher signals at the perifery of the subject. This attenuation anomaly can be reduced by using a CT image to measure the density of the subject and which can be used to correct the PET data.[246] However, in MRI, the tissue density is not exactly proportional to the electron density, so attenuation correction can not be directly derived from MRI data.[246] Several MRI attenuation correction algorithms (MRAC) have been developed, but these are limited by the anatomic heterogeneity of the study subjects, motion artefact and poor MR signal of bones. Bones do cause excessive bone tissue attenuation that distort the results. However, with the current MRACs, the PET-MRI images are comparable to PET-CT images in attenuation correction.[247,248] Indeed, with modern devices, a majority of false counts (scattered photons without corresponding annihilation) can be eliminated with window correction procedures. Lead and tungsten sheets absorbing scattered photons are also used in the construction of PET scanners to reduce attenuation. The study subject should also be placed in the center of the scanner to create an optimal field of view. Fourth, the detectors are not able to detect another count immediately after the previous photon; this is called dead time. To optimize the image quality, dead time can be adjusted. All these factors result in a maximal spatial resolution of ~2.5 mm for human PET scanner, which is less than that for CT or MRI.[249] This is why the kinetics of the radioligand of the tracer is usually linked to CT or MRI data to create precise PET images that enable the recognition of the exact anatomic location of tracer activity. Due to relatively poor spatial resolution, the chance of one voxel representing different anatomic structures increases, which is called the partial volume effect (PVE). The importance of PVE is most pronounced when the size of the anatomic structure of interest is less than 2 times the spatial resolution of the PET scanner.[250] Different partial volume-correction techniques have been developed to minimize this problem.[243,244,251]

PET data can be gathered statically, or dynamically. Dynamic PET data describe the change in radioactivity over time. In comparison, in static PET data only the radioactivity of a single time point is seen. Static PET data are often gathered after a longer time interval for tracer injection to scan, to enable adequate distribution of the tracer around the body. This is on the assumption of a stable radiotracer concentration and even distribution between blood circulation and other tissues. Dynamic data represent the change in tissue activity concentration and with different

models and calculations, this can be thought to visualize the translocation of a tracer from the blood circulation to the tissue of interest. Finally, after the accumulation of sinogram 2D data, an attenuation correction and a decay correction are applied. This results in a time-activity curve (TAC) where the decay of the radioactivity of the PET tracers radioisotope can be plotted as a function of time.[244,245]

2.5.3 Fusion imaging

When a PET scan is combined with MRI, the soft tissues can be visualized in more detail compared to CT. Additionally, there is no harmful ionizing radiation. The drawbacks of MRI compared to CT are a lower resolution and reduced slice thickness. The image acquisition time in CT is faster and causes less motion artefact resulting from breathing, cardiovascular system activity, and peristalsis. The fusion devices currently used have either CT or MRI scanner embedded in the same structural assembly as the PET scanner thus enabling sequential imaging without having to move the patient to a different scanner between scans.[246] Figure 8 shows a fusion PET-MRE image.

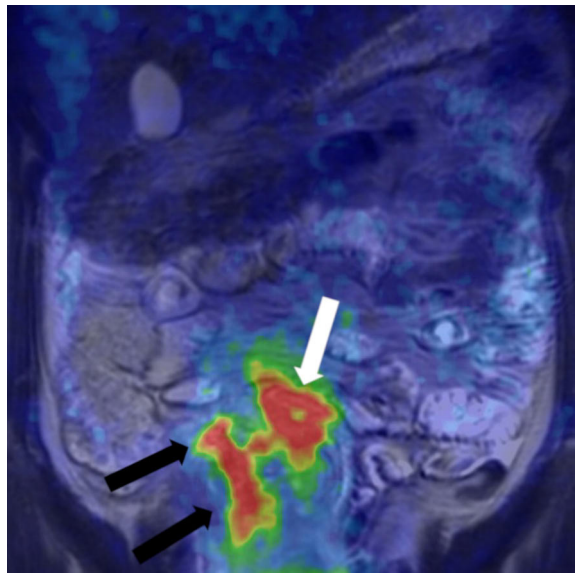

Figure 8. A fusion PET-MRE image of a 22-year-old-male patient who participated in Study II with active CD inflammation in ileum (black arrows) and an inflammatory phlegmon (white arrow). Modified from the original publication II with permission from Karger Publishing.

2.5.4 PET tracers

The tracers are labelled with positron emitting radioactive isotopes in cyclotrons or generators. In cyclotrons, negative hydride ions (e-) are accelerated in a strong electromagnetic field in a spiral motion, until they gain sufficient energy to escape. The beam of accelerated negative ions passes through a foil of graphite, which strips the electrons of the hydride ion thus producing the desired protons. The protons are then directed towards stable isotopes and the resulting collision produces the desired isotopes.[252]

[^{18}F]-FDG is a glucose analog that is widely used in visualizing energy metabolism in cells as the [^{18}F]-FDG molecule follows the same pathways as glucose in living tissues. In tumors or inflammatory cells, a metabolic shift towards glycolysis occurs, called the Warburg effect.[252] [^{18}F]-FDG is transported into the cell via GLUTs and is then phosphorylated intracellularly by hexokinase in the same process as occurs with glucose phosporylation to form [^{18}F]-FDG-6-phosphate. The activity and expression of glucose-6-phosphatase is low in other tissues except in the liver and kidneys. Due to [^{18}F] substituting a hydroxyl group, [^{18}F]-FDG-6-phosphate can not enter the glycolytic pathway nor the glycogen synthesis pathway. Nor is [^{18}F]-FDG-6-phosphate transported back to the blood, and due to this 'trapping' it thus accumulates in the tissue.[253] The trapping occurs until the decay of the [^{18}F]-positron. Due to this trapping mechanism, the intracellular concentration of [^{18}F]-FDG represents the glucose metabolic activity of the cell and can be quantified.[253]

Quantifying tissue specific glucose uptake with [^{18}F]-FDG *in vivo* using a hyperinsulinemic euglycemic clamp to mimic postabsorptive conditions and allow metabolic research was first described by Nuutila et al. in 1990s. [^{18}F]-FDG uptake is dependent on glucose transporters, which are indirectly affected by serum glucose level, insulin level and cellular demand. Inflammation causes increased glucose uptake due to recruitment of activated WBCs, which have high expression of GLUTs.[254] Inflamed tissue also expresses an abundance of cytokines and growth factors which together cause increased uptake of [^{18}F]-FDG.[255] However, increased uptake of [^{18}F]-FDG can not directly distinguish between inflammation and malignant tumors. Malignant cells have a decreased expression of glucose-6-phosphatase, which causes metabolic trapping of [^{18}F]-FDG. Compared to this, there is an increased expression of glucose-6-phosphatase and GLUTs in the inflammatory lesions, which leads to increased uptake and clearance of [^{18}F]-FDG.[256] [^{18}F]-FDG uptake in the intestine has been shown to correlate positively with the presence of the inflammatory cytokines IL-6 and TNF-alpha; both of which play a crucial role in the pathogenesis of CD.[257] This raises the question, as to whether [^{18}F]-FDG PET imaging could be used to monitor the efficacy of medical therapy in CD.

SUV is mostly used in quantifying [^{18}F]-FDG activity concentration. SUV is a semiquantitative variable that describes radiopharmaceutical accumulation in PET

studies. SUV is calculated using the radioactivity concentration measured by the scanner, the decay-corrected injected dose of the tracer and body weight.[258] This, however, only represents the [^{18}F]-FDG activity of a single moment and does not differentiate between the activity of [^{18}F]-FDG-6-phosphate of the targeted tissue and that of unbound [^{18}F]-FDG of the background. Dynamic imaging using multiple scans can bring more accurate information on the glucose metabolism of the target lesion, but studies on this are few.[259]

The confounding effect of background activity on SUVs may be decreased using prolonged scan times, but this also reduces the activity of the target lesion. Artifacts seen as increased [^{18}F]-FDG uptake may be caused by peristalsis of the muscular layer of the gut, bacterial uptake or luminal content of the gut. Lymphoid tissue inside the mucosa may also cause increased [^{18}F]-FDG uptake.[260] Benign lesions such as polyps and post-surgical changes may also cause false positive findings in PET imaging with [^{18}F]-FDG. Mild inflammation is also difficult to differentiate from physiological uptake. Bowel distension using large volume oral contrast and spasmolytics can be used to reduce background artefact.[261] Due to several sources of error in PET imaging of IBD with [^{18}F]-FDG, it is crucial that the extent, intensity and anatomic location of uptake is evaluated when interpreting imaging findings. For example, mild and diffuse uptake of [^{18}F]-FDG is more likely to be an artifact of normal physiologic processes, whereas segmental and intense uptake is more likely due to inflammation.

Glucocorticoids can also decrease the uptake of [^{18}F]-FDG as demonstrated in studies on giant cell arteritis and bacteremia. Even 7 days of glucocorticoid treatment can decrease SUVs and increase the risk of false negative findings.[262–264] In addition to the reduction of inflammation, this can also be explained by glucocorticoids decreasing glucose uptake and utilization by antagonizing the insulin response.[265] However, the use of glucocorticoids for 3 days or less does not reduce the sensitivity of PET imaging.[266,267] Glucocorticoids also generally increase plasma glucose levels. As [^{18}F]-FDG is a glucose analog, competition occurs between [^{18}F]-FDG and plasma glucose when entering cells via GLUTs. Thus, high plasma glucose levels decrease [^{18}F]-FDG uptake. High plasma glucose levels also result in higher intracellular glucose levels and thus more competition with the phosphorylation of [^{18}F]-FDG to [^{18}F]-FDG-6-phosphate, which in turn lowers the accumulation of intracellular [^{18}F]-FDG.[268] As elevated plasma glucose levels significantly interfere with [^{18}F]-FDG uptake, the EANM guidelines recommend a plasma glucose level of less than 11mmol/l for diagnostic [^{18}F]-FDG imaging and below 7-8.3mmol/l for research purposes.[269]

3 Aims

The aims of this thesis are to improve the accuracy of diagnostics and follow-up of small bowel CD, to thereby help avoid unnecessary procedures and to add to the knowledge of finding the correct treatment for patients with small bowel CD.

The specific aims were as follows:

I. To find predictive markers of CD in patients referred to SBCE with suspected small bowel CD, to avoid unnecessary procedures and make the differential diagnostics of CD easier.

II. To evaluate the value of [^{18}F]-FDG PET-MRE in diagnostics of small bowel CD by comparing the findings to conventional MRE, SBCE and ileocolonoscopy and further to compare diagnostic performance of [^{18}F]-FDG PET-MRE to MRE.

III. To assess whether [^{18}F]-FDG PET-MRE can be used in follow-up of small bowel CD by comparing its performance to MRE.

4 Patients and Methods

4.1 Patients

4.1.1 Patients with suspected small bowel CD scheduled for SBCE (Study I)

Data were collected retrospectively from all the patients who underwent SBCE in Turku University Hospital Department of Gastroenterology between 5 January 2012 and 22 October 2020. A total of 766 SBCE procedures were performed during that time. 374 patients who had SBCE for suspected small bowel CD were included in the study (Table 5). Patients had symptoms, imaging, endoscopy or laboratory findings suggestive for CD. Patients who had SBCE for previously established small bowel CD were excluded as were patients with only obscure GI-bleeding, suspected malignancy of small bowel or refractory coeliac disease. Only the first SBCE for each patient was included when a patient had multiple SBCE studies during the time of the study. The study plan was approved by the committee of ethics of Hospital District of Southwest Finland. Informed consent of patients were not needed in view of the retrospective nature of the study.

Table 5. Demographics of the patients included in Study I. CD-group = patients diagnosed with CD by SBCE. Non-CD group = patients not diagnosed with CD in SBCE. NSAID = nonsteroidal anti-inflammatory drugs. Modified from original publication I.

	CD-group, N=110 (%)	Non-CD-group, N=264 (%)	p-value
Age (years)			
Mean	40.5	40.9	0.69
Median [IQR]	36.1 [26.4-52.5]	38.1 [27.4-53.9]	
Age (min-max)	10.9–79.5	3.9–87.3	
Sex			
Male	54/110 (49.1)	104/264 (39.4)	0.086
Female	56/110 (50.1)	160/264 (60.6)	0.086
NSAID			
NSAID use	22/109 (20.2)	59/251 (23.5)	0.58
Smoking status			
Smokers	21/102 (20.6)	50/211 (23.7)	0.39
Ex-smokers	16/102 (15.7)	22/211 (10.4)	
Never smoked	65/102 (63.7)	139/211 (65.9)	

4.1.2 Volunteer patients with suspected small bowel CD recruited for a diagnostic [^{18}F]-FDG PET-MRE (Study II)

A total of 35 volunteer patients were recruited from Turku University Hospital's Department of Gastroenterology outpatient clinic. Of these, 30 patients completed the study and were included in analyses. All patients had colonoscopy done prior to recruitment with biopsies taken from the ileum, except for one patient who had an unpassable terminal ileum due to stricture and for whom no biopsies were thus taken. The inclusion criteria were 18-70 years of age and a suspicion of small bowel CD. Patients with previously known CD, metformin-medication, pregnancy, or unbalanced diabetes were excluded. A written informed consent was obtained. The included patients went through [^{18}F]-FDG PET-MRE. If the patients had no strictures in the [^{18}F]-FDG PET-MRE or symptoms suggestive for small bowel stenosis, the patients were scheduled for SBCE to confirm CD-diagnosis. Clinicians were allowed to start appropriate medication for CD after the [^{18}F]-FDG PET-MRE. Clinicians had access to all results for endoscopy, histology, laboratory, MRE, but were blinded to the PET-data (Figure 9). The study plan was approved by the committee of ethics of Hospital District of Southwest Finland. Informed consent was obtained.

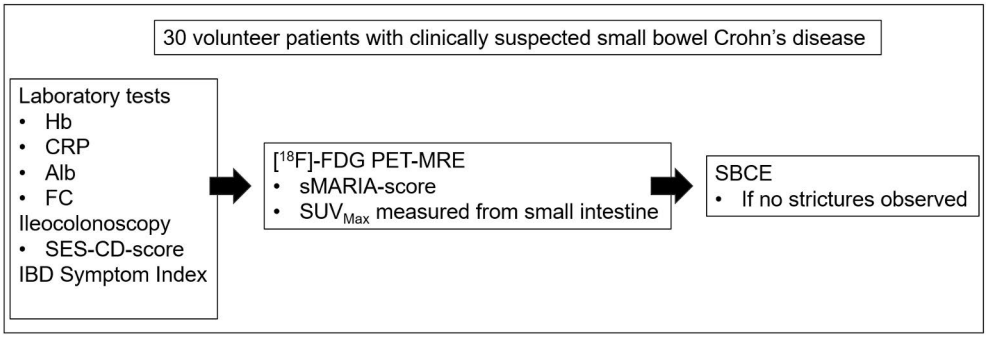

Figure 9. Study design outline of Study II. Modified from original publication II with permission from Karger Publishing.

4.1.3 Patients with small bowel CD diagnosed in [^{18}F]-FDG PET-MRE (Study III)

The patients diagnosed with small bowel CD in Study II, had the opportunity to take part in Study III. Of the 35 patients who initially were recruited for Study II, 18 had a follow-up [^{18}F]-FDG PET-MRE at approximately three months after the first diagnostic [^{18}F]-FDG PET-MRE. These patients had medical therapy started for CD by clinicians who were unaware of the PET results (Figure 10). The study plan was approved by the ethics committee of Hospital District of Southwest Finland. Informed consent was obtained.

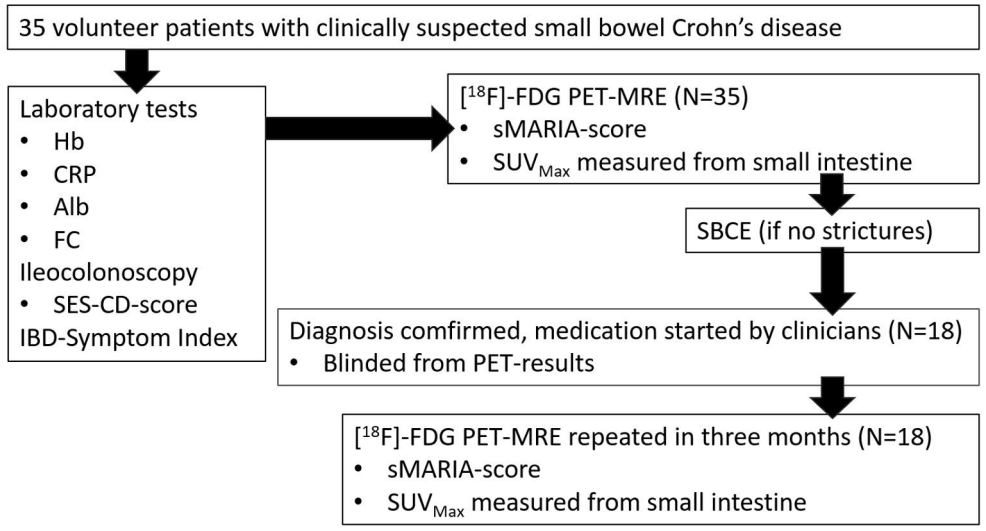

Figure 10. Study design outline of Study III. Modified from manuscript of Study III.

4.2 Methods

4.2.1 Data gathering (Study I)

The data for each patient were collected from the electronic patient database of Turku University Hospital and managed by using the REDCap electronic data capture tool hosted at University of Turku. Laboratory results (Hb, CRP, Alb, ESR and FC) prior to SBCE were collected for each patient. When no prior laboratory results were available, laboratory results from the closest date to SBCE were used. A text search tool in the electronic patient database was used to find the smoking status and the use of NSAIDs by each study patient. The GI-symptoms and signs (diarrhea, abdominal pain, weight loss, bloody stools) and indications of SBCE were collected from the electric patient database as the referring physician had described them. Medication data were retrieved for each patient. The CD medication was classified into one of four groups: 1) glucocorticoid course for less than three months, 2) glucocorticoids for more than three months, 3) immunomodulatory drugs (azathioprine, mercaptopurine or methotrexate), 4) biologics (adalimumab, infliximab, vedolizumab or ustekinumab). The date of the initiation of medication was noted and compared the date SBCE commenced. Data on CD related surgery and endoscopic dilatations were collected and the time between the operation and SBCE compared. Data on capsule retention were collected. Data were also collected on patients who had initially normal SBCE findings, but were later diagnosed with CD.

4.2.2 Data gathering (Studies II-III)

Data for each laboratory result (Hb, CRP, Alb, FC) were collected prospectively from the electronic patient database of Turku University Hospital. Medical records were used to extract medication data for each participating patient. The patients filled in a validated symptom questionnaire (IBD-Symptom Index)[270] with a score ranging from 0-22 at the date of each PET-MRE imaging.

4.2.3 Endoscopy (Studies I-III)

In Study I, the endoscopic findings were retrospectively collected from the electronic database. At the time of the study, there was no systematic grading (such as SES-CD) generally used in the patient population, thus the endoscopic findings had to be divided into two groups: ileitis and normal. Histopathology was also collected retrospectively in Study I and classified into three groups (normal, nonspecific ileitis and granulomas) based on descriptive morphology only.

In Studies II and III, endoscopic findings were retrospectively graded along the SES-CD-score by the researchers based on the written reports issued by the endoscopists. Still images stored in the electronic patient database were used when the endoscopists had not calculated SES-CD-score in their written endoscopy report.

4.2.4 SBCE-procedure (Studies I-III)

SBCE was performed with PillcamTM SB2 in 2012–2013, and PillcamTM SB3 from 2013 onwards (Medtronic, USA) (Studies I-III). PEG preparation was used prior the procedure from 2015 onwards (Study I) and hence for all the patients who participated in studies II and III. The SBCE findings for all the patients in these studies were reviewed by a single experienced clinician and graded according to the CECDAI-system. CECDAI was chosen over the Lewis-score, as it is easily reproducible and independent between readers of the study and as demonstrated in several studies.[17,138,153–155]

4.2.5 Imaging

4.2.5.1 Imaging in Study I

The data on imaging were retrospectively collected for the patients in Study I from the electronic patient database register of Turku University Hospital. The imaging modality was recorded (CT, MRE or no imaging prior to SBCE). The imaging findings were classified as normal, unspecific inflammation or active inflammation based on the radiologists written report.

4.2.5.2 PET-imaging protocol (Studies II-III)

Patients fasted for 6 hours before PET-MRE imaging to reduce artefact from bowel content and disturbances in glucose metabolism. Patients had venous cannulas bilaterally inserted in both antecubital veins: one for tracer injection and the contralateral vein for collecting blood samples. Fasting glucose of venous blood sample was determined before imaging. 1200 ml of diluted 3% oral mannitol solution was used to prepare the bowel for optimal small bowel wall visualization. Patients were positioned in the prone position to optimize image quality. 4MBq/kg of [^{18}F]-FDG tracer was injected into the antecubital vein. A static PET sequence was obtained at least 30 min after the tracer injection to allow sufficient distribution of [^{18}F]-FDG within the peripheral tissues.

4.2.5.3 MRE-protocol (Studies II and III)

A 3T-PET-MR scanner (SIGNA™ PET/MR, General Electric, Boston, MA, USA) was used for imaging. Patients received 10 mg of intravenous hyoscine butylbromide (Buscopan®, Boehringer Ingelheim International GmbH, Germany) during imaging to reduce artefact and background noise resulting from bowel motility activity, and 0.1 mmol/kg gadoterate meglumine (Dotarem®, Guerbet, France) as the MR contrast agent. The following MR sequences were used: 1) 2D breath hold (BH) fast imaging using steady-state acquisition (FIESTA) in coronal plane (slice thickness of 4 mm; TE/TR of 1.4 ms/3.7 ms), 2) 2D T2-weighed single shot fast spin echo (SSFSE) in coronal (slice thickness of 4 mm; TE/TR of 90 ms/3750-4615 ms) and in axial plane (slice thickness of 6 mm; TE/TR of 90ms/2100-2333 ms), 3) 2D diffusion weighted imaging (DWI) with fat saturation in axial plane (b-values of b50, b500 and b1000; slice thickness of 6mm; minimum TE) , 4) pre- and post-contrast BH fat saturated 3D T1 gradient echo in the coronal plane with 40 seconds post-contrast delay (slice thickness 2/4 mm; minimum TE) and 5) post-contrast BH fat saturated 3D T1 gradient echo in axial plane (slice thickness 2/4mm; minimum TE).

4.2.5.4 Image analyses (Studies II and III)

Endoscopists, PET-MRE readers and clinicians were blinded to each other's results. MRE analysis was done by a single experienced abdominal radiologist using sMARIA-score to grade the degree of inflammation of the small intestine. The segment with the highest sMARIA-score was included in the analyses. The lower edge of the liver and the kidneys were used as reference points to co-register the PET and MRE sequences for detailed matching of corresponding anatomical structures. Increased uptake was visually distinguished from background activity and anatomical location confirmed by the MR sequences using both axial and coronal MR slices. All lesions with signal intensity visually higher than that of liver and adjacent bowel loops were assessed. The highest SUV (SUV_{Max}) in the small bowel wall of each lesion was identified using the "Send cursor to max value" function in AW VolumeShare 5 11.3 (GE 2005-2010 General Electrics Company) software. For statistical analyses, only the lesion with the highest SUV measured was included. The severity of CD was thus graded according to the lesion with the most active inflammation. When comparing the SUV_{Max} between the first diagnostic and follow-up imaging, the SUV_{Max} of the same lesion was used to allow true paired analysis.

4.2.6 Statistical analyses (Studies I-III)

Continuous variables are summarized using mean and standard deviation (SD), range and 95% confidence intervals when normally distributed, and with median and lower

(Q1) and upper quartile (Q3) otherwise. Categorical variables are presented as counts and percentages.

Fisher's exact test was used to evaluate the association between CD-group and non-CD-groups and NSAID, biopsies, imaging findings, and smoking status. Laboratory results between CD-group and non-CD-groups were compared using Wilcoxon rank sum test. Wilcoxon rank sum test was also used in comparing males and females of age, CD, CECDAI, immunosuppressive medication, biologics, dilatation, Crohn's surgery, and FC. The same method was used in comparing CECDAI-scores between ileitis status (yes/no) or whether the inflammation was active or suspected, and biopsy results (normal, unspecific inflammation, granulomas).

Sensitivity, specificity, positive predictive value, negative predictive value were calculated for the following: FC (using cutoffs 50, 75 and 100 µg/g), ileitis in endoscopy, abnormal imaging finding, suspected inflammation in CT, active inflammation in CT, abnormal finding in CT, suspected inflammation in MRE, active inflammation in MRE, abnormal finding in MRE. Furthermore, area under ROC-curve were calculated using the trapezoidal rule and tested against 0.5 value.

One way analysis of variance (ANOVA) was used to study the associations between SUV_{Max}, SBCE-findings, Hb, Alb, FC and IBD Symptom Index. Logarithm transformation (natural logarithm) was used in SUV_{Max} to fulfill the assumption of normality of studentized residuals. To make the results more easily clinically applicable, model-based estimates were then back- transformed to the original scale (using formula e^x). Similar analysis method was used for patients later treated with oral budesonide only (yes/no), patients treated with immunomodulators (yes/no), and patients treated with biologics (yes/no). Nonparametric Wilcoxon rank sum test was used when the distribution of response variable did not follow a normal distribution.

With the logistic regression model, the area under the ROC-curve was estimated. In addition, the Youden index was used to find the optimal cutoff point for SUV_{Max} for diagnosing CD and defining the clinical response. After that we tested nearby SUV_{Max} -values for sensitivity and specificity for a diagnostic threshold. *p*-values less than 0.05 (two-tailed) were considered statistically significant. The data analysis for this study was generated using SAS software, Version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA)

5 Results

5.1 Predictive markers of CD in SBCE (Study I)

In SBCE, CD was diagnosed in 110 (29.4%) patients (CD-group) in the small bowel whereas 264 (70.6%) did not have CD in the small bowel (non-CD-group). We found no significant differences between the two groups for age (p=0.69), sex (p=0.086), NSAID use (p=0.58) or smoking status (p=0.39). Females were diagnosed with small bowel CD at a younger age than males (mean 37.8 vs 43.3yr). For the CD-group, there were 15 (13.6%) patients with previously diagnosed CD in the upper-GI-tract or colon compared to 21 (8.0%) for the non-CD-group. The most frequent indications for SBCE, as described in the patient registry, were as follows: suspected CD (59.9%), diarrhea (56.7%), abdominal pain (41.7%) and high FC (40.6%). Bloody stools and anaemia were less frequently mentioned as an indication. There were no significant differences in the indications for SBCE between the two groups.

97.9% of the patients included in the study had a colonoscopy prior to SBCE and the ileum was intubated in 93.4% of these patients. The median time between colonoscopy and SBCE was 140 days (IQR: 79-248). Ileitis was seen in 70.2% of the patients in the CD-group compared to 24.4% of the patients in the non-CD-group. Histology showed unspecific inflammation in ileal biopsies in 49.0% of the patients of the CD-group compared to 20.1% of the non-CD-group and granulomas in only 2.9% and 0.4% respectively. 79.5% of the patients in the non-CD-group had normal histology compared 48.0% in the CD-group. There was a significant difference between the histologic findings between the two groups ($p \leq 0.0001$). Macroscopic ileitis yielded in sensitivity of 66.3%, whereas specificity was 75.6%. Positive predictive value (PPV) was 54.3% and negative predictive value (NPV) 83.7% respectively.

75.1% of the patients included in the study had imaging of the abdomen done prior to SBCE. 59.9% had MRE and 15.2% had CT of the abdomen performed. Median time from imaging to SBCE was 85 days (IQR: 55-176). Active inflammation was observed more frequently in CD-group (24.7% vs 6.8% for the non-CD-group). Unspecific inflammation was reported for 29.2% of the patients in the CD-group compared to 20.8% in the non-CD-group. Normal imaging results were reported more often in the non-CD-group 72.4% vs 29.2% in CD-group. There was a significant difference in the imaging findings between CD- and non-CD-

groups (p≤0.0001). Combined sensitivity for MRE and CT was 65.2% and specificity 72.4% (PPV 52.2% and NPV 81.8%).

No statistically significant differences were observed in Hb- or CRP-levels between the patients in the CD and non-CD-group (p=0.59 and 0.24). ESR was slightly higher in the CD-group compared to the non-CD-group (mean 11.2 mm/h vs 13.7 mm/h, p=0.022). Alb levels were slightly lower for the CD-group compared to the non-CD-group (mean 37.6 g/l vs 38.3 g/l, p=0.025). The median time between laboratory tests and SBCE was 49 days (IQR: 8-93). Median FC was significantly higher in the CD-group (354 µg/g vs 132 µg/g), p≤0.001. FC also had a slight positive correlation for higher CECDAI-score (Spearman r=0.23, p=0.014). We also found a link between high FC and biologics, but not for immunosuppressants. (Table 6)

Table 6. Laboratory findings by group, CD-group stands for patients diagnosed with CD in SBCE whereas patients in non-CD group did not have findings suggestive for CD in SBCE. Modified from original publication I.

	All patients (n=374)	**CD-group (n=110)**	**Non-CD-group (n=264)**	**p-value**
	No. (%)	No. (%)	No. (%)	
CECDAI-score				
Data available	368/374 (98.4)	109/110 (99.0)	259/264 (98.1)	
Mean [95%CI]	3.8 [3.1-4.5]	11.6 [10.1-13.0]	0.49 [0.3-0.66]	<0.001
Median [IQR]	0[0-4]	9 [6-18]	0 [0-0]	
[Min-max]	[0-34]	[2-34]	[0-12]	
C-reactive protein				
Data available	263/374 (70.6)	77/110 (70.0)	186/264 (70.5)	
Mean (mg/l) [95%CI]	8.11 [5.9-10.3]	10.08 [5.4-14.7]	7.29 [4.9-9.7]	
Median [IQR]	2 [1-8]	3 [1-9]	2 [1-8]	0.24
[Min-max]	[1-131]	[1-131]	[1-122]	
Hemoglobin (g/l)				
Data available	365/374 (97.6)	109/110 (99.0)	256/264 (97.0)	
Median [IQR]	139 [130-149]	138 [127-149]	139 [131-148]	0.59
[Min-max]	[87-183]	[103-183]	[87-177]	
Erythrocyte sedimentation rate (mm/h)				
Data available	121/374 (32.4)	43/110 (39.1)	78/264 (29.5)	
Median [IQR]	7 [2.00-14]	9 [5-20]	6 [2-12]	0.022
[Min-max]	[1-54]	[2-48]	[1-54]	
Serum albumin (g/l)				
Data available	162/374 (43.3)	72/110 (65.5)	140/264 (53.0)	
Median [IQR]	38.8 [36.4-40.4]	38.1 [35.6-40.1]	39.4 [37.2-40.6]	0.025
[Min-max]	[12-46.8]	[18.7-46.8]	[15-45.3]	
FC (µg/g)				
Data available	358/374 (95.7)	109 (99.1)	249/264 (94.3)	
Median [IQR]	207 [57-527]	354 [195-802]	132 [50-413]	<0.001
[Min-max]	[20-6000]	[22-3165]	[20-6000]	

13 patients (11.8%) had FC <100 µg/g and yet were diagnosed with small bowel CD. Respectively, ten (9.1%) had FC <75 µg/g and only four patients presented with CD despite having FC<50 µg/g. 11 out of these 13 patients had earlier or later FC-results that were higher than 100 µg/g. For FC <100 µg/g, the sensitivity was 89% and specificity 45.4% (PPV 41.6%, NPV 90.4%). Sensitivity was higher for FC<50 µg/g (96.4%), but specificity lower 19.7%, as expected (PPV 34.6%, NPV 92.4%). Area under ROC-curve showed 0.69 for FC<50 µg/g.

To evaluate whether a combination of laboratory, imaging and endoscopic findings could predict CD in SBCE, a multivariate model was created. For simplicity and an easier application in daily practice, we created a combined model for patients with no ileitis in ileocolonoscopy and normal findings in imaging combined with FC. There were 27 patients with negative colonoscopy and imaging findings and FC<50 µg/g. Of these 27, only two (7.4%) were diagnosed with CD. Furthermore, in patients with FC <75 µg/g, 4 out of 60 (6.7%) had CD whereas in patients with FC <100 µg/g 6 out of 101 (5.9%) were diagnosed with small bowel CD.

Mean CECDAI-score was significantly higher in the CD-group than in the non-CD-group (11.6 vs 0.5), $p \leq 0.001$. A CECDAI-score of ≥3 was observed in 98.2% of the patients with small bowel CD. In the non-CD group, 90.5% of the patients had CECDAI-score of <3. The area under ROC-curve for CECDAI was 0.99 ($p \leq 0.0001$). Sensitivity and specificity were the highest between CECDAI 2.5 and 3.5. A cutoff at CECDAI-score 3 yielded PPV 81.1% and NPV 99.1%. There was a correlation between ileitis and CECDAI-score ≥3. Males had higher CECDAI-score compared to females (mean 13.3 vs 10.0), but the difference was not statistically significant. The patients with ileitis as detected by ileocolonoscopy prior to SBCE had higher CECDAI-scores (mean 6.8 vs 1.9, $p \leq 0.0001$) compared to those with no ileitis. Mean CECDAI-score was 3.4 for patients with normal imaging findings, 3.9 for patients with unspecific inflammation, 7.7 for those with active inflammation in imaging. Mean CECDAI-score was higher for patients with granulomas in their biopsies (10.8), compared to patients with unspecific inflammation (6.7) or no inflammation (2.7).

Capsule retention was observed in six (1.6%) of the patients included in the study. In CD-group, capsule retention was seen in three patients (2.7%) compared to non-CD group (three patients, 1.1%). The median time between FC measurement and SBCE was 94 days (IQR: 43-149).

The mean follow-up time for the whole study population was 1370 d (SD=937 d). Two patients (0.8%) were diagnosed later with small bowel CD despite initially presenting with normal SBCE findings. Four patients had surgery due to CD and three patients had endoscopic balloon dilatation. There was no difference between sexes and the surgery or dilatations. Females were diagnosed at a younger mean age compared to males (37.8 vs 43.3 yr) and males were more likely to have

immunosuppressive medical therapy (84% vs 69%) or biologics (40.8% vs 25.9%) than females, but the differences were not significant (p=0.11 and p=0.14). Looking at a subgroup (N=13) of patients diagnosed with CD and FC<100 µg/g, 12 (92%) required glucocorticoids for more than three months. Eight (62%) also needed immunosuppressants and three (23%) were started on biologics. None of these patients required endoscopic dilatations, but one patient had surgery due to perianal CD. For three patients, CD was diagnosed with FC<50 µg/g and two of these patients have presented clinically mild disease course and have neither needed immunosuppressive or biologic medication.

5.2 Combined [^{18}F]-FDG PET-MRE can be used in diagnostics of small bowel CD (Study II)

Thirty volunteer patients with suspected small bowel CD were recruited and underwent [^{18}F]-FDG PET-MRE. To confirm the diagnosis, 21 patients (70%) also had SBCE after [^{18}F]-FDG PET-MRE. 24 (80%) were diagnosed with small bowel CD (CD-group) and 6 (20%) did not have small bowel CD (non-CD-group). 21 patients (83%) had Montreal classification L1 disease (restricted to the small intestine) whereas 3 patients (17%) had ileocolonic (L2) disease. Moreover, 19 patients (79%) had non-penetrating disease phenotype (Montreal B1), three patients (13%) had stricturing disease (Montreal B2), and two patients (8%) had penetrating disease (Montreal B3). Patients in the CD- and the non-CD-group did not present statistically significant differences for age or sex. A significantly higher SUV_{Max} was measured in patients in the CD-group compared to the non-CD-group (model-based estimate mean 3.34 vs 1.84, p=0.022) (Table 7) (Figure 11). Area under ROC-curve for SUV_{Max} was 0.81. To find the optimal cutoff for diagnostics of small bowel CD, the highest sensitivity 79.2% and specificity 66.7% obtained from the ROC-curve were found at SUV_{Max} 2.76. For simplicity, SUV_{Max} was categorized with 2.5 and 3 and tested. SUV_{Max} cutoff 2.5 yielded the same agreement in our data where 22 out 30 (73%) were categorized correctly. sMARIA resulted in lower accuracy (sensitivity 75% and specificity 50%). When results of SUV_{Max} and sMARIA were combined to analyse the diagnostic performance of fusion imaging, the sensitivity increased to 87.5% but specificity was markedly weaker, 16.7%. Sample [^{18}F]-FDG PET-MRE images of the small bowel CD are shown in Figure 12.

Table 7. Patient demographics and differences between patients diagnosed with CD (CD-group) and not diagnosed with CD (non-CD-group), Study II. Modified from original publication II.

	CD-group	Non-CD-group	p-value
N	24	6	
Age, median [IQR]	30.5 [22.5-40.0]	39.5 [20.5-47.3]	0.59
Sex (M, F)	14 (58.3%), 10 (41.7%)	5 (83.3%), 1 (16.7%)	0.37
Hb (g/l), median [IQR]	144 [138-150]	150 [138-163]	0.35
Crp <10 mg/l, >10 mg/l	18 (75%), 6 (25%)	6 (100%), 0 (0%)	0.26
Serum albumin (g/l), median [IQR]	38.1 [36.5-39.7]	42.3 [39.3-45.3]	0.017
FC (µg/g), median [IQR]	563 [126-1610]	101 [45-271]	0.13
Plasma fasting glucose (mmol/l) (median) [IQR]	5.1 [4.8-5.5]	5.3 [4.8-5.3]	0.97
IBD Symptom Index, median [IQR]	5 [3-6.3]	2.8 [1.5-4.5]	0.12
SES-CD of terminal ileum, median [IQR]	3 [3-6]	1.5 [0-3]	0.025
CECDAI, median [IQR]	4 [2-7.5]	0 [0-2.25]	0.045
sMARIA, median [IQR]	1 [0.25-2]	0.5 [0-1.25]	0.14
SUV_{Max}, median [IQR]	3.1 [2.4-4.1]	1.9 [1.3-2.8]	0.022

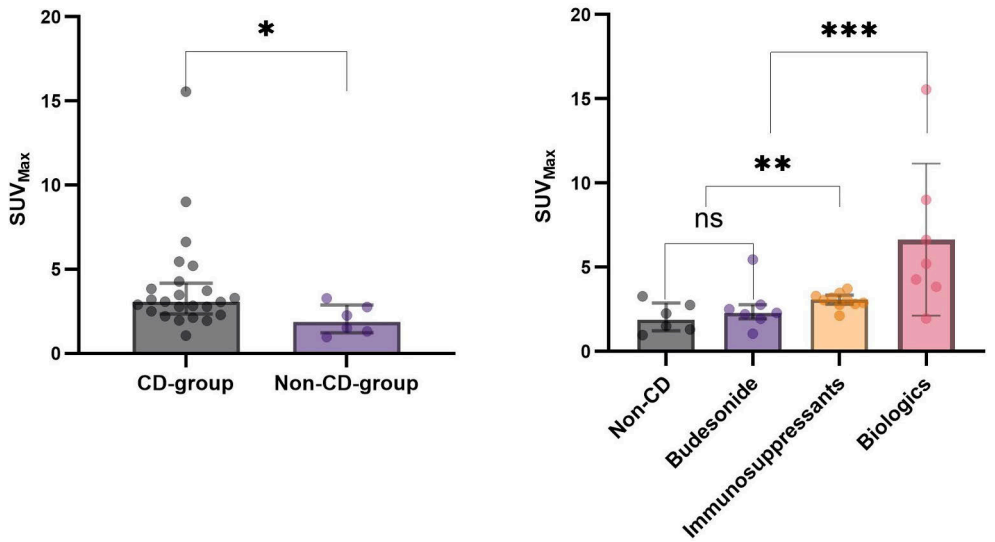

Figure 11. SUV_{Max} of the patients diagnosed with CD was significantly higher compared to those not diagnosed with CD, model-based estimate mean 3.34 vs 1.84 (left). Higher SUV_{Max} was also observed in patients treated later with biologics or immunosuppressants compared to patients who needed budesonide only or were not diagnosed with CD (right). Brackets show median with interquartile range. ns =non-significant,* p≤0.05, ** p≤0.005, ***p≤0.0005. Modified from the original publication II with permission from Karger Publishing.

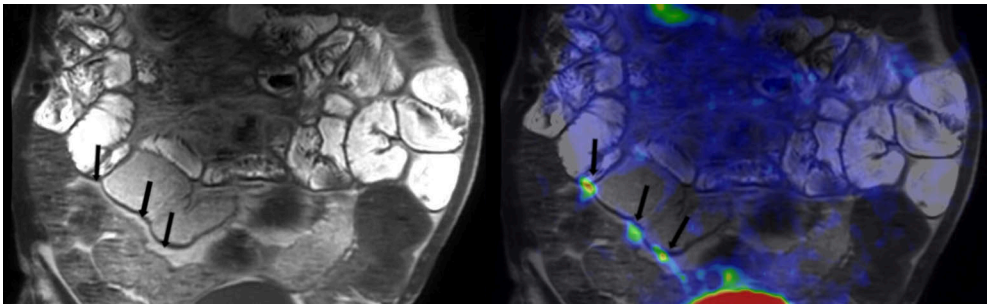

Figure 12. T2-weighted coronal slice of MRE showing the small bowel of a 64-year-old male presenting with small bowel CD in the jejunum beyond the reach of conventional endoscopy. No visible inflammation is seen in MRE alone (left), but when PET slices are fused with MRE slices (right), segmental inflammation is clearly seen with high FDG accumulation marked with arrows. Modified from the original publication II with permission from Karger Publishing.

When comparing the performance of PET scans to MRE, we found no significant difference in sMARIA between patients diagnosed with small bowel CD and those who did not have CD (p=0.14). However, a higher degree of inflammation was associated with a higher SUV_{Max}. Between sMARIA-score of 0 and 5 we found a significant difference in SUV_{Max} ($p \leq 0.001$), but not with lower sMARIA-score. Despite this, sMARIA had a significant correlation with SUV_{Max} (r=0.59, p=0.0007).

FC and CRP were positively correlated with SUV_{Max} (r=0.52, p=0.003 and r=0.60, p=0.0006) but there was no significant difference in FC or CRP (categorized as normal or abnormal) between patients with CD and those who did not have CD (p=0.13 and p=0.35). The IBD Symptom Index did not differ between the two groups either (p=0.12). With Hb and Alb, a negative correlation was found with SUV_{Max} (r=-0.51, p=0.039 and r=-0.58, p=0.0013). Albumin was the only laboratory parameter that showed significant difference being lower in CD-group compared to the non-CD-group (p=0.017). Hb did not have a significant difference between the two groups (p=0.35).

The endoscopic findings were significantly different between the CD-group and the non-CD-group; SES-CD-score was higher in the CD-group (p=0.02) and CECDAI-score was higher in CD-group as well (p=0.05). The CECDAI-score did not correlate with SUV_{Max} (r=0.23, p=0.32), but SES-CD did show a significant positive correlation with SUV_{Max} (r=0.63, p=0.0002).

CD-patients that were divided into subgroups by their medication were compared together and to the non-CD-group. SUV_{Max} was significantly higher in patients treated later with biologics (adalimumab or infliximab) than in patients not treated with biologics (5.51 vs 2.45, p=0.0005, model-based estimate means) (Figure 11). A significant difference was also found between patients treated with immunomodulators (thiopurines or methotrexate) and those who did not receive

immunomodulators or biologics (model-based estimate mean 3.86 vs 2.1, p=0.0026). SUV_{Max} did not show any significant difference for CD-patients treated with corticosteroids only, and for patients without CD (model-based estimate mean 3.2 vs 2.1 p=0.16). Median follow-up time of the study population was 27.5 months (IQR 19-35 months).

5.3 Combined [^{18}F]-FDG PET-MRE can predict clinical remission in small bowel CD (Study III)

A total of 35 patients were initially recruited for this study and had a diagnostic [^{18}F]-FDG PET-MRE scan (Study I). 26 (75%) of these patients were diagnosed with small bowel CD. Of these 26 patients, 18 (69%) had a follow-up [^{18}F]-FDG PET-MRE in median109 d (IQR 91-128d).

Six (33%) patients declined to participate in follow-up imaging and two (11%) had an uncertain diagnosis and were not started on CD-medication and were thus excluded from Study III. Median SUV_{Max} decreased significantly between the first diagnostic imaging and follow-up imaging after starting CD-medication (median 3.2 vs 2.1, p=0.0025). sMARIA was also significantly lower in the second imaging (p=0.001). In order to relate these imaging findings to intestinal inflammation, FC was analysed and there was also a significant decrease (451 µg/g vs 165 µg/g, p=0.004). (Figure 13) FC normalized in 61% of the patients (11/18). In contrast, there were no significant differences in CRP, Hb, Alb or IBD Symptom Index. We also found a positive correlation between SUV_{Max} and FC (r=0.56 at the diagnostic and r=0.46 at follow-up). Sample images are shown in Figure 14. Median plasma fasting glucose was not significantly different between the diagnostic and follow-up imagings (p=0.21). (Table 8)

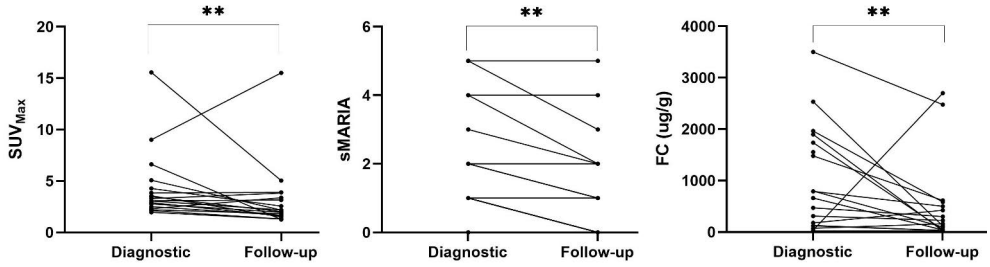

Figure 13. SUV_{Max}, sMARIA and FC measured at both the diagnostic and follow-up. Each parameter shows a statistically significant decrease over time. ** p ≤0.005. From manuscript of Study III.

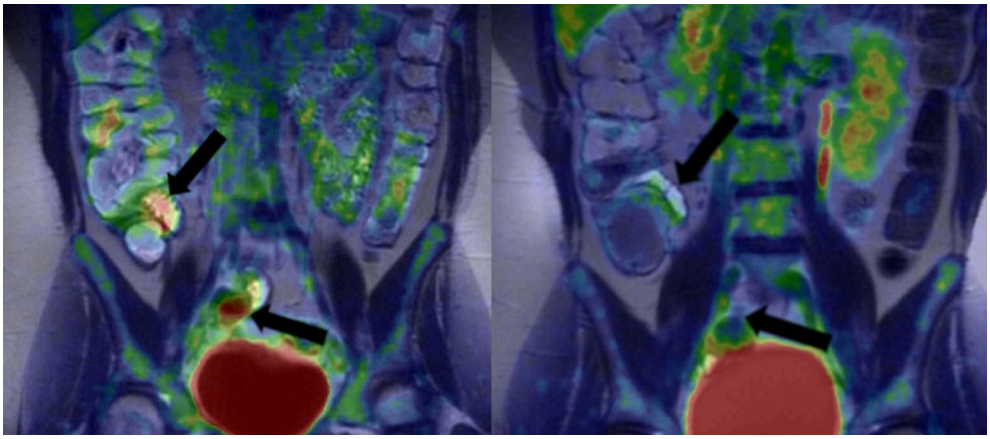

Figure 14. A fused PET-MRE coronal slice taken from a 27-year-old female presenting with small bowel CD in the terminal ileum and another segment in the proximal ileum in the first diagnostic imaging (left). The patient was started on triple therapy, prednisolone, mercaptopurine and infliximab and a follow-up [^{18}F]-FDG PET-MRE performed 98d later is shown on right with markedly decreased inflammatory activity. From manuscript of Study III.

A decrease in SUV_{Max} was observed in 13 (72%) patients and sMARIA decreased in 11 (61%) patients. We found a tendency of association between SUV_{Max} change and sMARIA-change (p=0.065). The average decrease in SUV_{Max} was 5.23 when sMARIA changed by -2 points, which reached statistical significance (p=0.027). However, with smaller changes in sMARIA, the changes in SUV_{Max} were not significant.

Table 8. Results of Study III showing laboratory and imaging findings in both first diagnostic and 3 month follow-up imaging (median 109d, IQR [91-128]) Modified from original publication III.

	First diagnostic imaging	Follow-up imaging	p-value
SUV_{Max} (median) [IQR]	3.2 [2.5-4.5]	2.1 [1.6-3.5]	0.0025
sMARIA-score (median) [IQR]	1.5 [1-3.3]	1 [0-2]	0.001
FC (µg/g) (median) [IQR]	725 [127-1779]	163 [36-545]	0.004
CRP (mg/l) (median) [IQR]	1.5 [1-12.8]	2 [1-10.8]	0.9
Hb (g/l) (median) [IQR]	142 [130-156]	140 [133-150]	0.17
Serum albumin (g/l) (median) [IQR]	38.4 [37.1-40.7]	39.8 [36.7-42.3]	0.59
Plasma fasting glucose (mmol/l) (median) [IQR]	5.1 [4.9-5.5]	5.2 [5.0-5.8]	0.21
IBD Symptom Index (median) [IQR]	4.5 [2.3-7.8]	3 [2.0-5.0]	0.52

To assess long time remission, laboratory samples were collected later (median 407d, IQR 316-454d). 85% of the patients had normalized CRP and FC was less than 250 µg/g in 92% of the study population. In follow-up imaging, there was no significant difference in SUV_{Max} or sMARIA between patients who had FC <250 and >250 (p=0.36 and 0.62 respectively). The area under ROC-curve for SUV_{Max} was 0.66 compared to 0.69 for sMARIA. Using the FC cutoff 250 µg/g, area under ROC was 0.64 for natural logarithm of SUV_{Max}. The ideal cutoff was 3.1 at the time of follow-up imaging where sensitivity was 60% and specificity was 86%. Defining long time remission as normal CRP and FC <250 µg/g at 12 months, area under ROC-curve for SUV_{Max} was 0.63 measured from follow-up images. Sensitivity and specificity were highest at SUV_{Max} 3.3 (58.3% and 100%).

We also found a strong correlation between SUV_{Max} and FC at both the diagnostic and follow-up imaging (r=0.56 and 0.46). Patients who had a decrease in SUV_{Max} also had a lower FC at the time of follow-up imaging (p=0.019). In contrast, patients who did not have a decrease in SUV_{Max} did not have significant differences in FC between the two imagings. When SUV_{Max} decreased in follow-up imaging, there was also a significant decrease in FC (p=0.039). In patients without an initial decrease in SUV_{Max}, there was no statistically significant difference in FC at the 12-month laboratory tests (p=0.0625).

All of the 18 patients completing the study had glucocorticoids started before follow-up imaging occurred. Indeed, five (28%) patients were already on glucocorticoids even before the diagnostic imaging. During the follow-up period, 6 (33%) patients were started on anti-TNFs, 7 (39%) were treated with immunomodulators combined with glucocorticoid induction and 5 (28%) were treated with a course of glucocorticoids only. There were no significant differences in SUV_{Max} at follow-up imaging between patients treated with different medications. When comparing changes in SUV_{Max} between diagnostic and follow-up imaging, the only significant difference was found in patients who were started on anti-TNFs (p=0.027).

Conclusions of the results of the Studies I-III

I. Patients with FC <50 µg/g with no endoscopic ileitis and negative MRE can be followed safely without SBCE. A CECDAI-score of 3 could be used as a cutoff for small bowel CD in SBCE.

II. [^{18}F]-FDG PET-MRE can be used in diagnostics of small bowel CD, as patients with CD had a higher SUV_{Max} than patients who did not have CD (p=0.0022). [^{18}F]-FDG PET-MRE outperformed MRE alone in diagnostic accuracy. SUV_{Max} 2.5 could be used as a diagnostic threshold for small bowel CD.

III. A decrease in SUV_{Max} in patients with newly diagnosed small bowel CD measured by [^{18}F]-FDG PET-MRE predicts clinical remission and early response to medical therapy. [^{18}F]-FDG PET-MRE is a promising tool for follow-up of small bowel CD.

6 Discussion

6.1 Low FC, no endoscopic inflammation and negative imaging results predicts normal findings in SBCE in patients suspected of small bowel CD (Study I)

Differential diagnostics of luminal small bowel CD and functional disorders of the gut such as IBS is difficult, as the symptoms are often alike. This is underlined by our results, which found no statistically significant differences between the indications (and thus symptoms) of the patients with negative SBCE findings and those who had CD as detected by SBCE. Small bowel imaging and colonoscopy had a relatively low sensitivity (65.2%) and NPV (81.8%) for small bowel CD, which is in line with previous studies that compared SBCE and MRE.[4,159]

Laboratory tests that had significant differences between CD and non-CD patients were FC, Alb and ESR whereas Hb and CRP commonly used in diagnostics of CD did not differ between the two patient groups. The non-significant differences in Hb and CRP may be explained by the number of patients with luminal CD, which in general does not elevate CRP compared to more penetrating forms of CD. In view of this, our results are different from those of Egea-Valenzuela and his colleagues, who reported that in addition to elevated FC, elevated CRP is associated with CD in SBCE.[271] The diagnostic cutoff for FC remains somewhat controversial, as values ranging from 100-250 µg/g have been proposed.[134–138] In this study, we found that clinically relevant (i.e. requiring immunomodulators, biologics or surgery) small bowel disease can be found in patients with FC concentrations of less than 100 µg/g which is a cutoff suggested by Koulaouzidis et al.[272] However, for a FC <50 µg/g, the sensitivity was 96.4%, but demarking the FC cutoff at these low levels is inevitably associated with poor specificity (19.7%), which results in markedly lower clinical utility. This is further emphasized by area under ROC-curve of 0.69 for FC, which is most likely caused by high median FC concentration found in the non-CD-group. The reason for this high FC in patients without CD remains unclear, but supports the widely known fact that FC is not specific for IBD as verified by other studies on small bowel CD and SBCE.[138,271,273]

Combining a FC cutoff concentration <50 µg/g to negative endoscopic and imaging findings could be a reasonable combination used to rule out clinically significant small bowel CD and instead of SBCE, a follow-up seems to be a safe alternative and FC <50 µg/g could be used as a threshold for patient selection for SBCE. In patients with symptoms suggesting for more functional disorders, FC <50 µg/g can quite effectively rule out CD even without endoscopy or small bowel imaging, which is a finding that especially helps clinicians in primary healthcare, as functional disorders such as IBS are very common and cause major burden to healthcare system.[274,275] In fact, more than a third of patients with CD suffer from IBS-type symptoms despite being in clinical remission, which emphasizes further the difficulties in differential diagnostics.[8]

In addition to the burden on the healthcare system and the costs it imposes, SBCE is also time consuming for the patients and there is the risk of capsule retention. The retention rate was 1.6% for all the patients in this study and 2.7% for patients diagnosed with CD, which correlates well with recent systematic reviews.[152,276] Patients in this study presumably did not have symptoms, imaging, or endoscopic findings suggestive of strictures, which increases the proportion of patients with mild luminal small bowel CD in the study population. Diagnostics of mild, luminal disease in general is more difficult compared to more penetrating phenotypes of CD. This also explains the low number of endoscopic dilatations and CD related surgery during the follow-up period in this study population.

Females were diagnosed with CD at a younger age compared to males, and males were more likely to be treated with immunomodulators and advanced therapies. In general, females have a higher incidence of CD after childhood[277], but these findings together may imply that males in this study sought medical help later in disease progression than females.

Although CECDAI-score is validated and correlates with the Lewis-score commonly used in evaluating CD-findings in SBCE, there was no previously established cutoff for CD in CECDAI.[138,153,154,278] Omori and Koulaouzidis suggested values that ranged from 3.8 to 5.8.[138,279] Our results showed that the specificity and sensitivity were the highest at approximately CECDAI 3, which could therefore be used as a cutoff for small bowel CD and is slightly lower than the cutoff points previously suggested. For example, a CECDAI-score of 3 corresponds to a few aphtae with a diameter of less than 5 mm in a single bowel segment. Again, drawing the cutoff lower may increase the number of false positive findings, so the SBCE findings in each patient must be individually considered together with clinical, laboratory, endoscopic and imaging findings.

6.2 [^{18}F]-FDG PET-MRE can be used in diagnostics of small bowel CD (Study II)

The patients diagnosed with small bowel CD had significantly higher SUV_{Max} than patients who did not have CD findings in SBCE, a finding which shows the potential of [^{18}F]-FDG PET-MRE in the diagnostics of CD. Sensitivity (81%) and specificity (69%) were the highest at SUV_{Max} 2.76, but equal accuracy was found at SUV_{Max} 2.5. Based on these results, a cutoff at SUV_{Max} 2.5 could be used as a diagnostic threshold for small bowel CD in [^{18}F]-FDG PET-MRE. PET-MRE seems to yield somewhat lower SUV_{Max} than PET-CT, as Ahmadi et al. suggested, a SUV_{Max} cutoff at 4.77 for small bowel CD which is a value that is clearly higher than our results.[280] Their imaging protocol was different to ours however, as the [^{18}F]-FDG injection was administered 90 min prior to image acquisition compared to 39 min in this study. In comparison, Pellino et al. compared the impact of PET-CT and PET-MRE in clinical management of small bowel CD, and suggested a SUV_{Max} cutoff 2.95 for active inflammation, which is again close to our results, even though the [^{18}F]-FDG injection was administered 80 min before imaging began in their study.[194] However, their study included only patients with known small bowel CD scheduled for surgery and thus the study population did not include patients with mild CD. In a study by Li et al. ileal and colonic bowel segments without active inflammation had a median SUV_{Max} 2.34 compared to 4.18 with mild to moderate inflammation.[281] Defining a universal cutoff is difficult though as there are differences in imaging protocols, e.g. time between tracer injection and imaging. Standardized protocols have been recommended by EANM guidelines.[269] Different characteristics of PET scanners also affect SUVs including: image acquisition times, scanner sensitivity, attenuation correction, noise-to-signal ratio, reduction of PVE and dead time; all of which can add to the differences in diagnostic SUV cutoffs presented in previous studies. Indeed, using universal cutoffs is not yet recommended in EANM guidelines.[198]

We found a clear correlation between endoscopic findings (SES-CD-score) and an increased SUV_{Max} which supports our conclusions that SUV_{Max} measured by [^{18}F]-FDG PET-MRE can also be used in the grading of inflammation. The study population though was too small to suggest cutoffs for mild, moderate and severe inflammation.

[^{18}F]-FDG PET-MRE also outperformed MRE alone in diagnostic accuracy of finding active small bowel CD. We found no significant difference in sMARIA between patients with small bowel CD and those who did not have small bowel CD. This lack of difference highlights the difficulties in diagnostics of mild small bowel CD, as only severe inflammation in MRE (sMARIA >4) correlated with increased SUV_{Max}. Catalano et al. found an increased sensitivity for active CD inflammation in patients with known CD for [^{18}F]-FDG PET-MRE 88% vs 80% in MRE. The specificity was also superior for [^{18}F]-FDG PET-MRE compared to MRE alone

(91% vs 83%).[193] Compared to these, the sensitivity of [^{18}F]-FDG PET-MRE in our study was 87.5%, but the specificity was poor (16.7%) due to the large number of false positives in the non-CD-group. A false positive was defined as sMARIA ≥1 or SUV_{Max} ≥2.5. If a positive SUV_{Max} was required for the diagnosis, then the specificity for combined [^{18}F]-FDG PET-MRE was 66.7%.

Small bowel CD can not be diagnosed based on laboratory findings alone, which is also confirmed by our results, as there were no significant differences in Hb, CRP, or FC between the patients with CD and patients not diagnosed with CD. Albumin, however, was lower in patients with CD. Higher FC and elevated CRP correlated with an increased SUV_{Max} and low Alb and Hb correlated negatively with SUV_{Max}. This is likely due to more severe disease phenotype, where Alb and Hb are commonly decreased. These findings support the reliability of [^{18}F]-FDG PET-MRE in assessing the inflammation in small bowel CD even in the milder cases.

There are no previous studies on the use of PET imaging to predict disease outcomes in terms of medication. In this unique setting, we found that a higher SUV_{Max} predicts the need for more advanced therapies as SUV_{Max} was higher in patients requiring biologics or immunomodulators compared to patients who did not have CD or only had a mild phenotype responding to a course of glucocorticoids. To avoid bias, the clinicians were blinded to the PET results and medication were chosen on an individual patient basis that followed the 2020 guidelines of ECCO[203], i.e. based on disease phenotype and extent. In the future, these results could help the clinicians to identify the patients who need more advanced therapies earlier, which in turn can lead to better disease outcomes in the long term. This would also reduce the risk of overtreating the patients, as advanced therapies are both expensive and increase the risk of adverse effects.[23,126] Furthermore, these findings may help the clinicians in assessing the inflammatory component in patients with CD strictures. It is important to be able to distinguish between a chronic inactive stricture that needs surgery and an active inflammation in stricture, which can be treated with a step-up in medication.[194,195] An RCT in which patients were divided into groups according to low and high SUV_{Max} and randomized into different treatments would be the ultimate study design to confirm these results, but setting up this kind of study would also have ethical issues.

The strengths of this study are that it is the first to study prospectively [^{18}F]-FDG PET-MRE in relation to diagnostics, inflammatory activity and response to treatment. In addition to [^{18}F]-FDG PET-MRE, the patients went through all the other standard diagnostic measures[17] and to avoid bias, the clinicians making the diagnosis and treating the patients were unaware of the PET results. The clinicians, however, did have access to endoscopy, histology, laboratory and MRE results just as they normally do in everyday clinical practice.

[^{18}F]-FDG PET-MRE can be used safely in diagnostics of small bowel CD as the main risk is the radiation dose. Recently however, the radiation doses have decreased[199] as imaging techniques and scanners have developed. The procedure does not involve bowel preparation with PEG-solution: a factor which adds to patient comfort in comparison to SBCE or ileocolonoscopy. Unlike for SBCE, there is no risk of capsule retention, should there be any strictures in the small bowel. In the severe acute setting, [^{18}F]-FDG PET-MRE can also be obtained safely without the risk of perforation linked to ileocolonoscopy.[185,194,282]

6.3 [^{18}F]-FDG PET-MRE in assessing response to medical therapy in small bowel CD (Study III)

A decrease in SUV_{Max} predicts clinical remission at followup imaging after starting medical therapy that is targeted at small bowel CD. A cutoff SUV_{Max} of 3.1 at follow-up imaging predicts a response to medical therapy. We found a strong link between SUV_{Max} and FC, which further supports that [^{18}F]-FDG PET-MRE can be useful to assess inflammatory activity of CD in the small bowel. To our knowledge, this is the first study to investigate the performance of [^{18}F]-FDG PET-MRE in assessing response to medical therapy in patients with newly diagnosed CD. The accuracy of PET-imaging was close to that of MRE using the sMARIA-scale, although direct statistical comparison was difficult due to sMARIA being a categorical variable and SUV_{Max} a continuous variable. sMARIA decreased in fewer patients than SUV_{Max}, which may indicate that the resolution of inflammation (and hence reduced glucose metabolism) can be detected faster in PET sequences compared to MRE. sMARIA needed to change by two points for the SUV_{Max} to significantly decrease, which further supports this hypothesis.

In Study II, we found that blood sample markers (Hb, CRP and Alb) do not correlate well with changes in inflammation in the small intestine. Possibly this is mostly due to a high percentage of study patients with luminal CD rather than penetrating disease, as penetrating CD affects the whole body more drastically. The results are in line with Ahmadi et al. in their study with [^{18}F]-FDG PET-CT.[280] FC was chosen for comparison of imaging due to a high number of studies that support its ability to follow IBD and being used widely in clinical diagnostics and follow-up around the world. The cutoff value of 250 µg/g for FC was chosen according to STRIDE II-consensus. However, this cutoff value is debated and may not be accurate enough for CD that is isolated to the small bowel. Lower thresholds have been suggested as patients with isolated small bowel CD have lower FC levels than patients with colonic or ileocolonic disease.[283] FC does correlate well with endoscopic inflammatory activity, both SES-CD[134] and CECDAI.[138] FC has also been shown to correlate with clinical indexes such as Crohn's disease Activity Index

(CDAI)[284], but in this study we did not find any significant link between IBD Symptom Index and FC or imaging findings.

Fasting glucose levels have a strong impact on [^{18}F]-FDG metabolism and glucocorticoids often increase plasma glucose levels. Patients fasting glucose were thus determined before PET-MRE and there was no significant difference between the diagnostic and follow-up imaging concentrations of fasting glucose.[285] None of the patients in either study II or II had diabetes and the highest measured glucose was 6.3 mmol/l which is well below the suggested threshold for PET-imaging.[269] For this reason, we did not do a separate glucose normalized SUV-analysis.

Setting up universal thresholds for SUV_{Max} is difficult. There are a few studies with small series and mostly used the [^{18}F]-FDG PET-CT modality rather than [^{18}F]-FDG PET-MRE. Ahmadi et al. suggested a cutoff SUV_{Max} for 4.77. in active CD in patients with known CD.[280] One explanation for this may be a more complicated attenuation correction in PET-MRI compared to that of PET-CT. Pellino et al. suggested a cutoff value for active CD inflammation at SUV_{Max} 2.95, which is in range of our results from both Study II and III.[194] The only previous studies on PET imaging and response to treatment were done on patients with known CD.[194,196]

6.4 Limitations and future directions

The major drawback in Study I is that it was retrospective and a single centre study. This can give rise to unreliability in the laboratory, endoscopy and imaging data, as there were delays between SBCE and other parameters. The follow-up time also differs between the patients, as those who underwent SBCE earlier had a longer follow-up period. Some data were missing, due to heterogenous laboratory measurements, and some patients may have moved to other areas during the follow-up period. As SBCE is only available in Turku University Hospital in Southwest Finland, most patients with small bowel CD are treated in Turku University Hospital's Department of Gastroenterology outpatient clinic and hence the clinical data were available, but it is also possible that some patients have been treated by some other institution and the researchers of Study I did not have access to those medical records. All the patients in the study had a clinical suspicion of small bowel CD, so the non-CD-group does not represent healthy controls per se as they were also symptomatic for various and often unclear reasons. 13.6% of the patients had previously been diagnosed with CD in the colon, stomach or mouth, which may bias the laboratory findings. There were also a few uncertainties in laboratory tests that were not considered in our study model. The FC assay has changed during the study period, initially the range was 50-2000 µg/g, but later changed to 20-6000 µg/g. The CRP range has also changed over the course of the study. The minimum CRP was

initially 10 mg/l and later 1 mg/l. Hb and Alb also have different normal ranges for different age and sex.

The small sample size in Studies II and III limits the power of our results and especially the assessment of long term outcomes is difficult with a population this small (Study III). Glucocorticoids use is also a confounding factor, as some of the patients were already on glucocorticoids before the first diagnostic imaging occurred, which may have decreased the SUV_{Max}, sMARIA-score, CRP or FC values of those patients. Glucocorticoid induction was used for all the patients diagnosed with CD, which may distort the results in comparing SUV_{Max} of the patients with different medication groups (glucocorticoids only, immunomodulators or anti-TNFs) in Study II. Not all the patients included in both Studies II and III had CD limited to the small bowel, which possibly causes bias in the laboratory findings.

The main limitation of [^{18}F]-FDG PET-MR imaging in diagnostics of small bowel CD is the limited specificity for different kinds of inflammation such as infections and tumors that also cause increased tissue glucose uptake and hence increased SUV in PET imaging[179]. Several conditions that affect glucose metabolism also alter [^{18}F]-FDG metabolism and are a possible source of error. Elevated blood glucose levels decrease SUV rates whereas metformin medication markedly increases glucose uptake.[9,285] Glucocorticoids have been shown to decrease the diagnostic yield of [^{18}F]-FDG PET imaging.[262–264] Despite the paradigm shift in the treatment of CD towards an earlier introduction of advanced therapies, glucocorticoids are still a critical part of the treatment of CD. The use of glucocorticoids makes the interpretation of [^{18}F]-FDG uptake more complex. One way of tackling this problem is to calculate glucose normalized SUVs, but it is uncertain how this affects the results in [^{18}F]-FDG imaging and this is not routinely suggested by the guidelines of EANM.[269]

In studies II and III, only the segment with the highest rate of [^{18}F]-FDG uptake was included in the analyses. This does not give any information on the extent of the disease overall, but only the inflammatory activity of a single lesion. The extent of the disease naturally affects patients' symptoms and thereby the treatment decisions of the clinicians. However, the scales used for assessing CD lesions in imaging such as sMARIA[163] or PET-MR-index[286] only assess a single lesion, the latter using SUV_{Max}. Palatka et al. also created a global PET-score in which the highest activity of each bowel segment are added together. However, they also defined the small bowel as a single segment.[196] Endoscopic scores (SES-CD[144] and CECDAI[153,154]), in turn, do include the disease extent and hence give a better estimate of the total disease burden. One could speculate as to whether evaluating a mean SUV from a large volume of interest could provide more accurate information on disease severity. There are, however, several potential sources of error, as large volumes of interest inevitably include bowel content and background activity and do not

represent the true inflammatory activity of the bowel wall. Furthermore, the current treatment goals for CD include transmural healing of the whole gastrointestinal tract.[141]

The lack of true specificity for CD has been a key issue in the diagnostics of small bowel CD. To date, no single gold standard method exists. Molecular imaging could possibly address this problem if an inflammatory marker specific for CD could be identified. Such a hypothetical marker in turn, could be a target for the development of a tracer that could quantify this marker. The problem though is the complexity of pathogenesis including numerous cytokines and WBCs, none of which are precisely specific for CD or IBD overall. However, currently there are several potential tracers under research, which may be superior to [^{18}F]-FDG in terms of accuracy, as they are, at least at the theoretical level, less prone to errors caused by glucose metabolism.

In fact, several radiotracers other than [^{18}F]-FDG have already been developed and tested in the field of IBD. The imaging of fibrosis in CD has been under research using gallium 68 ([^{68}Ga]-FAPI), which has been designed to target fibroblast activation proteins (FAPs). Scharizer et al. showed that a higher SUV was associated with histopathologically confirmed fibrosis.[287] [^{68}Ga]-FAPI also correlates well with endoscopic activity in CD as shown in a study by Chen et al.[288] Immuno-PET offers an interesting target for future research; fragments from monoclonal antibodies targeted at the innate immune mediators such as CD11b, Il-1ß and CD4-positive T-cells are proven to correlate with IBD in murine models.[289,290] Imaging of IL12/23 using ^{89}Zr-radiolabeled anti IL12/23p40 antibody has been documented to correlate with serum IL12/23 levels in mice with induced colitis in addition to ^{89}Zr-radiolabeled infliximab, which correlates with IBD activity.[289,291] These biomarkers could possibly be used to predict drug response, as nearly half of the patients treated with anti-TNFs eventually lose the response to therapy, which is possibly due to IL-23 mediated resistance developing.[292] Imaging of x_C^- expressed in activated macrophages may be more specific to IBD than [^{18}F]-FDG and hence could solve one of the key issues of imaging of IBD.[293,294] [^{68}Ga]Ga-DOTA-Siglec-9 is another radiotracer that has the ability to assess intestinal inflammatory activity in murine model, however, the role of vascular adhesion protein-1 it targets is somewhat unclear in IBD.[295] IL-8 can also be radiolabeled to quantify inflammation, but no data on IBD exist.[296] Recent data shows that both cytotoxic CD8-cells and IL-17 producing CD8 (Tc17)-cells contribute to the pathogenesis of CD and may be assessed with recently developed radiotracers.[297,298] CD69-cells can be considered as negative regulators of IBD and inflammation can be assessed in joint arthritis using CD69-targeting PET agent, [^{68}Ga]Ga-DOTA-Z_{CAM241}. This could prove to be a potential tool for assessing treatment response in IBD as well.[299,300] Future research

on these potential tracers is, however, needed, as most of the studies are preclinical and conducted on murine models instead of human patients.

PET-MRI scanners are few in number, and the imaging is more expensive than MRI only, which naturally limits the clinical use of [^{18}F]-FDG PET-MRE. Compared to PET-CT, the radiation dose is though smaller. In PET-MRI, the image acquisition times are longer than in PET-CT causing more motion artefact. In this study setting, the motion artefact was reduced by using a mannitol bowel preparation, injection of butylbromide and breathing gated imaging. In PET imaging, there is always background noise due *to inter alia* motility of the small bowel, which needs to be appreciated when interpreting PET images and diagnostics should only be based on the inflammatory foci with clearly increased glucose uptake by carefully comparing them to that of the liver and the MRE sequences to find the representative anatomic location. The high number of false positive findings seen in the follow-up MRE lead us to suspect, that $SUV_{Max,}$ normalizes faster than the MRE-findings subside. Our hypothesis is that luminal inflammation due to infection or other types of inflammation that are not related to CD, but self-resolving, can be seen for longer in MRE than in PET sequences thus increasing the risk of a false diagnosis.

In mild luminal small bowel CD, the diagnostics will still be difficult with [^{18}F]-FDG PET-MRE, as the accuracy may not be sufficient, but moderate and severe small bowel can be diagnosed with confidence based on the results of this study. However, studies with larger samples could provide more accurate comparisons between MRE and [^{18}F]-FDG PET-MRE. Larger studies with a longer follow-up could also bring more information on prognostics and long term-outcomes of patients with different inflammatory activity. Whether this could affect the clinicians' treatment decisions, is yet to be investigated.

Previously the indications for [^{18}F]-FDG PET-MRE in CD have been evaluating the disease activity and the extent of the disease and extraintestinal manifestations. It has previously been reported that fusion imaging can predict early response postoperatively and can be useful in assessing fibrotic strictures.[195] This provides useful information, as symptomatic, fibrotic inactive strictures or fistulae require surgery, whereas lesions with high inflammatory activity may respond better to medical therapy. However, this remains speculation and targeted prospective studies on these special patient groups need to be carried out. Perhaps, the novel PET radiotracers can improve the specificity of current diagnostic measures and predict response to medical therapy or even the loss of response.

7 Summary and conclusions

The main aim of this thesis was to improve the accuracy of diagnostics of small bowel CD, which is often difficult to achieve as most of the small bowel can not be reached by conventional endoscopy. As CD often causes debilitating symptoms, there is an urgent need for better diagnostics to start medical therapy early as early medical interventions are well-linked to better long-term outcomes. On the other hand, considering the current global economy, there is an inevitable need to avoid unnecessary costs by identifying the right patients eligible for small bowel examinations, as the number of people suffering from GI-symptoms is enormous.

In this thesis I have demonstrated 1) that patients with low FC, no endoscopic inflammation or inflammation in small bowel cross sectional imaging can be safely observed without performing an SBCE, and that a cutoff of 3 in CECDAI-score in SBCE is diagnostic for small bowel CD, 2) [^{18}F]-FDG PET-MRE can be used in diagnostics of small bowel CD even with better accuracy than MRE, and a high SUV_{Max} predicts the need for advanced therapies, 3) [^{18}F]-FDG PET-MRE can be used to assess the response to medication for small bowel CD and a decreased SUV_{Max} predicts clinical remission.

The major issue to be addressed in the future is still the number of people suffering from GI-symptoms. Examining the small bowel thoroughly remains time consuming for both clinicians and patients. More simple and non-invasive tests are needed for the differential diagnostics of functional disorders of the gut and IBD. Research must be done using larger study populations to improve the accuracy of [^{18}F]-FDG PET-MR imaging especially in predicting clinical outcomes. Novel tracers, other than [^{18}F]-FDG could be investigated for both diagnostics and follow-up of CD. Indeed, several novel tracers in PET-imaging have already been tested in preclinical models, and some of these tracers have shown higher specificity for IBD compared to [^{18}F]-FDG.

At the present time, PET-MRE can provide useful information on inflammatory activity of a CD stricture or fistula and in the future PET-MRE can aid in the decision making between operative and medical treatment. However, no single gold standard exists for the diagnostics of small bowel CD as the specificity [^{18}F]-FDG PET-MRE was relatively low.

The findings presented here bring more confidence to the differential diagnostics between: 1) functional GI-disorders and small bowel CD, and 2) small bowel CD and self-resolving unspecific ileitis. The novel value of this thesis is the presentation of a new, non-invasive method to evaluate the treatment response in small bowel CD.

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