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ASSESSMENT OF DISEASE ACTIVITY OF INFLAMMATORY BOWEL DISEASE USING POSITRON EMISSION TOMOGRAPHY / MAGNETIC RESONANCE IMAGING AND KERATIN 7 -ANALYSIS

Mervi Tenhami

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ISBN 978-951-29-9591-2 (PRINT)
ISBN 978-951-29-9592-9 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2024

To Meri-Tuuli, Valtteri and Venla

UNIVERSITY OF TURKU
Faculty of Medicine
Department of Surgery
Division of Digestive Surgery
MERVI TENHAMI: Assessment of disease activity of inflammatory bowel disease using positron emission tomography / magnetic resonance imaging and keratin 7 -analysis
Doctoral Dissertation, 111 pp.
Doctoral Programme in Clinical Research
January 2024

ABSTRACT

The clinical course of inflammatory bowel disease (IBD) is characterized by relapses and spontaneous or drug-induced remissions, but it cannot be predicted by currently known parameters. The priority of this thesis was to improve the accuracy of IBD diagnostics and to find methods in evaluating the course and the prognosis of IBD to facilitate individualized medication and to improve the outcome of the disease.

Positron emission tomography and magnetic resonance imaging (PET/MRI) is a hybrid imaging modality in which MRI provides high spatial resolution and soft tissue contrast while PET using fluorodeoxyglucose (^{18}F-FDG) detects metabolic activity related to inflammation. We compared the data of PET/MRI of altogether ten ulcerative colitis (UC) and Crohn's disease (CD) patients to intestinal histological samples to find out the ability of PET/MRI to detect and grade the inflammation activity of bowel wall. The analysis showed that the glucose uptake in bowel wall had significant association to inflammation activity. Also, the standardized uptake values from areas of severe inflammation activity emerged significantly from the background. Consequently, ^{18}F-FDG-PET/MRI is a promising method to detect especially severe inflammatory lesions non-invasively.

The second aim was to investigate the expression of keratin 7 (K7) in IBD affected intestinal epithelial cells, and to evaluate its potential as prognostic biomarker of IBD. The study showed that K7 was expressed in the colonic epithelium of patients suffering from UC and CD, but not in healthy colon. The expression of K7 was the most pronounced in drug-resistant colitis. The analyses suggested also that K7 expression varies based on the anatomical location in colon. To evaluate the prognostic value of K7, the state of 16 patients was followed for three years. As a result, the severity of the disease during the following three years was not related to expression of K7 at the time of enrollment. Consequently, K7 could be used as a specific diagnostic marker for severe IBD, indicating especially disease with poor drug response and major pathological changes in epithelium.

KEYWORDS: inflammatory bowel disease, ulcerative colitis, Crohn's disease, positron emission tomography/magnetic resonance imaging, keratin 7

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Kirurgia
Mervi Tenhami: Tulehduksellisten suolistosairauksien aktiivisuuden arviointi yhdistettyä positroniemissiotomografia / magneettiresonanssikuvausta ja keratiini 7-määritystä käyttäen
Väitöskirja, 111 s
Turun kliininen tohtoriohjelma
Tammikuu 2024

TIIVISTELMÄ

Tulehduksellisten suolistosairauksien (IBD) taudinkulku on vaihteleva. Käytössä olevilla menetelmillä ei pystytä ennustamaan remissiovaiheen kestoa eikä tulevia relapseja. Tutkimuksen tavoitteena oli parantaa IBD:n diagnostiikkaa ja löytää taudin kulun ennustamiseen uusia menetelmiä, joiden avulla pystyttäisiin suunnittelemaan potilaan hoitoa yksilöllisemmin ja parantamaan taudin kulun ennustetta.

Yhdistetty positroniemissiotomografia/magneettiresonanssikuvaus (PET/MRI) on hybridimenetelmä, jossa yhdistyvät MRI:n hyvä resoluutio ja pehmytkudoskontrasti, sekä PET-kuvauksen tuottama tieto kudoksen aineenvaihdunnasta. Tutkimuksessa selvitettiin PET/MRI:n soveltuvuutta IBD-muutosten havaitsemiseen suolen seinämässä sekä haavaista paksusuolitulehdusta (UC) että Crohnin tautia (CD) sairastavilla potilailla. Lisäksi tutkimuksessa verrattiin PET/MRI-tutkimuksen antamaa merkkiainekertymää ja suoliston histologisten näytteiden tulehdusaktiivisuutta toisiinsa. Tuloksista havaittiin, että suoliston seinämän glukoosikertymällä ja seinämän tulehdusaktiivisuudella on merkitsevä yhteys, ja että ^{18}F-FDG-PET/MRI:llä pystytään havaitsemaan erityisesti suolen vaikeat tulehdusmuutokset, jotka erottuvat selkeästi normaalista kudoksesta.

Tutkimuksen toisena tavoitteena oli tutkia, ilmeneekö keratiini 7:ää (K7) IBD-potilaiden suoliston epiteelisoluissa, ja tutkia sen ennusteellista merkitystä IBD:n vaikeusasteen arvioinnissa. Tutkimus osoitti, että K7:ää ilmenee sekä UC että CD potilaiden paksusuolen epiteelissä. K7:n ilmentyminen oli suurinta lääkeresistentissä paksusuolitulehduksessa. Tutkimus antoi myös viitteitä siitä, että suolen anatominen kohta vaikuttaa K7:n ilmentymiseen. K7:n ennusteellista arvoa tutkittiin seuraamalla 16 potilaan taudinkulkua kolmen vuoden ajan, jolloin K7:n ilmentymisen perusteella ei kuitenkaan voitu ennustaa taudin vaikeusastetta. Tuloksista voidaan päätellä, että K7:ää voidaan käyttää erityisesti vaikean IBD:n merkkiaineena.

AVAINSANAT: tulehduksellinen suolistosairaus, haavainen paksusuolitulehdus, Crohnin tauti, positroniemissiotomografia, magneettiresonanssikuvaus, keratiini 7

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Abbreviations

ANCA	antineutrophilic cytoplasmic antibody
ANOVA	analysis of variance
Anti I2	an antibody against *Pseudomonas fluorescens*
ASA	acetylsalicylic acid
ASCA	anti-*Saccharomyces cerevisiae* antibody
ASUC	acute severe ulcerative colitis
AZA	azathioprine
BMI	body mass index
BWT	bowel wall thickness
Calpro	calprotectin
CBir1	bacterial flagellin
CC	collagenous colitis
CCAI	Clinical Colitis Activity Index
CD	Crohn's disease
CDAI	Crohn's Disease Activity Index
CI	confidence interval
CMV	cytomegalovirus
CRC	colorectal cancer
CRP	c-reactive protein
CT	computed tomography
CyA	cyclosporine A
DAB	3,3'-diaminobenzidine
ECCO	European Crohn's and Colitis Organization
EIM	extraintestinal manifestation
EHEC	enterohemorrhagic *Escherichia coli*
ESR	erythrocyte sedimentation rate
^{18}F-FDG	18-fluoro-fluorodeoxyglucose
FFPE	formal-fixed, paraffin embedded
FL	fecal lactoferrin
GER	gastroesophageal reflux
GI	gastrointestinal

Hb	hemoglobin
HBI	Harvey-Bradshaw Index
HE	hematoxylin eosin
HGD	high grade dysplasia
HLA	human leukocyte antigen
hr	heart rate
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IF	intermediate filament
IFX	infliximab
IgA	immunoglobulin A
IHC	immunohistochemistry
IL	interleukin
im	intramuscular
IPAA	ileal pouch anal anastomosis
IRA	ileorectal anastomose
IUS	intestinal ultrasound
iv	intravenous
JAK1/2/3	Janus kinase 1/2/3
K	keratin
kDA	kiloDalton
LC	lymphocytic colitis
LGD	low grade dysplasia
LIFT	ligation of intersphincteric fistula
MBq	megabecquerel
MP	mercaptopurine
MRE	magnetic resonance enterography
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MTX	methotrexate
na	not available
NOD2	nucleotide-binding oligomerization domain-containing-2
NSAID	non-steroidal anti-inflammatory drug
OmpC	*Escherichia coli* outer membrane porin C
PACS	Picture archiving and communication system
PBC	primary biliary cirrhosis
PD	percutaneous drainage
PET	positron emission tomography
po	per oral
pr	per rectum

PSC	primary sclerosing cholangitis
QoE	quality of evidence
QoL	quality of life
RBC	red blood cell
RCT	randomized controlled trial
ROI	region of interest
SBCE	small bowel capsule endoscopy
sc	subcutaneously
SCT	stem cell therapy
SES-CD	simple endoscopic score for Crohn’s disease
SD	standard deviation
SIBDQ	short inflammatory bowel disease questionnaire
SICUS	small intestinal contrast enhanced ultrasound
STC	subtotal colectomy
SUV	standard uptake value
Th	T-helper
TNF	tumor necrosis factor
TOF	time-of-flight
UC	ulcerative colitis
VOI	volume of interest
wk	week

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 Tenhami M., Virtanen J., Kauhanen S., Koffert J., Kemppainen J., Saunavaara V., Kujari H., Hurme S., Teperi S. and M. Voutilainen. The value of combined positron emission tomography magnetic resonance imaging to diagnose inflammatory bowel disease: a prospective study. *Acta radiologica*, 2021, Vol. 62(7): 851–857.

II Polari L., Tenhami M., Anttila S., Helenius T., Kujari H., Kallajoki M., Voutilainen M. and D.M. Toivola. Colonocyte keratin 7 is expressed de novo in inflammatory bowel diseases and associated with pathological changes and drug-resistance. *Scientific reports*, 2022, 23;12(1):22213.

III Tenhami M., Polari L., Kujari H., Löyttyniemi E., Toivola D.M. and M. Voutilainen. Keratin 7 expression in distinct anatomical parts of inflamed colonic epithelium in inflammatory bowel diseases and its prognostic value – a three-year follow-up. *Scientific reports*, 2023, 24:13(1):11979.

1 Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic inflammatory bowel diseases (IBD) that affect over 0.3 % of population in the countries of the highest prevalence and the incidence of IBD is currently increasing especially in developing countries (Ng et al. 2017; Windsor and Kaplan 2019). UC affects the mucosa of colon and rectum, while CD is a transmural disease that may affect the whole gastrointestinal tract, albeit it is most often found in ileocecal and perianal areas.

The major manifestations of IBD are diarrhea and rectal bleeding, and in CD also intestinal strictures, fistula formation and abscesses that occur especially in perianal area. The clinical course of IBD is characterized by relapses and spontaneous or drug-induced remissions. The disease is intermittent in about 90% of the cases and relapses are unpredictable. The disease activity in foregoing years indicates with 70–80% probability that the disease will continue the following year (Ebbe Langholz et al. 1994), but the course of IBD cannot be predicted by currently known tests or parameters.

The pathogenic factors of IBD include environmental changes, numerous susceptibility gene variants, abnormal gut microbiota and dysregulated immune response but profound understanding of IBD pathogenesis is still missing (de Souza and Fiocchi 2016; Mentella et al. 2020). It has been recently established that CD in small intestine is genetically different to CD affecting colon and they should be separated into two different entities. (Palmieri et al. 2017)

The diagnosis of IBD is based on endoscopic observation and mucosal biopsies in combination with clinical history, laboratory tests and radiological imaging (Maaser et al. 2019). The follow-up scheme for patients with IBD consists of health maintenance and colorectal carcinoma (CRC) surveillance using the previous procedures.

Integrated positron emission tomography and magnetic resonance imaging (PET/MRI) using fluorodeoxyglucose (^{18}F-FDG) is a hybrid imaging modality (Muzic and DiFilippo 2014), in which MRI provides high spatial resolution and has excellent soft tissue contrast while PET using fluorodeoxyglucose detects metabolic activity related to inflammation (Wehrl et al. 2015). Both ^{18}F-FDG-PET and MRI

have been shown to be useful in diagnostic evaluation of a variety of inflammatory and infectious processes (Jadvar and Colletti 2014; Spier, Perlman, and Reichelderfer 2009), but their utility to detect inflammation in bowel wall is unknown. In this thesis we compared intestinal histological samples of both UC and CD patients to data obtained using PET/MR imaging to find out the ability of PET/MRI to detect and grade the inflammation activity of bowel wall.

Currently, there is no good molecular marker to indicate IBD-associated disruptions of epithelial integrity, such as erosion, edema, crypt damages, and existing markers, likee neutrophil derived fecal calprotectin (F-Calpro), do not predict the outcome of the disease (de Souza and Fiocchi 2016; Garrett, Gordon, and Glimcher 2010).

Keratins (K) are intermediate filament proteins expressed in epithelial cells. They are major components of the cytoskeleton and they have been shown to protect the cell from mechanical and non-mechanical stress. They also regulate electrolyte transport in colonocytes, participate in cellular differentiation and proliferation and they have a role in inflammatory signaling (Coulombe and Omary 2002; Majumdar et al. 2012; Omary, Coulombe, and McLean 2004). Healthy intestinal epithelium expresses keratins 8, 18, 19, and 20 (Moll et al. 1982). Keratins are associated with the pathogenesis of various colorectal diseases, including cancer and IBD, and their expression and post-translational modifications are altered in colonic stress (Asghar et al. 2015; Corfe et al. 2015). Keratin 7 (K7), not expressed in normal colon, was found to be expressed in the epithelium of patients suffering from colitis linked adenocarcinoma or serrated adenoma. (Stenling et al. 2007; Tatsumi et al. 2005).

The aim in this work was to investigate the expression of K7 in IBD affected intestinal epithelial cells and its potential role as prognostic biomarker in the course of IBD as keratins are easy to detect due to high cellular concentration and recognizable cytoplasmic expression pattern (Zhong et al. 2004).

The overall priority of this study was to improve the accuracy of IBD diagnostics and to find new methods in evaluating the course and the prognosis of the disease.

2 Review of the Literature

2.1 Epidemiology and clinical presentation of inflammatory bowel diseases

2.1.1 Incidence and prevalence

IBD is a global disease and the incidence and the prevalence of both UC and CD are the highest in developed countries. However, there is a lot of variation in both based on geographic region, environment, and ethnic groups. The total IBD prevalence exceeds 0.3% in many European countries and North America. The incidence increase of both UC and CD have stabilized in US and Western Europe but their incidence continue to increase in developing countries. (Ng et al. 2017; Windsor and Kaplan 2019)

The highest annual incidences of UC have been 24.8 per 100 000 in Europe, 19.2 per 100 000 in North America and 9.3 per 100 000 in Asia where recent studies have revealed a remarkable emergence of IBD (Jussila et al. 2012; Mak et al. 2020; Molodecky et al. 2012). The highest incidences of CD are 29.3 in Australia, 20.2 in Canada and 10.6 per 100 000 person-years in Northern Europe (Torres et al. 2017). The highest reported prevalence values for IBD were in Europe (UC: 505 per 100 000 persons and CD: 322 per 100 000 persons) and North America (UC: 249 per 100 000 persons and CD: 319 per 100 000 persons) (Torres et al. 2017). In time-trend analyses, 60% of UC studies and 75% of CD studies had an increasing incidence of statistical significance ($P < 0.05$) (Molodecky et al. 2012). In Finland, between years 2000 and 2020, the mean annual incidence raised from 8 to 13 in CD and from 20 to 35 in UC (Kontola et al. 2022).

In Finland 55 925 persons (the total prevalence of IBD 55 925/5 549 599 * 100% = 1.01 %) got compensation for their costs in IBD medication (identification number 208 in their Social Insurance Institution (Kela) card) at the end of the year 2021, and a new diagnosis of IBD was placed for 2 746 persons (the total incidence of IBD 2 746/5 549 599 * 100 000/y = 49 per 100 000 person-years). In the Hospital District of Southwestern Finland, the corresponding numbers were 4 936 persons and 293 newly diagnosed IBDs in that same particular year. (https://tilastot.kela.fi/)

2.1.2 Etiopathogenesis

It is hypothesized that IBD is caused by an aberrant immune response to the microbes in gut, and genetic susceptibility of an individual catalyzes the process. Environmental factors and certain nutrients have also been shown to predispose a genetically susceptible individual to IBD. Genome-wide association studies and other analysis have ascertained 240 nonoverlapping susceptibility gene loci for IBD and thirty of them are shared with UC and CD (Dipasquale and Romano 2022; Guan 2019). The first susceptibility gene *NOD2* was found in 2001 and it codes for an intracellular receptor protein that recognizes muramyl dipeptide (MDP) that is a peptidoglycan structure present in both gram positive and gram negative bacteria (Ogura et al. 2001). The bacterial MDP induces autophagy in the invaded epithelial cells and as a consequence prevents the replication and further invasion of the bacteria (Cooney et al. 2010). *NOD2* also participates in MDP-independent pathway that regulates T-cell response to viral ssRNA invasion (Sabbah et al. 2009). Genetic analyses have also revealed other autophagy associated genes like *ATG16L1*, and its coding mutation T300A is associated with an increased risk of CD. (Zhang and Li 2014). In cells homozygous for the CD-associated *NOD2* frameshift mutation, mutant NOD2 failed to recruit ATG16L1 to the plasma membrane and wrapping of invading bacteria by autophagosomes was impaired (Travassos et al. 2010). The further discovery of other genes and their products that participate in bacterial sensing, innate immunity, Th17 function and changes in mucus layer (LRRK2, IRGM, HLA, JAK2, IL23R, STAT3, Th17 pathways and MUC2) has widened the understanding of the disease pathogenesis (Torres et al. 2017), and consequently opened possibilities to design and develop new therapeutic agents.

Genetic factors explain only a portion of the disease. The prevalence of IBD is 5 to 15% in diseased families (Monsén et al. 1991; 1987; Torres et al. 2017), while the prevalence of IBD is around 0,3% in the general population (Ng et al. 2017). The studies suggest that IBD is caused by a complex interplay of genetic factors, immune dysregulation, and environmental factors, including alterations in the fecal microbiome. Meanwhile, the adaptive immune response has been considered to play a significant role in the pathogenesis of IBD, recent studies have also clarified the major role of the innate immune response in the intestinal inflammation. (Jiang et al. 2022; Zhang and Li 2014)

Approximately 99% of the intestinal microbiota consists of four bacterial phyla: Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria. The biodiversity of intestinal microbiota is altered in the intestine of IBD patients (=dysbiosis): number of pathogens is increased and amount of Firmicutes species is diminished. Consequently, dysbiosis may lead to diminished amount of mucosal protective bacteria and increased amounts of hostile bacteria. As a result, the hostile bacteria

may invade the epithelial cells and activate the inflammation cascade. (Nishida et al. 2018)

As part of inflammation, the role of T helper (Th) cells is particularly important in the pathogenesis of IBD (Jiang et al. 2022). Interleukin-23 (IL-23) plays an important role in controlling the Th1/Th17 cell balance in both UC and CD (Kobayashi et al. 2008), and Th17 lymphocytes are a major component of Th cells (Jiang et al. 2022). The Th17 lymphocytes protect mucous membranes and epithelial tissue against hostile microbiota, and secrete interleukin-17 (IL-17), which is a key effector driving colitis in animal models. (Gajendran et al. 2018). If the immune system is dysfunctional, Th17 cells proliferate inappropriately and produce excessive amounts of proinflammatory cytokines and consequently induce exaggerated immune response which may lead into the development of an autoimmune disease. Th17 cells are shown to have a major role in the pathogenesis of IBD (Jiang et al. 2022). Although UC and CD share many common clinical symptoms and signs, they are in fact two separate clinical entities characterized by different immunopathogenesis (Park et al. 2017).

Well-established environmental influences on the risk of UC include cigarette smoking and a history of appendectomy. Current cigarette smokers are significantly less likely than never smokers to develop UC (Mahid et al. 2006). According to a recent study, smokers have fewer UC related hospitalizations than non-smokers, but their risk of CRC is increased (Chen et al., 2022). Nicotine causes changes in rectal blood flow and to production of colonic mucus and also to cytokine and eicosanoid production, which may explain the protective effect. However, other nicotine products (chewing gum, transdermal nicotine) have shown just limited benefit (Rubin and Hanauer 2000). Appendectomy in childhood for appendicitis also appears to diminish the risk of UC, suggesting that removing the appendix might influence the mucosal immune system (Koutroubakis, Vlachonikolis, and Kouroumalis 2002).The role of dietary factors in UC remains controversial. High vegetable intake may be protective and increased intake of fat, polyunsaturated fatty acids, omega-6 fatty acids and meat may be harmful. Acute gastroenteritis may trigger IBD. The increased risk may be explained by IL-6 production, nonspecific blockage of regulatory T-cells, and activation of self-reactive T-cells (van der Sloot et al. 2017). Cytomegalovirus (CMV) infection may delay remission by causing steroid-resistance. (Ayre et al. 2009)

In Crohn's disease, smoking increases the risk for the disease twofold, causes need for more immunosuppressants and for more surgical interventions and higher rates of post-operative disease recurrence (Chen et al. 2022; J. Cosnes et al. 1996; Mahid et al. 2006; van der Sloot et al. 2017). The potential mechanisms may be changes in humoral and cellular immunity, epithelial permeability and blood flow, and colonic mucus. Nicotine is assumed to be the active moiety. (Birrenbach and

Böcker 2004). Appendectomy increases the risk of CD, and the increase is dependent on the patient's sex, age, and the diagnosis at operation (Andersson et al. 2003). Also medications such as oral contraceptives and non-steroidal anti-inflammatory drug (NSAID) and the usage of antibiotics especially in early childhood have been implicated as potential risk factors for CD (Cornish et al. 2008; Godet, May, and Sutherland 1995; van der Sloot et al. 2017).

The western diet is also correlated with a higher risk of IBD. Refined proteins, processed carbohydrates and an excessive intake of saturated fatty acids may cause intestinal dysfunction and changes in the composition of intestinal microbiota. Western diet has also increased amount of food additives like artificial sweeteners and preservatives which modulate gut microbiome and lead to enrichment of mucosal inflammatory cells, consequently leading to an undesired immune response and induction of IBD (Liu et al. 2022).

2.1.3 Presentation and symptoms

Ulcerative colitis

UC has peak incidence between 20–30 years of age followed by a second peak between 50 and 80 years, respectively. (Gajendran et al. 2019; E. Langholz et al. 1991). The most common symptoms of UC are diarrhea and passage of blood and mucus. Colicky abdominal pain is also a frequent manifestation in patients with acute severe colitis. There may be just minor impairment in general health if the beginning of the disease is mild.

The course of UC varies in severity and ultimate prognosis. In approximately 90% of the cases the UC is a chronic condition that is characterized by remissions and relapses (Ebbe Langholz et al. 1994). In few patients the onset of UC may be fulminant, and symptoms typically become most severe in the first few months after diagnosis. (Dignass et al. 2012). Some patients are troubled by continuous symptoms without remission and few patients have only one attack with no subsequent symptoms. According to an early study where 1 161 UC patients were followed 25 years after the diagnosis, 25% of the patients were in remission after 3–7 years of diagnosis and 18% had activity every year and 57% had intermittent relapses. Activity in the first two years after diagnosis correlated significantly with increased probability of having an active disease in the forthcoming five years ($P = 0.00001$). After ten years, the colectomy rate was 24%. The probability of maintaining working capacity after ten years was 92.8% (range, 90.8–94.8%). (Ebbe Langholz et al. 1994)

Remission of UC is defined as complete resolution of symptoms and endoscopic mucosal healing. In clinical practice, “remission” means a stool frequency less or equal to three per day with no bleeding and no urgency (Dignass et al. 2012). Relapse

is a flare of symptoms in a patient with established UC who used to be in clinical remission, and relapse is defined "early" if period of remission lasts less than 3 months after achieving remission on previous therapy. (Dignass et al. 2012)

Crohn's disease

Typical onset of CD is aged between second and fourth decades of life. Common symptoms include diarrhea, midabdominal pain and weight loss (Torres et al. 2017). Although diarrhea is a dominant feature of both UC and Crohn colitis, colonic bleeding is less common with CD (Sands 2004). Other symptoms and clinical findings include fever, bleeding, anemia, nausea, and vomiting. Approximately one third of the patients present with perianal manifestations, which can be classified into skin lesions (maceration, erosion, ulceration, abscess formation, and skin tags), anal canal lesions (fissures, ulcers, and stenosis of anal canal), fistulas, and hemorrhoids. (Buchmann and Alexander-Williams 1980; Torres et al. 2017).

The onset of the CD may also present symptoms of an acute abdomen. An ileocolic disease may mimic the symptoms of appendicitis. Toxic megacolon may complicate Crohn colitis as well as other forms of colitis. Extraintestinal manifestations (EIM) like arthralgia, arthritis, sacroiliitis, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis (PSC) and iritis/uveitis/episcleritis complicate up to 20–50% of the cases, depending on the definition of EIM. (Gajendran et al. 2018)

In terms of distribution and behavior of the disease, approximately 50% of patients have ileocolitis, 25% have colitis only and 25% of the patients have ileitis. One-third of the patients have perianal involvement and 5–15% have involvement of oral or gastroduodenal area. (Gajendran et al. 2018). The location of the disease remains relatively stable over the course of the disease. Only 16% of patients have had a change in location after ten years of follow-up (Louis et al. 2001; Torres et al. 2017). However, most of the patients have a change in the behavior of the disease over the years, and over 60% the patients develop either fistulizing or stricturing complications within the next 20 years after diagnosis (Jacques Cosnes et al. 2002; Louis et al. 2001; Torres et al. 2017). Ileal CD is more often stricturing, instead colonic or ileocolonic CD is more often penetrating. (Louis et al. 2001)

Remission is defined as a Crohn's Disease Activity Index (CDAI) ≤ 150. Relapse is a flare of symptoms in an established CD patient who was in clinical remission. The suspected relapse is confirmed by laboratory parameters, imaging, or endoscopy in clinical practice. The relapse is considered as early relapse if the patient relapses within three months of achieving remission on a particular therapy. Relapses could be categorized into infrequent (<1/yr.), frequent (>2 /yr.) or continuous (persistent active CD without period of remission) based on the pattern of their existence.

In luminal CD at any point of time about half of all patients will be in clinical remission. If a patient is in remission for one year, there is an 80% chance that the disease will remain in remission in the forthcoming year. If a patient had an active disease in the past year, there is a 70% chance of having the active disease in the next year (Gajendran et al. 2018). In terms of prognosis, one fifth of the patients will have relapse every year and 13% of the patients will have relapse-free course of CD. In addition, 67% of the patients will have a combination of years with relapse and years in remission after the first eight years of initial diagnosis (Gajendran et al. 2018). In addition to previous facts, according to a prospective study, where CD patients were followed 5 years from the diagnosis, 22% received surgical treatment, 36% were hospitalized because of CD, and 49% of the patients that were diagnosed with non-stricturing non-penetrating CD developed stricturing and/or penetrating form of CD during the 5-year follow up period. (Burisch et al. 2019)

2.1.3.1 Extraintestinal manifestations

EIM are autoimmune-related disorders in other organs, and they affect 25 to 40% of the patients suffering from IBD within ten years after the onset of the disease (Sairenji, Collins, and Evans 2017). There are two types of EIM. First, immune-related manifestations of IBD are associated with inflammatory activity in the intestine. These manifestations include aphthous stomatitis, peripheral arthritis, iritis/uveitis, pyoderma gangrenosum and erythema nodosum. Second, autoimmune disorders associated with IBD are independent of the intestinal state of inflammation. These diseases, like primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and alopecia, are related to increased susceptibility to autoimmunity disorders of the individual. (Sange et al. 2021; Torres et al. 2017)

The intestinal microbiota has a crucial role in the etiopathogenesis of EIM. The microbiota activates patient's immune system against bacterial antigens and, due to cross-reactivity, also against the colonic mucosa. Bacteria that leak through damaged intestinal barrier can also trigger an adaptive immune response that attacks against bacterial epitopes and also against those epitopes that structurally resemble those of bacteria like the epitopes of joints and skin in the patients that have genetic susceptibilities to autoimmune disorders associated with human leukocyte antigen (HLA) system. HLA-A2, HLA-DR1 and HLA-DQw5 cell surface proteins are associated with CD complications and UC complications are associated with HLA-DR103 genotype. (Sange et al. 2021)

The existence of untreated EIM along with IBD prolongs the course of the disease and decreases the quality of life (QoL). The primary therapeutic target is the bowel and the other management of EIM depends on the diagnosis, behavior and location of the EIM. (Sange et al. 2021)

2.2 Diagnosis and classification

According to ECCO guidelines, currently there is no single reference standard for the diagnosis of UC or CD. (Maaser et al. 2019)

Ileocolonoscopy and biopsies in combination with clinical history, laboratory tests and radiological imaging, especially in CD, are used for the diagnosis of IBD. A minimum of two biopsies should be taken from the inflamed mucosal regions along with the routine biopsies (Maaser et al. 2019). Typical features in investigations are presented in **Table 1.** A complete ileocolonoscopy is not usually recommended in case of acute severe colitis due to increased risk of bowel perforation. However, a flexible sigmoidoscopy can be safely performed to establish the diagnosis of UC. (Maaser et al. 2019)

Table 1. Typical findings in laboratory, endoscopic and histological examinations in UC and CD. (Sipponen 2018a).

Laboratory parameters	**UC**	**CD**
anemia	in extensive colitis	often
elevated CRP or ESR	in extensive colitis	often
elevated F-calpro	often	often
hypoalbuminemia	in severe colitis	often
Endoscopy		
location of inflammation	continuous from rectum	anywhere in GI-tract
proctitis	almost always	30 – 50 %
ileitis	rarely (backwash ileitis)	often
ulcers	wide, superficial	deep, branching, longitudinal
skip-lesions	absent	present
strictures	absent	often
pseudopolyps	present	present
Histology		
transmural inflammation	absent	present
altered crypt architecture	present	rarely
cryptitis	present	present
granulomas	absent	present (rarely in mucosal biopsies)
fissures and skip-lesions	rarely	often

UC = ulcerative colitis, CD = Crohn's disease, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, GI = gastrointestinal.

The classification of UC is recommended to be done by the extent of the disease because of following reasons: 1) the extent of inflammation influences the choice of treatment given for the patient, 2) the extent of the colitis indicates the need and the timetable for the surveillance of each patient (Dignass et al. 2012). Consequently,

ECCO prefers that the classification of UC is based on findings in endoscopy and division should be made into proctitis, left-sided colitis and extensive colitis (**Table 2).**

Table 2. Montreal classification of UC (Silverberg et al. 2005).

Term	Distribution	Description
E1	Proctitis	involvement limited to rectum
E2	Left-sided	involvement limited to the proportion of the colon distal to the splenic flexure
E3	Extensive	involvement extends beyond the splenic flexure, including pancolitis

The key feature for diagnosing CD includes a combination of endoscopic and histological findings along with imaging studies that demonstrate focal, asymmetric, transmural or granulomatous features in gastrointestinal tract. Differential diagnosis for CD includes UC, infectious colitis, ischemic colitis, radiation colitis, medication-induced colitis (especially NSAID), microscopic colitis, celiac disease, and irritable bowel syndrome (IBS). Additional serologic tests in the case of differential problems include antibodies against saccharomyces cerevisiae (ASCA), antineutrophil cytoplasmic antibodies (ANCA), and antibodies directed against CBir1 and OmpC (Gajendran et al. 2018).

The classification of CD (**Table 3.**) provides a tool for patient counselling, assessing disease prognosis, and particularly with choosing the most appropriate therapy for each disease subtype. (Satsangi et al. 2006)

Table 3. Montreal-classification of Crohn's disease (Satsangi et al. 2006).

Variable	
Age at the time of dg	A1 < 17 years
	A2 17–40 years
	A3 > 40 years
Localization of disease	L1 ileum
	L2 colon
	L3 ileum and colon
	L4 upper GI tract (orally from terminal ileum) *
Behavior of the disease	B1 inflammatory (non-stricturing, non-penetrating)
	B2 stricturing
	B3 penetrating
	p perianal disease (adds to previous if contemporary)**

A = age, L = localization, B = behavior. * L4 is a modifier that can be added to L1–L3 when concomitant upper gastrointestinal disease is present. ** ''p'' is added to B1–B3 when concomitant perianal disease is present.

2.2.1 Clinical examination and endoscopy

Ulcerative colitis

The severity of UC is classified mainly by the severity of the clinical symptoms according to the Montreal classification (**Table 4**).

The severity of clinical symptoms of UC may be estimated by using clinical colitis activity index (CCAI) (Walmsley et al. 1998). The most common scoring systems used to measure clinical disease activity include CDAI, HBI- Harvey-Bradshaw index (HBI), short inflammatory bowel disease questionnaire (SIBDQ) and Lehmann score. (Best et al. 1976; Gajendran et al. 2018)

The common endoscopic features of UC are represented in **Table 5.,** and they are seen within an extent which is categorized into proctitis, left-sided colitis, and extensive colitis (**Table 2.**). Inflammation of terminal ileum (backwash ileitis) can be associated with extensive colitis in about 20 % of the cases (Magro et al. 2013). Although proctitis is an essential finding in UC, rectum may appear normal in endoscopy and histological specimen in patients, who receive topical or systemic treatment, in untreated children, and in patients who suffer from fulminant colitis. (Kleer and Appelman 1998; Magro et al. 2013). The argument has been challenged in a study of colectomy patients, where 56 patients underwent pre-operative endoscopy with biopsies and had colectomy for non-neoplastic complications. None of those patients had complete absence of rectal involvement after all tissue sections (pre-operative biopsy specimen and colectomy specimen) were evaluated after colectomy, even though endoscopic view and pre-operative biopsies of rectum appeared normal (Joo and Odze 2010). The distribution of the disease may also change over time, and the usual progress is from proctitis to extensive colitis (Magro et al. 2013). The Mayo-score (**Table 6.**) combines the clinical and endoscopic findings, but it is mainly used in research purposes.

Table 4. The clinical severity of UC, the Montreal-classification (Dignass et al. 2012)

Severity (S)	Definition
Clinical remission (S0)	no symptoms
Mild colitis (S1)	loose stools < 4 times a day (bloody stools +/-), no general symptoms, normal inflammatory markers in laboratory tests
Moderate colitis (S2)	loose stools ≥ 4 or more times a day, bloody stools, no more than mild general symptoms
Severe colitis (S3)	loose bloody stools ≥ 6 times a day and tachycardia (hr > 90/min) or fever (> 37,5 °C) or anemia (Hb < 105 g/l) or elevated inflammatory markers (CRP > 30 mg/l or ESR > 30 mm/h)

Table 5. The endoscopically estimated severity of UC in colonic mucosa (Sturm et al. 2019; S. P. L. Travis et al. 2013).

Degree	Features	Clinical estimate
0	normal mucosa, normal vascular pattern, minor nodules	normal
1	reduced vascular pattern, mucosal erythema, friability, erosions, mucosal bleeding	mild colitis
2	complete loss of vascular pattern, significant erythema, superficial ulcers, mild luminal bleeding	moderate
3	complete loss of vascular pattern, ulcers, spontaneous luminal bleeding, mucopurulent secretion	severe colitis

Table 6. A combined endoscopic and clinical scale (Mayo-score) to assess the severity of ulcerative colitis (Schroeder, Tremaine, and Ilstrup 1987). The points will be calculated.

A. frequency of bowel function	0 = normal
	1 = 1–2 times more than normally
	2 = 3–4 times more than normally
	3 = 5 or more times more than normally
B. bloody stools	0 = no visible blood
	1 = occult blood
	2 = clearly visible blood
	3 = only blood
C. endoscopic features	0 = normal or inactive
	1 = mild: minor erythema, reduced vascular pattern, friability
	2 = moderate: erythema, no vascular pattern, friability
	3 = severe: ulcers, spontaneous bleeding
D. clinical estimate	0 = normal
	1 = mild
	2 = moderate
	3 = severe

Crohn's disease

The endoscopic appearance of CD colitis is usually different from that of UC (**Table 7.**). The lesions are segmental, and the mucosa of rectum appears unaffected in approximately 50 % of patients with colitis. There may be isolated aphthous ulcers with normal surrounding mucosa and there may be irregular erythema, edema, cobblestone appearance, and deep linear ulcerations and fistulas.

All newly diagnosed CD patients should have MR enterography and/or a capsule endoscopy for small bowel assessment (SBCE). In addition, if ileocolonoscopy is normal and there still is a clear suspicion of CD, the SBCE or the MRI studies should

be considered. Prior to SBCE, the risk of capsule retention should be assessed if stenosis of small bowel is of doubt. (Maaser et al. 2019)

According to ECCO guidelines, a gastroscopy is recommended for those patients who have upper GI symptoms, like dyspepsia, nausea and vomiting, but not routinely for all newly diagnosed adult IBD patients. (Maaser et al. 2019)

Table 7. SES-CD endoscopic classification of Crohn's disease. (Daperno et al. 2004)

Variable	0	1	2	3
size of ulcers	none	aphthous	large	very large
ulcerated surface	none	< 10 %	10-30 %	>30 %
affected surface	unaffected	< 50 %	50-75 %	>75 %
presence of narrowing	none	single	multiple	cannot be passed

	ileum	right colon	transversum	left colon	rectum	points
size of ulcers						
ulcerated surface						
affected surface						
presence of narrowing						
SES-CD						_____

SES-CD = simple endoscopic score for Crohn's disease

2.2.2 Biomarkers

Biomarkers are measurable indicators of normal and pathogenic processes and their level changes as response to given therapeutic intervention. They have an essential role in early diagnosis and in monitoring the disease activity.

As serological markers, antibodies have been described in IBD for over the last 60 years (Broberger and Perlmann 1959). The Anti-*Saccharomyces cerevisiae* antibodies (ASCA) have high prevalence in patients with CD (Main et al. 1988), and perinuclear anti-neutrophil cytoplasmic antibodies (pANCAs) are found in up to 65% of patients with UC, respectively (Dignass et al. 2012; Rump et al. 1990). There are also other antibodies especially against bacterial epitopes, like anti-OmpC, anti-CBir1 and Anti I2. However, ASCA and pANCA are the most widely studied serological markers of IBD. Because of limited sensitivity of these markers, their routine use for the diagnosis and for therapeutic decisions is not reasonable. (Dignass et al. 2012)

Of the fecal markers, the neutrophil derived proteins, such as calprotectin, elastase, lysozyme and lactoferrin, have been evaluated as biomarkers of inflammatory activity in IBD (Poullis et al. 2002; Vermeire, Van Assche, and Rutgeerts 2006). Fecal calprotectin turned out to be the most sensitive marker and its quantity correlates well with endoscopically and histologically detected inflammation (Konikoff and Denson 2006). Later studies have proved calprotectin valuable in assessing the severity of disease, in diagnosing relapse and in response to treatment. (Gisbert et al. 2009; Schoepfer et al. 2009; von Roon et al. 2007). A cut-off value of 150 µg/g provides a reasonable diagnostic accuracy in distinguishing IBD from IBS. (Maaser et al. 2019). However, IBD cannot be differentiated from other etiologies of inflammatory conditions by using F-calprotectin as biomarker (Dignass et al. 2012).

According to a recent comprehensive meta-analysis, the most sensitive biomarkers for distinguishing IBD from non-IBD were fecal calprotectin and fecal lactoferrin (FL). The most specific tests were pANCA and FL. ASCA, IgA was the best test to distinguish CD from UC. (Shi et al. 2022)

A novel approach to biomarkers is the introduction of omics technologies, which means objective identification of gene products like genes (=genomics), mRNA (=transcriptomics), proteins (=proteomics) and metabolites (=metabolomics) in a biological sample. By integrating the information obtained from different stages of gene expression may improve the understanding of normal physiology and its alternations leading to pathological disease processes. (Feakins et al. 2022)

2.2.2.1 Simple epithelial keratins and keratin 7 (K7)

Simple epithelial keratins are heterodimer proteins consisting of one type I acidic intermediate filament (IF) protein component and one type II neutral/basic IF protein component. In humans their molecular weight ranges from 44 to 66 kDa. Dimers form tetramers and consequently filamentous structures in cytoplasm of mammalian epithelial cells. Keratin protein expression depends on the cell type and their expression pattern also varies between different species. Keratins form part of the cytoskeleton which provides structural integrity in the cellular cytoplasm. In addition to their structural role, they also provide dynamic functions by participating in processes including barrier function, ion transport, differentiation and inflammatory signaling. They are also part of the filamentous bundles that connect cells to each other by desmosomes and to extracellular matrix by hemidesmosomes. As keratins participate in maintaining homeostasis in cells, their expression and post-translational modifications are altered in stressful conditions. (Corfe et al., 2015; Helenius et al., 2016; Polari et al., 2020).

Human colonic epithelial cells express keratins 8, 18, 19 and 20. Keratin 7 (K7) is a type II intermediate filament protein that is part of cytoskeleton in several glandular and ductal epithelia. In humans, K7 is not expressed in healthy colonic epithelium, and K7 expression is rare in sporadic CRCs. Anyhow, 45–70% of UC-related adenocarcinomas have been reported K7 positive (Stenling et al., 2007; Tatsumi et al., 2006). K7 expression in colonic epithelial cells in UC and CD associated inflammation is unknown.

At present, there is no biomarker that would indicate IBD-associated disruptions of epithelial integrity. Since K7 is expressed in UC-related neoplasia, but not in physiological state in colonic epithelium, its de novo expression could possibly indicate alternations in epithelial cells and in their integrity. A similar mechanism has recently been described in keratinocytes, where inflammation induces keratin 17 expression, which on behalf is related to tumor promoting circumstances (Nair et al., 2021).

2.2.3 Histopathology

Ulcerative colitis

An adequate number of biopsies taken during ileocolonoscopy provides the histologic diagnosis of IBD. Several other etiologies may cause histological features present also in IBD, including bacterial, viral, and fungal infections, vascular disorders, diverticular disease, immune disorders, radiation proctitis and drug-induced disease. Basal plasmacytosis, granulomas and changes in crypt architecture support the diagnosis of IBD albeit any of them can also exist in other circumstances (Feakins et al. 2022). At least two biopsies from five sites along the colon, including the rectum and the terminal ileum, should be taken to achieve a reliable diagnosis. If the patient suffers from fulminant colitis, a minimum of two samples from at least one site should be taken. In the follow-up examinations, a smaller number of biopsies may be enough to confirm the diagnosis. However, when the patient is screened for dysplasia, multiple biopsies are needed again. (Magro et al. 2013)

In ulcerative colitis, biopsies discriminate between inactive disease, quiescent disease, and different grades of disease activity. The macroscopic view in endoscopy and the features in histological specimen match in approximately 65% of the samples (Kleer and Appelman 1998).

The histological diagnosis of UC is based on a widespread crypt distortion (branching and dilation), and diffuse transmucosal inflammatory infiltrate composed of neutrophils, lymphocytes, and basal plasmacytes. The number of inflammatory cells is usually higher in mucosa than in submucosa and the degree of inflammation usually increases towards the rectum. If there is an active component of

inflammation, cryptitis (neutrophilic infiltration in mucosa) and crypt abscesses (neutrophil aggregates in distended crypt lumina) can also be found. In fulminant colitis, ulcers may penetrate even into the muscularis propria. At an early stage of the disease, the presence of basal plasmacytosis appears to be the highest predictive value for the diagnosis of UC, and the absence of transmucosal inflammatory cell infiltrate or the existing normal crypt architecture does not rule out UC. Consequently, a repeated endoscopy with biopsies is recommended not earlier than six weeks after the first assessment of the disease. (Magro et al. 2013)

Crohn's disease

Typical histological features of CD are focal crypt architectural abnormalities (like distortion and withering), granulomas, mucin depletion, focal lymphoid cell aggregates (chronic inflammation) without crypt atrophy, transmural fissure formation, and on the other hand, mucin at active focuses. Epithelial damage along with amount of neutrophils are the markers of the activity of the disease. (Maaser et al. 2019; Tanaka et al. 1999). However, a single finding present in biopsy is not considered to be diagnostic (Feakins et al. 2022; Tanaka et al. 2000). It is not known how many features must be found in endoscopically derived biopsies to propose a plausible diagnosis. On surgical samples three histological features of CD are suggestive of firm diagnosis, and when an epithelioid granuloma is present, one additional feature is enough, respectively. (Maaser et al. 2019; Tanaka et al. 2000; 1999)

2.2.4 Cross sectional imaging techniques

2.2.4.1 Computed tomography (CT)

Abdominal CT enterography has been the first-line radiologic study in the assessment of small bowel CD with a sensitivity of 95.2% (Gajendran et al., 2018; Siddiki et al., 2009). CT that covers abdomen and pelvis requires luminal distention and intravenous contrast agent to assess small intestine. Findings associated with active inflammation include mucosal enhancement, wall thickness, comb sign, and presence of enlarged lymph nodes (Chiorean et al., 2007). The sensitivity of CT in CD colitis ranged from 60% to 90% and the specificity from 90% to 100%, respectively (Andersen et al., 2006). Compared with endoscopy, the colonic wall thickness on CT correlated with the presence of ulceration, pseudopolyps and fistulae, whereas increased vascularity correlated with mucosal inflammation (Andersen et al., 2006). CT guidance is used in interventional procedures like percutaneous intra-abdominal abscess drainage (Jensen et al., 2011). The major

limitation of CT is the radiation exposure. An emergency CT scan of a young female UC patient shows pipe-like sigmoid colon in **Figure 1**.

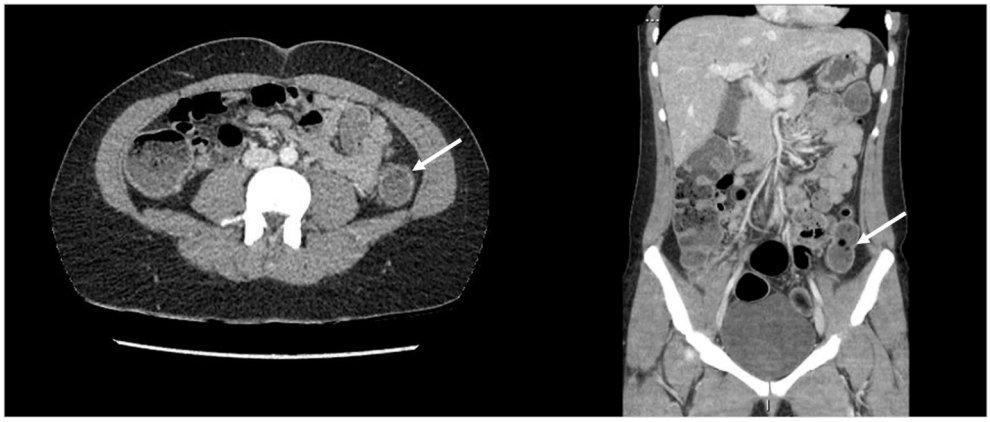

Figure 1. An axial and a coronal CT scan of a young female UC patient in ER due to inflammatory symptoms in joints and rise of CRP. The sigmoid colon is pipe-like, but the activity of UC was ruled out by CT.

2.2.4.2 Magnetic resonance imaging (MRI)

MR imaging provides high spatial resolution and has an excellent soft tissue contrast (Wehrl et al. 2015). The use of intravenous contrast medium improves the assessment of inflammatory changes in bowel wall like deep ulcers, wall thickening, wall hyperenhancement, and enlarged mesenteric lymph nodes. The findings correlate from modestly to well with the disease activity. (Gourtsoyiannis, Papanikolaou, and Karantanas 2006; Kaushal et al. 2017; Rimola et al. 2022)

The typical features of active inflammation in small bowel include high mural signal intensity in T2-weighted images, arterial phase hyperenhancement in T1-weighted images, restricted diffusion, vasa recta engorgement and reactive lymphadenopathy. Fibrostenotic disease shows as hypointense or intermediate signal in T2-weighted images depending on the used examination pattern while in T1-weighted images the enhancement is restricted to mucosa. There is no restricted diffusion in purely fibrostenotic lesion of the disease. Fistulizing and perforating disease is seen with linear tracts of hyperintense signal from serosal surface of bowel leading to another structure or bowel segment. In regeneration phase, pseudopolyps show with low signal intensity and fat deposition within bowel wall shows with intermediate signal intensity in T2-weighted images. There is no hyperenhancement in contrast-enhanced T1-weighted images and diffusion is unrestricted. (Kaushal et al. 2017)

MRI of small bowel and colon requires fast imaging sequences and luminal distention with a contrast compound so as to acquire T1 and T2 weighted images during a single breath hold. (Feuerbach 2010; Kuehle et al. 2006). In the assessment of small bowel CD the diagnostic accuracy of MR enterography (MRE) is similar to that of CT scan and it does not expose the patient to ionizing radiation (Gajendran et al. 2018). The sensitivity and specificity of MRI in the detection of small bowel disease is 78% (95% CI 67–84%) and 83% (95% CI 76–90%) respectively (Panés et al. 2011).

In the assessment of CD colitis, the sensitivity of MRI is 78−100% and the specificity is 46−100%. Significant correlation has been observed between MRI and endoscopic activity markers in CD colitis (Oussalah et al. 2010; Rimola et al. 2022). In clinical practice MRI is a useful imaging modality to assess CD colitis after incomplete colonoscopy in patient's not requiring biopsy, those with severe comorbidities, especially in cases where extraluminal complications (**Figure 2.**) are suspected (Pilleul et al. 2005; Rimola et al. 2022; Sollini et al. 2018). Several activity scores have also been developed to depict the activity of IBD assessed by MRE. (Rimola et al. 2022)

Motility of bowel can also be quantified by MR-imaging. Especially, evaluation of alterations in motility may help to increase the sensitivity and specificity of MRI of the small bowel. However, further studies are needed to establish classifications of motility disorders as assessed by MRI. (Maccioni et al. 2012; Rimola et al. 2022)

In perianal area, MRI is the golden standard imaging technique for diagnosis and classification of fistula and abscesses. Pelvic MRI depicts precisely the structures of anal sphincter and pelvic floor muscles, and it characterizes fistula tracts and abscesses with an accuracy of 76–97%. (Barker et al. 1994; Koelbel et al. 1989; Torres et al. 2017)

When IBD or its potential extraintestinal manifestations are concerned, MRI has several strengths compared with CT, including functional imaging capability through specialized techniques such as diffusion-weighted imaging, functional MR imaging, MR elastography, MR spectroscopy, perfusion weighted imaging and the availability of some targeted MR imaging contrast agents. (Torigian et al. 2013)

The limitation of MRI is availability. It is also more time consuming and more expensive than CT scanning. Anyhow, the choice of imaging method depends on clinical questions, patient’s characteristics, and availability of local imaging modalities.

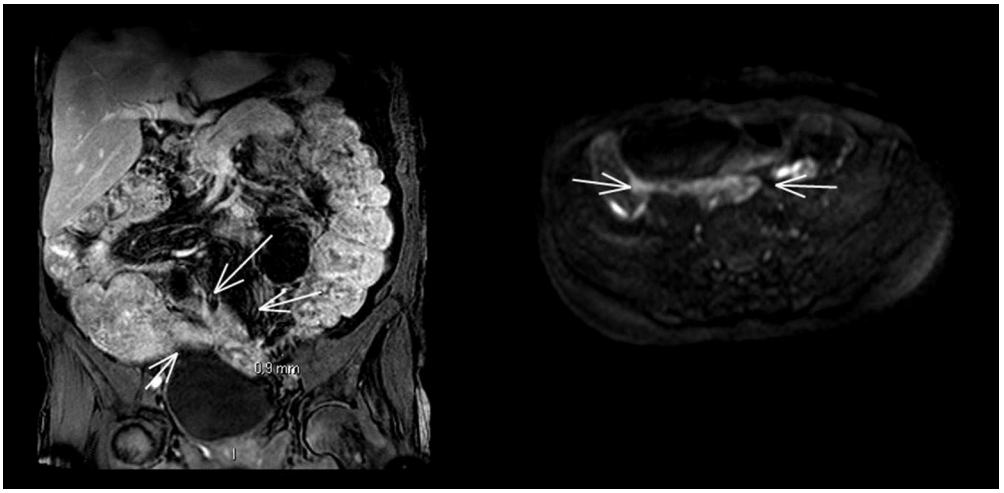

Figure 2. A coronal and an axial MR-image of a male patient with CD and a fistulizing structure (arrows) in distal ileum with attached caecum, sigmoid colon, and small bowel. There is also a pre-stenotic dilation in the small bowel because of passage problem.

2.2.4.3 Intestinal ultrasound

Intestinal ultrasound (IUS) is used to estimate both bowel wall thickness (BWT) and enhancement, and to look up extraintestinal manifestations of CD. Small intestinal contrast enhanced ultrasound (SICUS) improves intraluminal distension and visualization, but it is more time consuming and less applicable in clinical practice. According to meta-analyses, IUS is accurate in diagnosis and staging of CD. Anyhow, compared with endoscopy, the usefulness of IUS in assessing the response to treatment is still limited. When expertise is available, IUS can be done at the point-of-care aiding real-time decision making. (Rimola et al. 2022; Torres et al. 2017)

2.2.4.4 Positron emission tomography and hybrid imaging

18-fluoro-fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET) is a three-dimensional imaging modality that utilizes a radioactively labelled structural analogue of glucose molecule and consequently reveals the sites of active glucose metabolism, like tumors and areas of inflammation and infection. The cells that express increased amounts of glucose transporters bind more FDG-molecules than normal cells and consequently produce elevated radioactive signal which is detected by PET-camera. The activity in the volume of the interest (VOI) is quantified using standardized uptake value (SUV). The activity in the target tissue, the injected FDG dose and the weight of the subject must be considered when calculating SUV's. (Catalano et al. 2018)

PET can be integrated with CT or MRI and consequently create high-resolution hybrid-images with metabolic data of PET and exact anatomical information produced by CT or MRI (Catalano et al. 2018). Integrated PET/TT has been shown useful and feasible when estimating the location and extent of the inflammation in bowel (Sollini et al. 2018). It is particularly beneficial in CD when extra-intestinal manifestations are suspected or when strictures in the bowel prevent endoscopy (Catalano et al. 2018). Anyhow, according to European guidelines by ECCO, FDG-PET is not recommended for diagnostics in IBD because of a lack of evidence (Brodersen and Hess 2020). Integrated PET/MRI may offer advantages in diagnosis and follow-up of IBD since both ^{18}F-FDG PET and MRI have been shown useful in diagnostic evaluation of a variety of inflammatory and infectious processes (Jadvar and Colletti 2014; Rimola et al. 2022; Sollini et al. 2018; Spier, Perlman, and Reichelderfer 2009), and according to a recent systematic review, MRE is a sensitive and specific imaging method for disease activity in IBD (Shi et al. 2022). The capability of combined PET/MRI to detect pathological lesions in bowel wall has been studied, but the group of subjects was heterogeneous including mainly malignant processes (Beiderwellen et al. 2016). In addition to previous, relationship between SUV and degree of inflammation activity in CD has been suggested (Pellino et al. 2016; Sollini et al. 2018) therefore raising interest on grading the severity of inflammation in bowel wall based on the calculated SUV's.

2.3 Treatment

The aims of medical treatment are induction and maintenance of remission, mucosal healing, prevention of complications and improvement of the quality of life. The location and the severity of the disease and especially in CD, the progression risk of the disease, determine the choice of treatment. An efficient medication that has been initiated without delay is likely to have a beneficial influence on the course of the disease.

2.3.1 Medication

Medical agents available for the treatment of IBD include 5-ASA (mesalazine), local steroids, systemic steroids, thiopurines (azathioprine (AZA), and mercaptopurine (MP)), methotrexate (MTX), and biologic therapies such as anti-TNFa-agents (infliximab (IFX), adalimumab, golimumab), anti-integrins (vedolizumab), anti-kinase JAK1/2/3 (tofacitinib, upadacitinib (UC, CD) and filgotinib (UC)), anti-interleukin 12/23 (ustekinumab) and anti-interleukin 23 (risankizumab). (Torres et al. 2020)

Ulcerative colitis

The treatment presented here is based on the ECCO Guidelines on Therapeutics in Ulcerative Colitis (**Table 8 a. and 8 b.**) and ECCO Guidelines on Therapeutics in Crohn's Disease (**Table 9.**) and national recommendations. The treatment of induction and the treatment of remission are presented in separate sections. They should be used within the framework of local resources and the individual needs and expectations of each patient.

Table 8 a. The treatment of induction of ulcerative colitis according to ECCO guidelines (Raine et al. 2022; Spinelli et al. 2022) and Finnish recommendations (Sipponen 2018b).

Induction of remission

Severity	**medication**	**QoE/recommendation**
Mild colitis[+]	5-ASA ≥ 2.0g /d po corticosteroids* po	low / strong low / weak
Moderate colitis	5-ASA ≥ 2.0g /d po corticosteroids** po/pr	low / strong very low / strong
Moderate-Severe colitis	corticosteroids** po anti-TNF-agents*** vedolizumab**** tofacitinib**** ustekinumab****	very low / strong moderate / strong low / strong moderate / strong moderate / strong
Severe colitis	corticosteroids cyclosporine 2mg/kg/d iv infliximab 5 mg/kg iv	40-60 mg/d po/iv
Location		
Distal colitis[+]	5-ASA ≥ 1.0g/d pr steroids pr	low / strong very low / strong
Rectosigmoid (at least)[++]	5-ASA ≥ 2.0g/d po+pr	low / weak

*Colonic-release corticosteroid budesonide MMX 9 mg x 1 / d

**Oral/topical prednisolone (non-hospitalized patients)

***Infliximab, adalimumab, golimumab; if response to conventional therapy is inadequate or if patient is intolerant

****If response to conventional therapy is inadequate or if patient is intolerant. Vedolizumab is preferred to adalimumab.

[+] Topical 5-ASA may be used as sole medication if there is only distal colitis. ECCO recommends the usage of topical 5-ASA over the topical steroids for induction of remission in patients with active distal UC (weak recommendation, very low QoE).

[++]In case of left-sided colitis, the combination of oral and topical 5-ASA is recommended.

A corticosteroid sparing medication should be initiated if the patient has corticosteroid-refractory disease or has contraindication to corticosteroids (Raine et al. 2022). In addition, thiopurine treatment is often initiated at the beginning of the

disease if the patient has severe colitis. Mesalazine treatment is usually kept along with other medical agents (Sipponen 2018b).

Colitis is considered severe if the patient is suffering from general symptoms (fever, tachycardia) along with symptoms of either a newly diagnosed acute colitis or a relapse of a previously diagnosed colitis. The estimate of the severity is also based on the laboratory results (elevated inflammatory markers, anemia, hypoalbuminemia, significantly elevated fecal calprotectin) and findings in endoscopy. CT is recommended to rule out complications like perforation and toxic megacolon. Infections (cytomegalovirus, *Clostridium difficile*, enterohemorrhagic *Escherichia coli* (EHEC)) must also be excluded. The supportive care of severe colitis consists of rehydration, red blood cell (RBC) transfusions (if Hb < 80 g/l), nutrition (enteral or parenteral) and prophylaxis of thrombotic events. Medical treatment depends on the general state of the patient, the previously used medication, and the duration and the extent of the disease. (Färkkilä 2018b)

Third of patients suffering from acute severe UC (ASUC) fail to respond to conservative treatment and need to be evaluated for short term colectomy (Spinelli et al. 2022; Turner et al. 2007). According to Truelove and Witts criteria (S. P. Travis et al. 1996), after three days of treatment of ASUC, the indicators referring to failure (likelihood 85%) of sole corticosteroid treatment are a stool frequency more than eight per day, or a stool frequency between three and eight with CRP more than 45 mg/l. Further, if the stool frequency is more than three bloody stools per day on the seventh day of the treatment, the risk of ending up with colectomy in the following months is 40%. If the corticosteroid therapy fails to induce remission, the second line treatment, either IFX or cyclosporine, should be considered at the third day of treatment (Spinelli et al. 2022). Choy et al. (2019) stated that the colectomy-free survival with IFX salvage was 80% at three months and 70% at 12 months (Choy et al. 2019). However, there is no definitive difference between their efficiencies in RCTs (Narula et al. 2016) and the long-term outcomes are not different either (Szemes et al. 2020). In addition, no significant difference has been detected regarding adverse events and mortality between the groups. (Narula et al. 2016; Szemes et al. 2020). Consequently, the hospital experience and the further maintenance therapy should be considered when choosing between IFX and cyclosporine (Spinelli et al. 2022). The infliximab dosage is 5–10 mg/kg iv at 0., 1.–2., and 4.–6. weeks, although there still is lack of evidence to determine an optimal regimen of rescue therapy (Spinelli et al. 2022), and the dosage of cyclosporine is 2 mg/kg intravenously for seven to ten days or 4–5 mg/kg per orally divided into two dosages per day The target level of CyA in serum is 150–250 ng/ml. If the second line treatment fails, the third line medication may postpone a need for colectomy, but it is often associated with adverse effects and its usage is recommended only in specialized centers. However, if there is no significant improvement after seven days

of intensive treatment, a colectomy is highly recommended since the only thing that has been shown to increase the amount of perioperative complications is the delay of operative treatment (Spinelli et al. 2022). Tofacitinib is a promising novel immunosuppressant for steroid refractory ASUC, but the results of its efficacy and safety profile are preliminary and prospective studies are needed (Gilmore et al. 2022).

Table 8 b. The treatment of ulcerative colitis during remission according to ECCO guidelines (Raine et al. 2022) and Finnish recommendations (Sipponen 2018b)

Maintenance of remission

Severity	**medication**
Mild colitis	5-ASA ≥ 2.0g /d pr or/and po
Moderate colitis	5-ASA ≥ 2.0g /d po, pr AZA 2–2,5 mg/kg/d or MP 1–1,5 mg/kg/d anti-TNF-agent** vedolizumab*** tofacitinib**** ustekinumab*****
Severe colitis	AZA 2–2,5 mg/kg/d or MP 1–1,5 mg/kg/d anti-TNF-agent** vedolizumab*** tofacitinib**** ustekinumab*****

**Infliximab, adalimumab, or golimumab; for patients who responded to induction therapy with the same drug; strong recommendation, QoE high.
*** For patients who responded to induction therapy with vedolizumab; strong recommendation, QoE moderate. Vedolizumab rather than adalimumab is recommended.
**** For patients who responded to induction therapy with tofacitinib; strong recommendation, QoE moderate.
***** For patients who responded to induction therapy with ustekinumab; strong recommendation, QoE moderate.

Crohn's disease

For CD, three domains for medical treatment are presented: induction therapy, maintenance therapy and therapy of fistulizing perianal disease.

Table 9. The medical treatment of Crohn's disease (induction and maintenance of remission) according to ECCO guidelines (Torres et al. 2020) and Finnish recommendations (Sipponen 2018b).

Severity	medication	QoE / recommendation
mild active inflammation limited to terminal ileum and ascending colon	budesonide 9 mg	moderate / strong
moderate active inflammation	budesonide 9 mg* or systemic corticosteroid**	moderate / weak
severe active inflammation	budesonide 9 mg* or systemic corticosteroid**	moderate / weak
maintenance of remission	AZA 2–2,5 mg/kg/d*** or MP 1–1,5 mg/kg/d***	moderate / strong

*In case of ileocecal inflammation

**For corticosteroid dependent or resistant patients: AZA 2–2,5 mg/kg/d or MP 1–1,5 mg/kg/d OR MTX 15–25 mg/wk. im/sc or anti-TNFa-therapy (infliximab or adalimumab or certolizumab pegol; QoE: moderate, strong recommendation). Vedolizumab or ustekinumab is recommended if previous recommendations do not induce remission. Combination therapy with AZA or MP is recommended when starting infliximab to induce remission (QoE: moderate, strong recommendation).

***If the patient is steroid dependent. Second line treatment (in case of contraindication or intolerance to thiopurines): MTX 15–25 mg/wk; infliximab or adalimumab; vedolizumab; ustekinumab. If the patient achieved remission with anti-TNF agents, the maintenance treatment using the same treatment is recommended (QoE: moderate, strong recommendation).

ECCO Guidelines does not recommend an early introduction of thiopurine therapy in patients with newly diagnosed Crohn's disease for maintaining remission (QoE low, weak recommendation).

In case the response to thiopurines during maintenance of remission is inadequate, and anti-TNF therapy is planned, there is no evidence that continued use of immunomodulator therapy after starting anti-TNF therapy is beneficial. However, immunogenicity should be considered and individualized approach should be considered (Torres et al. 2020).

The usage of antibiotics is indicated for septic infections. However, in luminal CD, none of the several studies have demonstrated efficacy of antibiotics to induce clinical remission or mucosal healing compared to placebo. In addition, considering the potential adverse effects, antibiotics are not routinely recommended to treat luminal CD. (Torres et al. 2020)

In complex perianal fistulizing CD, infliximab is recommended as the first-line choice for the induction and maintenance of remission (QoE low, strong recommendation). Also, another anti-TNF-agent adalimumab may be used, but the QoE of its benefits is very low and so the recommendation is weaker than with infliximab. The evidence regarding the benefits of immunomodulators (AZA, MP, MTX) alongside anti-TNF-agents is insufficient. Consequently, no recommendation

for or against their usage in treatment of perianal CD exists at this point. However, thiopurine monotherapy should not be used for fistula closure in patients suffering from CD. Further, there is not enough evidence to recommend the use of either ustekinumab or vedolizumab for fistula healing. A sole antibiotic treatment is not recommended for fistula closure either (QoE low, weak recommendation). (Torres et al. 2020)

2.3.2 Surgical management

Ulcerative colitis

Approximately one fourth of patients suffering from UC need surgical intervention during their lifetime (Spinelli et al. 2022). The reasons for surgery are acute severe UC (ASUC) that does not respond to conservative treatment and threatens patient's life; chronic UC that responds poorly to conservative treatment or requires repeated corticosteroid cures and UC related cancer or high-grade dysplasia or repeatedly detected low-grade dysplasia. (Spinelli et al. 2022)

Medically refractory UC is defined as steroid dependent, and immunomodulator- or biologic-refractory disease, and it is the most common indication for UC related surgery. A half a year regimen of corticosteroids, and burden of adverse effects it causes, induce the operative treatment. Roughly, if the patient is aged less than 65 years, a proctocolectomy and ileal pouch anal anastomosis (IPAA) with protective loop-ileostomy should be performed (**Figure 3**). Although the rate of early and late complications is high, restorative surgery improves the Qol of most of the patients. If the patient is aged more than 65 years, or if there are multiple risk factors, a proctocolectomy and permanent end-ileostomy is preferred since it has lower morbidity and the QoL of the patient is still comparable to that of the patients with J-pouch (Spinelli et al., 2022). Pre-and post-operative treatment should consist of adequate nutritional corrections, iron supplementation for the patients with iron deficiency and prophylactic anticoagulation during hospitalization (Spinelli et al. 2022).

UC related cancer or high-grade dysplasia or repeatedly detected low-grade dysplasia indicate colectomy. The risk of CRC is increased by one percent after ten years of diseased history and increases one percent every year thereafter. The dysplastic changes are often multifocal and difficult to detect macroscopically during endoscopic investigation. The histologic diagnosis of dysplasia is also demanding because of contemporary inflammatory changes. Therefore, it is generally recommended that two pathologists specialized in gastropathology estimate the histology of the biopsies suspicious of UC related dysplasia (Färkkilä 2018a).

The operative procedure consists of either two or three separate phases: first, in the acute phase, a colectomy and temporary ileostomy are performed; second, in a stable phase after sufficient nutrition, an IPAA is constructed either with or without a loop-ileostomy. Finally, in the third phase, the loop ileostomy is secluded. If the rectum is only minimally affected, an option of an ileorectal anastomose (IRA) can also be considered in the second phase of the procedure. (Spinelli et al. 2022)

Nowadays, the robotic approach offers advantages in narrow pelvis with endowristed instruments and visual advantages. Robotic subtotal colectomy (STC) has longer operative time than laparoscopic STC, but it also has lower conversion rates, and the bowel function returns earlier. Robotic IPAA has proved with less blood loss and shorter hospitalization than laparoscopic IPAA. (Anderson and Grucela 2022)

The usual early complications (≤ 30 days after surgery) related to proctocolectomy or IPAA are bowel obstruction or ileus, wound infection, pouchitis, anastomotic leak, and fistula formation. Frequent late complications (> 30 days after surgery) are ileus, fecal incontinence, loss of pouch, chronic pouchitis, Crohn's like disease of the pouch and fistula formation. (Spinelli et al. 2022) According to a systematic review performed by Fradet et al. (2020), the early complications occurred in 0–53% of patients, and the late complications occurred in 3–58% of patients. (Fradet et al. 2020). The overall mortality rate after surgery is 0.1%. (Spinelli et al. 2022)

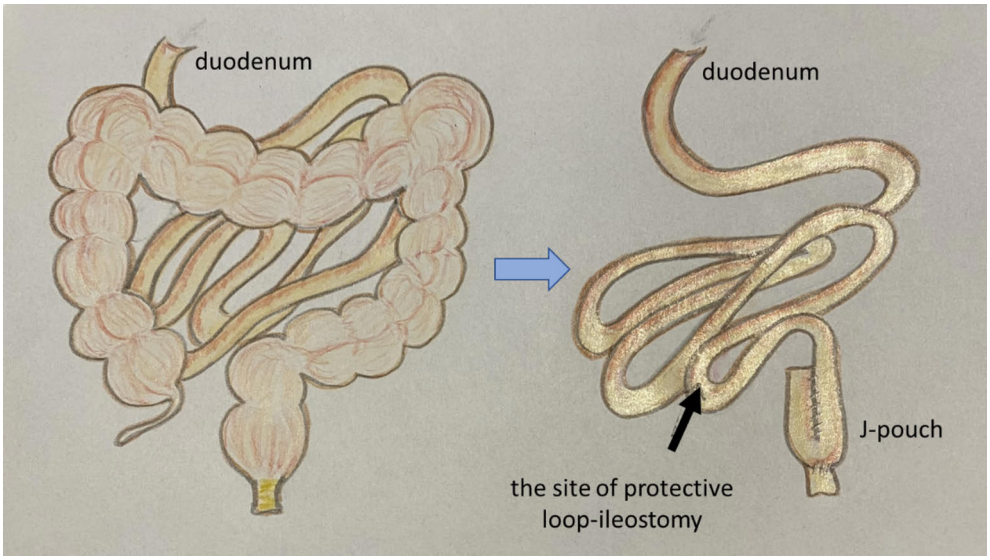

Figure 3. A schematic representation of the situation before panproctocolectomy (on the left) and after ileal pouch anal anastomosis (IPAA) reconstruction and stoma seclusion (on the right). The site of the protective temporary loop-ileostomy is sketched by an arrow on the right. (An own illustration)

Crohn's disease

Approximately half of the patients suffering from CD experience one or more surgical procedures during their lifetime. Surgical management of CD can be divided into the management of abdominal disease and perineal disease. In chronic and complex situations, a multidisciplinary approach and careful pre- and perioperative optimization leads to best results. Considering especially CD, correct nutrition, iron supplement, drug management, minimally invasive techniques, bowel- and sphincter-sparing techniques, and thrombosis prophylaxis are especially important. (Adamina et al. 2020)

For patients with intra-abdominal abscesses accompanied by sepsis, a treatment with antibiotic therapy and percutaneous image-guided drainage (PD) is recommended prior to abdominal surgery. In the case of large well-defined intra-abdominal abscesses, percutaneous drainage is also recommended as primary treatment. PD allows for nutritional optimization thereby improving outcome of a possible surgical treatment following the PD. After the drainage, medical treatment may be considered. However, if conservative treatment is not successful, a low threshold for surgery is recommended. An emergency operation without preceding percutaneous drainage and control of septic situation leads to higher rate of complications and stoma (Adamina et al. 2020; He et al. 2015).

If the patient is on a prolonged high dose steroid cure (prednisolone 20 mg daily or equivalent > 6 weeks), a staged procedure with a temporary stoma should be considered. Preoperative treatment with infliximab, vedolizumab or ustekinumab does not increase the risk of post-operative complications (Adamina et al. 2020; Law et al. 2018; Xu et al. 2019).

In case of acute small bowel obstruction, without ischemia or peritonitis, conservative management including nasogastric decompression and rehydration, is the preferred option. If there is active inflammation, iv steroids should be considered to diminish the edema in bowel wall. However, deferred surgery is recommended. If a resection is performed, an ileocolic side-to-side anastomosis or stapled small-bowel anastomosis is preferred over an end-to-end anastomosis since they have been proved to result in less postoperative complications than an end-to-end anastomosis (Adamina et al. 2020; Guo et al. 2013). Strictureplasty is a safe procedure and may be preferable to resection of long segments. For stenotic segments up to eight cm Heineke-Mikulicz technique if recommended (**Figure 4**). Longer or multiple strictures should be treated with Finney and side-to-side isoperistaltic techniques. Also, endoscopic balloon dilatation is an eligible choice when treating strictures less than five centimeters in terminal ileum. However, recurrence after the dilation is common, and need for surgery is frequent in five years. (Adamina et al. 2020; Ambe, Campbell, and Cagir 2012). In colonic strictures, a segmental colectomy is recommended if only a single segment of colon is affected. Multiple strictures in

different segments of colon indicate a subtotal colectomy as general. Strictureplasty of colon is not recommended (Bemelman et al. 2018). In the case of chronic refractory CD, a defunctioning stoma may postpone or avoid the need for colectomy. If there is no history of perianal disease, a proctocolectomy with IPAA can be considered in selected patients with pancolonic CD, albeit there is a high risk of pouch failure. (Adamina et al. 2020)

For overall decision-making, ECCO recommends the following lines: laparoscopic surgery is preferred as the primary approach if appropriate expertise is available. Primary anastomosis may be constructed if anti-TNF therapy, vedolizumab or ustekinumab is ongoing given that the other risk factors have been considered. Anyhow, a temporary stoma may be preferred option if a high dose (> 20 mg prednisolone or equivalent) corticosteroid cure is used for six or more weeks. (Adamina et al. 2020; Huang et al. 2015; Rizzo et al. 2011).

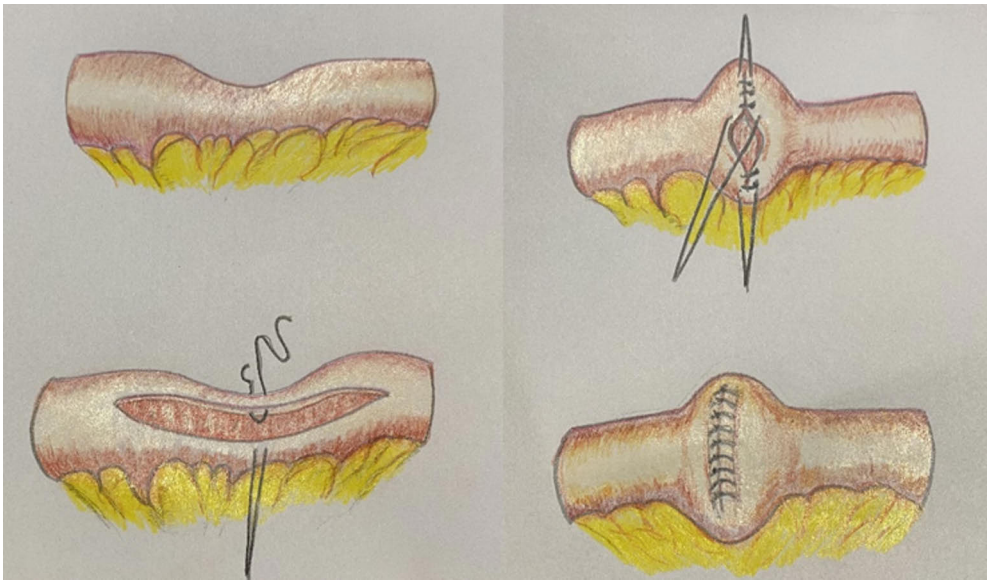

Figure 4. Heineke-Mikulitz stricturoplasty. A longitudinal incision is made up 2 cm over the stenotic segment on the antimesenteric side of the bowel. Enterotomy is sutured in transverse fashion. (An own illustration)

In perineal disease with high fistulas with a single internal opening, there has been three standard options for treatment: a chronic seton drainage, a medical approach by anti-TNFa antibodies, and a surgical closure with or without anti-TNF induction treatment. According to a recent study, chronic Seton treatment was associated with significantly higher re-intervention rate as compared to sole anti-TNFa-treatment and combined anti-TNFa-treatment followed by surgical closure.

Consequently, a chronic Seton should not be recommended as the sole treatment for fistulas in CD. Anyhow, the preference of the patient should be considered when making decision. (Wasmann et al. 2020)

The observational studies prefer a combined medical/surgical treatment for septic patients suffering from complex perianal fistulae, but there are no prospective studies that compare isolated or combined medical and surgical treatments. CD related fistulae that penetrate from anus or rectum to genital area, are very complex and they are recommended to be treated by an experienced multidisciplinary team. From the selection of different procedures, a ligation of an intersphincteric fistula (LIFT) and advancement flaps are eligible options for treating complex perineal fistulae in CD patients. Fibrin glue has only a limited efficacy and fistula plugs should not be used routinely (Adamina et al. 2020). An adipose-derived stem cell therapy (SCT) may be an effective treatment for complex perianal fistulae in patients with CD and stem cells that are autologous have better tolerability and they are safer to use. However, the evidence of both the fistula closure and the effect of SCT to cause no difference in number of adverse events is of low-certainty (El-Nakeep et al. 2022). Also, a diverting stoma is an option to control pelvic sepsis and symptoms from complex perineal disease that is refractory to medical or surgical interventions. (Adamina et al. 2020)

2.3.3 Follow-up

Ulcerative colitis

The aims of follow up for patients with UC are health maintenance and CRC surveillance since the cumulative risk of CRC in extensive colitis is 5% after twenty years of disease history (Al Bakir et al. 2022). The efficacy of medical treatment requires an individually tailored contact to gastroenterological outpatient clinic (doctor or IBD nurse) in forms of reports of general well-being and safety laboratory tests based on patient's medication.

The UC patients with over 30% of the colon involved should have surveillance colonoscopy every 1–5 years starting eight to ten years after the diagnosis. The surveillance interval depends on the CRC risk level based on clinical risk factors like extension and inflammatory activity of colitis, presence of pseudopolyps or dysplasia, family history of CRC and patients comorbidities (Maaser et al. 2019). In case of sole proctitis there is no increased risk for CRC and consequently those patients should follow the national CRC screening guidelines. The patients with both UC and PSC have five times higher risk for CRC and the surveillance is recommended to begin at the time of diagnosis and continue annually. (Maaser et al. 2019; Ungaro et al. 2017; Färkkilä 2018a)

The endoscopic observation should be performed as chromoendoscopy (0.2% indigocarmin), and the disease should be in remission at the time of examination. If dysplasia is detected, the PAD should be based on the statement of two pathologists specialized in gastropathology. Even though adenocarcinoma may develop without preceding dysplasia, established dysplasia is still the best sign of the risk of cancer. Detected low grade dysplasia (LGD) will proceed into high grade dysplasia (HGD) in 16–54% of the cases. HGD is associated with 40% risk of metachronic adenocarcinoma, and consequently a colectomy is recommended.

Crohn's disease

In CD, the follow-up strategy aims for sustained remission and prevention of complications. The early introduction of immunosuppression, and for high-risk patients, the combination therapy accompanied by frequent/personalized controls of inflammation, are essential to achieve the target. In a case of an extensive involvement of colon (> 30–50% of the colonic surface) there is higher risk for CRC and these patients should follow the guidelines for colorectal neoplasia surveillance like patients with UC (Torres et al. 2017). The follow-up takes place at gastroenterological outpatient clinic (doctor or IBD nurse) with reports of general well-being, laboratory tests, required endoscopy and imaging studies.

Since the therapeutic repertory for IBD is expanding, it would be beneficial to find suitable biomarkers that could predict the outcome of the disease and response to the personally selected therapy.

2.4 Prognosis

Ulcerative colitis

UC is most often diagnosed between ages 20 and 30 years. At the onset, 14–35% of patients have pancolitis, 16–45% have left-sided colitis and 30–60 % have proctitis. After five years from the onset, the disease has progressed proximally in 10–19% of the patients, and at ten years in up to 28% of the patients (Ungaro et al. 2017). The disease may have a widely variable course of disease and UC may worsen the quality of life in several ways, but it does not affect the prognosis of living (Sipponen 2018a). If the disease onset is after 60 years of age, the course of the disease often appears milder compared to younger patients (Ungaro et al. 2017).

Crohn's disease

Over 80% of the patients suffering from CD get the diagnosis before age of 40. The pediatric form of CD is usually more extensive, and the clinical course is more

severe. The location of the disease usually remains the same (Torres et al. 2017), but the disease often progresses over time and causes stricture formation and fistulizing from the bowel (Freeman 2014; Lichtenstein 2010). Within eight years from diagnosis, approximately 20% of the patients experience relapse every year, 67% of the patients have variable clinical course of disease with years in remission and 13% of the patients experience no relapses (Gajendran et al. 2018). Within ten years of diagnosis, about half of the patients develop complications that require surgical intervention, and the need for repeated intervention is recurrent in 30% of the cases (Torres et al. 2017). Altogether, CD is a chronic disorder and albeit the periods of remission may last for decades, relapses may still appear (Freeman 2014).

3 Aims

The aim of this thesis was to develop new tools for diagnostics, follow-up and prognostics of UC and CD.

The specific aims were as follows:

I: To evaluate the value of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET/MRI in the diagnostics of IBD and further compare the data obtained using PET/MR imaging to histological findings.

II: To evaluate the potential of epithelial keratin 7 as a novel IBD biomarker and analyze pathological changes and clinical characteristics potentially related to K7 expression in colonic epithelium.

III: To explore the association between K7 expression and epithelial inflammation in distinct segments of colon and terminal ileum and to estimate the prognostic value of K7 in the clinical course of IBD by a three year follow up study.

4 Patients and Methods

4.1 Patients

4.1.1 Patients with drug responsive IBD (Study I)

Ten patients with known or suspected IBD were recruited (**Figure 5.)** prospectively either from gastroenterology outpatient clinic or from a hospital ward at Turku university hospital between years 2014–2015. They had symptoms referring to either a relapse of UC or CD, or an undiagnosed IBD. Exclusion criteria were pregnancy, diabetes mellitus, aged less than 18 years and mental retardation. (Clinicaltrials.gov NCT02364973)

4.1.2 Samples of patients with non-drug responsive IBD, mRNA samples and additional patients with drug responsive IBD (Study II)

Formalin fixed; paraffin embedded (FFPE) patient samples (56 pieces) were obtained from Auria Biobank (Turku, Finland). 15 UC and 11 CD samples had been harvested from resected intestine specimen after colectomy or ileal resection. The samples of collagenous colitis (CC) (8) and lymphocytic colitis (LC) (10), and 12 control biopsies were harvested during ileocolonoscopy. The exclusion criteria for the control biopsies were inflammatory and neoplastic intestinal diseases. The medical history of the patients, relevant to IBD, was filed and the information was stored in encoded format and kept anonymous. The research project was authorized by the Auria Biobank's Scientific Steering Committee (AB17- 6901) and Hospital District of Southwestern Finland (T05/032/19).

Data on the K7 mRNA expression in colon of IBD patients was acquired from publicly available biogps.org gene annotation portal.

Altogether 15 patients with drug responsive IBD with terms mentioned above were included into the study II since the recruitment process had been ongoing until year 2018 (**Figure 5.**).

4.1.3 Patients of the three-year follow-up study (Study III)

16 patients (**Figure 5.**) were recruited according to the protocol described in Study I between years 2014 and 2018. 14 patients met the criteria of follow up and they were included in Study III. The inclusion criteria were available medical history for three years after the enrollment and the patient's commitment to recommended treatment and follow-up. The patient characteristics are shown in **Table 10**.

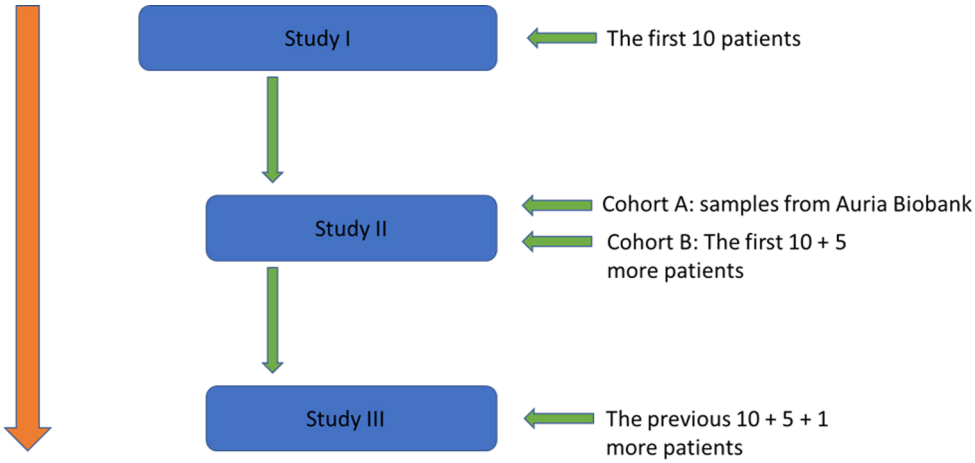

Figure 5. The flow chart of patient recruitment. All the recruited patients went through the same protocol including ileocolonoscopy ja PET/MRI-imaging. The first 10 patients formed the Study I cohort. Five more patients were recruited, and all the 15 patients formed the Study II Cohort B. Thereafter one more patient was recruited and fulfilled the follow-up criteria. The 16 patients formed the Study III group that was followed for three years after the enrollment. (An own illustration)

Table 10. Patient characteristics of Study III.

Sex	Age (years)	F-Calpro (µg/g)	UC/CD	Endoscopic diagnosis	Medication	Severity of the disease during the 3-year follow up
F	39	>2000	UC	proctitis	prednisolone + azathioprine 150 mg	severe
F	19	1400	UC	pancolitis	methylprednisolone + azathioprine 25 mg x 2 + 5-ASA 1600 mg x 2	severe
F	28	>2000	UC	pancolitis	prednisolone + 5-ASA 1600 mg x 2	mild/moderate
M	27	>2000	CD	jejunitis, ileitis	methylprednisolone	severe
F	19	>2000	UC	pancolitis	prednisolone + 5-ASA 1600 mg x 2	severe
F	19	>2000	I	pancolitis	methylprednisolone + 5-ASA 2 g + 1 g	severe
F	31	>2000	UC	pancolitis	methylprednisolone + azathioprine 150 mg + 5-ASA 1600 mg x 2	severe
F	30	1294	CD	ileitis, sigmoiditis	methylprednisolone + azathioprine 25 mg x 2	mild/moderate
F	36	393	CD	ileitis	prednisolone + mercaptopurine 50 mg x 1	mild/moderate
F	54	557	CD	ileitis	budesonide 9 mg x 1 + azathioprine 25 mg x 1	mild/moderate
M	37	5860	UC	pancolitis	prednisolone + azathioprine 25 mg x 2	severe
F	19	3178	CD	ileitis, segmental colitis	methylprednisolone	mild/moderate
F	85	45	CD	segmental colitis	budesonide 9 mg x 1	mild/moderate
M	79	607	CD	jejunitis, ileitis	budesonide 9 mg x 1	mild/moderate

F = female, M = male, F-calpro = fecal calprotectin, QoL = quality of life, UC = colitis ulcerosa, CD = Crohn's disease, I = intermediate colitis, N =normal. 5-ASA = mesalazine. From the original publication III.

4.2 Methods

4.2.1 Recruitment

Prior to participating in the study, all patients were informed about the course and the details of the study. They had a minimum of three days of time to consider their decision and they signed an informed consent, with the protocol approved by the

ethics committee of the Hospital District of Southwest Finland (66/1801/2014; §264).

4.2.2 Ileocolonoscopy, laboratory tests and clinical state estimation

The patients had ileocolonoscopy performed by senior or junior gastroenterologist. Routine biopsies were taken at least from terminal ileum, ascending colon, transverse colon, descending colon, and rectum. Laboratory markers of inflammation and relevant to treatment were routinely recorded. To estimate the clinical state of the patient the CCAI (Walmsley et al. 1998) or the CDAI (Best et al. 1976; Sostegni et al. 2003) were calculated provided that all the relevant data was available.

4.2.3 Positron emission tomography / magnetic resonance imaging (Study I)

The PET/MRI was performed as closely as possible before or after the ileocolonoscopy. The timetable of the imaging study depended on the available imaging slots which had to be scheduled before the individual recruitment process.

The abdomen and pelvis of each patient was scanned with a 3T Philips Ingenuity TF PET-MR scanner in the national PET Centre. The patient fasted for at least six hours and had 1.2 liter 3% mannitol dilution in 45 minutes as intestinal contrast medium prior to imaging. At the beginning of the imaging algorithm the patient was injected intravenously ^{18}F-FDG at the dose of 4 MBq/kg. During the procedure the patient received intravenously 10 mg hyoscine butylbromide to reduce bowel movements and 0,1 mmol/kg gadoterate meglumine as intravenous contrast agent. Images of abdomen and pelvis were acquired in prone position**.**

The MR enterography was performed first and the acquisition time was 50 minutes. The PET imaging was performed after MRI sequences to allow for FDG tracer uptake. The PET series covered abdominal area from diaphragm to groin and transaxial field of view was 576 mm. The PET imaging was conducted in a single bed position and with ten minutes acquisition time. The images were reconstructed using a default reconstruction algorithm and a 3D ordered subset iterative TOF reconstruction technique with 3 iterations and 33 subsets. Using 144 × 144 matrices, the final voxel size was $4 \times 4 \times 4$ mm^3. The dose activity, the weight of the patient and the time from the injection were considered when computing standard uptake values. All reconstructions included the necessary corrections for image quantification: attenuation, randoms, scatter, dead-time, decay, and detector normalization.

4.2.4 Interpretation of PET/MRI scans (Study I)

PET/MRI scans were interpreted by an abdominal radiologist with several years of experience in both MRI and PET fusion imaging. The analyses were performed using AW workstation version 2.0 and Carestream PACS.

In each patient the SUV_{max}'s were measured and reported in six regions of intestine: terminal ileum, ascending colon, transverse colon, descending and sigmoid colon and rectum. The VOI was manually placed in the bowel region containing the voxel with SUV_{max}. If there was no visible activity (i.e., the activity was at the level or below the blood pool level) the VOIs were obtained constantly in the same manner from the bowel region to be analyzed.

4.2.5 Immunohistochemistry and pathological evaluation (Study I–III)

HE-staining was performed according to the routine protocol of histology laboratory of the Hospital District of Southwest Finland, and the inflammation activity in each histological sample was graded into four classes by a pathologist specialized in gastrointestinal pathology: no activity; mild (ad cryptitis); moderate (crypt abscesses); and severe (erosions/ulcers) according to ECCO guidelines (Magro et al.).

For the K7 and keratin 20 (K20) expression analyses (Study II and III) the immunostaining was conducted from 5 µm rehydrated tissue sections with antibodies to K7 and K20. The visualization was carried out using anti-mouse antibody and 3,3'-diaminobenzidine (DAB) as a chromogen and hematoxylin as counterstain.

The slides were scanned to digital images using Pannoramic 1000 (3D HISTECH) digital slide scanner. The expression of K7 in epithelial cells was measured using mean optical density of cellular DAB staining, and it was followed by quantification of K7 positive cells. The bioimage analysis was performed using QuPath software version 0.2.3 for Windows.

The regions of interest (ROIs) consisting of selected epithelial cells were drawn manually. Epithelial ROIs contained at least 1000 cells per single biopsy, and ROIs including full crypts were prioritized. Each epithelial ROI was identified and annotated by QuPath cell detection tool. Each cell in ROI was classified from 0 to 3 based on the measured K7 expression using QuPath positive cell detection tool (0: no expression; 1: low expression; 2: moderate expression, and 3: high expression). The lowest threshold was based on barely visible cytoplasmic DAB staining and the upper value on intensity surpassing respective nuclear hematoxylin staining. The cells classified as 1–3 for K7 are referred as K7 positive (K7+) and the cells classified 0 for K7 as K7 negative (K7-) respectively.

The pathological analysis in cohort A was carried out from HE stained samples by an analyst not aware of K7 expression. Inflammatory activity was graded into four classes as in Study I. The mean top to bottom crypt length was measured as an average of at least four crypts per sample. Presence of ulcers, erosion, or granulomas (including microscopic granulomas) were assessed. Epithelial changes including crypt deformation, atrophy and crypt loss were graded to three classes: low, modest, and severe. Neutrophil content in epithelium was graded into four classes: none, topical, cryptitis and crypt abscesses. Neutrophil presence in lamina propria was graded to three classes: none, modest and severe infiltration.

4.2.6 Statistical analysis

Study I: According to a consultation of a professional statistician, the relationships between SUV and independent variables (inflammation activity, region of biopsy and the time between endoscopy and PET/MRI) were studied with descriptive statistics and repeated measurements ANOVA. Continuous variables were characterized using means and standard deviations (SD) for normally distributed variables and median and range of values for variables that were not normally distributed. Normality of variables were evaluated visually and tested with Shapiro-Wilk test. Logarithmic transformation was used to make SUV variable normally distributed. Main statistical analyses for SUV were performed using repeated measurements ANOVA. Measurements from different biopsy regions for the same person were considered repeated measurements. Region of biopsy, inflammation activity and time between endoscopy and PET/MRI were included in all models. The Tukey-Kramer -method was used to correct the p-values in pairwise comparisons. Results are presented using model-based means with 95% confidence intervals (95% CI), which are transformed back to original scale. Statistical significance level was set at 0.05 in all tests (two-tailed). The analysis was performed using SAS system, version 9.4 for Windows (SAS Institute Inc., Cary, NC, US).

Study II: The difference between more than two groups was measured using Kruskal–Wallis's test, followed by Dunn's test. The difference between two variables was evaluated using the Mann-Whitney test. The correlation between two variables was studied using linear regression analysis.

Study III: The relationship between the percentage of K7-positive cells and inflammatory activity was examined with one-way analysis of variance separately for each region of biopsy. For K7-positive cells square root transformation was used to fulfill assumption of normally distributed residuals. To study the association of the severity of illness (mild/moderate and severe) and the expression of K7 at the time of enrollment, the K7 expression in ascending colon and descending colon, and in the biopsy site with the greatest K7 expression was evaluated using log-binomial

model, including one K7 feature at a time. To illustrate the percentage of epithelial cells that express K7 in different grades of inflammation four boxplots were drawn (Figure 1a–d), and the Spearman correlation coefficient was calculated to study association between K7 and K20 expressions. The data analysis for this paper was performed by a professional statistician and generated using SAS software, Version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA).

5 Results

5.1 PET/MRI in the diagnosis and follow-up of IBD (Study I)

Ten patients were included in this prospective study. Five of them suffered from UC, three patients had CD and one had intermediate colitis. One patient had normal findings in both histological samples and imaging studies and the inflammation detected in terminal ileum at the time of recruitment was likely to be caused by appendicitis. Due to technical problems PET data was not obtained from one subject. The F-Calpro measured in early phase of the symptoms was significantly (>500 ug/g) elevated in all the patients. The CCAI indicated mild symptoms in four patients (CCAI > 5) and moderate symptoms (CCAI > 9) in two UC patients. The CDAI suggested relapse (CDAI > 150) in one patient with CD and no relapse in two patients with symptoms of CD. The patient with both the normal histological samples and the normal imaging studies had no active IBD according to CDAI test.

The time gap between the endoscopy and the PET/MR-imaging varied between three and 19 days. In endoscopy terminal ileum was reached in all the patients. The histology in biopsies was used as reference standard when the severity of the disease was graded. The inflammation activity in each biopsy was graded into four classes: no activity, mild (ad cryptitis), moderate (crypt abscesses) and severe (erosions/ulcers) according to ECCO guidelines. (Magro et al. 2013)

In total fifty samples of intestine were included in this semi-quantitative analysis. There was no inflammatory activity in 28 samples, and inflammation was detected in 22 samples. The lowest SUV detected was 0.7 in a bowel segment with mild inflammation of UC and the highest SUV was 7.2 in a segment with moderate inflammation of CD (**Figure 6**). The highest SUV calculated in severe inflammation was 6.2. **Figure 7.** shows MR-, PET- and fused MRI/PET -image of a young female patient suffering from UC.

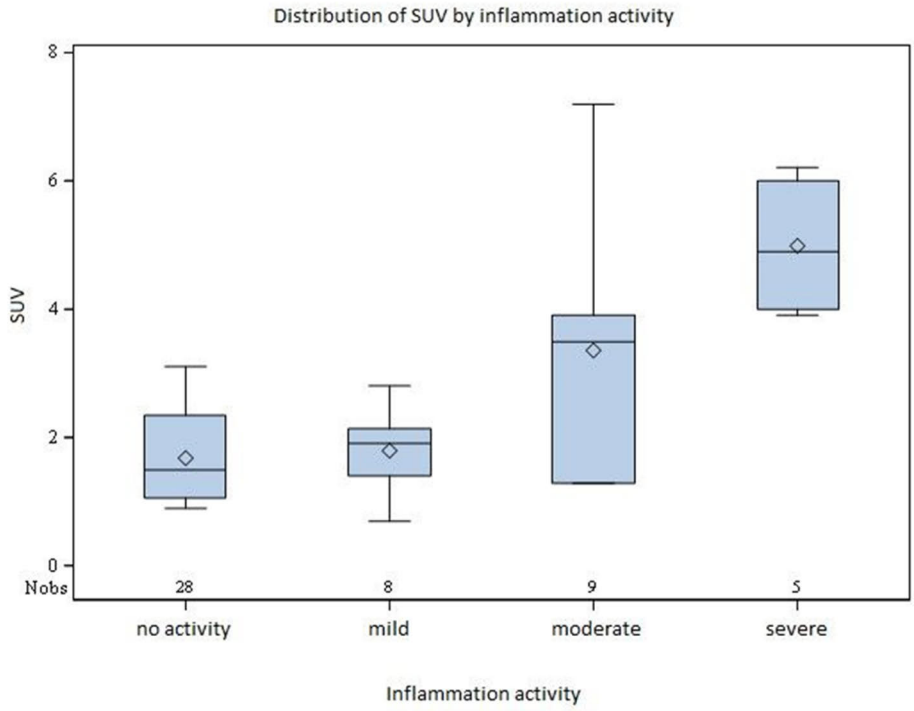

Figure 6. The distribution of detected SUV_{max}s in bowel segments graded according to histological activity of inflammation. N_{obs}, number of observations in each class of inflammation; SUV, standard uptake value. From the original publication I with permission from SAGE publishing.

The relationship between SUV and independent variables (inflammation activity, region of biopsy and the time between endoscopy and PET/MRI) was studied with repeated measurements ANOVA. The analysis showed that both the inflammation activity (p=0.008) and the region of biopsy (p=0.015) had statistically significant association to SUV while the time between endoscopy and PET/MRI did not seem to affect SUV (p=0.993).

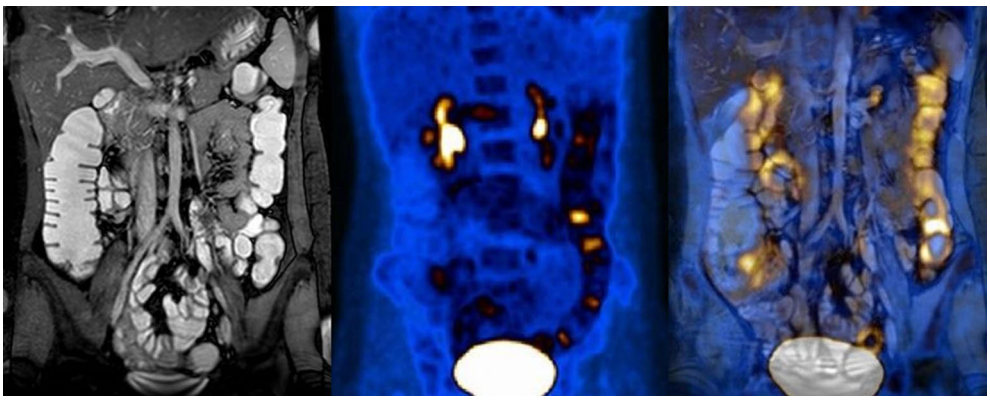

Figure 7. A coronal MR image (on the left), a PET image (in the middle) and a fused PET/MR image (on the right) of bowel of a 19-year-old woman suffering from UC. The inflammation was estimated moderate by endoscopic visualization and histological analysis showed moderate to severe inflammation activity in bowel mucosa. In the PET and the PET/MR images the moderately and severely inflamed segments point out as bright yellow light. The SUV_{max} of each bowel segment was calculated between 3.6 and 6.2. From the original publication I with permission from SAGE publishing.

The subsequent analyses showed that SUV_{max}'s calculated from severely inflamed bowel segments differed statistically significantly from SUV_{max}'s calculated from mild inflammation (p=0.007) and from normal bowel wall (p=0.006). SUV_{max}'s that were calculated from moderate inflammation raised above baseline, but 95% CI was wide and there was no statistically significant difference compared to normal (p=0.083) or mildly inflamed bowel (p=0.093). SUV_{max}'s from mild inflammation did not differ from the baseline (p=0.988). (**Table 11.)**

Table 11. Model based means of SUV_{max} in different inflammation classes and the p-values of pairwise comparisons.

Inflammation activity	SUV_{max}	95% CI	Severe	Moderate	Mild
No activity	1.6	1.2–2.2	0.006*	0.083	0.988
Mild	1.5	1.0–2.2	0.007*	0.093	-
Moderate	3.0	1.9–4.7	0.452	-	
Severe	4.3	2.6–7.0	-		

SUV_{max} = Model based estimated mean (transformed back to original scale) of SUV in inflammation classes. 95% CI = 95% confidence interval of SUV_{max} (transformed back to original scale). From the original publication I with permission from SAGE publishing.

5.2 Keratin-7 expression is associated with severe form of IBD (Study II)

Two cohorts were included into this study. Cohort A consisted of 26 colonic samples from Auria Biobank that represented tissue samples of patients with non-drug responsive IBD that had ended up in colectomy or other operative treatment. This cohort included also colonic biopsies from eight patients suffering from collagenous colitis (CC) and biopsies from ten patients suffering from lymphocytic colitis (LC). Also, twelve control patients were included and the samples in this group were biopsies and these patients had no inflammatory or malignant bowel disease. The 15 patients in cohort B suffered from IBD that was drug responsive. The demographics of the patients are represented in **Table 12**.

Table 12: Demographics of cohorts A and B.

Group	Number	Sex F/M	Age at harvest (median)	Years from onset to harvest (median)	# of drug receivers: Mesalazine	Corticosteroids	TNFα blockers	Azathioprine	Sulfasalazine	Methotrexate	Other
Cohort A											
Control	12	6/6	34–80 (57)	–	1	0	0	0	0	0	
UC	15	6/9	15–85 (47)	0–29 (6)	12	14	5	6	2	0	***
CD	11	6/5	17–78 (35)	0–22 (6)	5	5	3	3	1	2	
CC	8	6/2	40–85 (78)	0–9 (0)	2	3	0	0	1	0	****
LC	10	5/5	63–88 (78)	0–4 (0)	0	0	0	0	0	0	
Cohort B											
UC	7	6/1	19–39(28)	0–14(1)	5	7	0	4	0	0	
CD	8	5/3	20–85(34)	0–12(0)	1	8	0	3	0	0	++

* only 4 values available
** only 2 values available
*** single users of allopurinol, ursodiol and anabolic steroids
**** single users of leflunomide and hydroxychloroquine
+ only 5 values available
++ single user of mercaptopurine
UC = ulcerative colitis, CD = Crohn's disease, CC = collagenous colitis, LC = lymphocytic colitis. Cohort A samples were obtained from Auria Biobank and Cohort B samples consisted of mucosal biopsies acquired during colonoscopy from the patients of clinical trial no NCT02364973.The numbers in the medical treatment columns indicate the number of patients receiving this medication within one month prior to tissue or biopsy harvest. From the original publication II with permission from Nature Springer publishing.

In both cohorts, K7 was expressed in the colonic epithelium of patients suffering from UC and CD, but not in healthy colon. LC samples had similar negligible K7 levels as controls while some of the CC samples showed a few focal K7 expressing areas, failing to reach statistical significance (**Figure 8a**).

In cohort B, the patients with UC had a significantly higher percentage of K7+ epithelial cells compared to CD (**Figure 8b**). When single biopsies were studied a statistically significant difference was only seen between inflamed and non-inflamed samples as K7 expression did not increase along with severity of inflammation (**Figure 8c**).

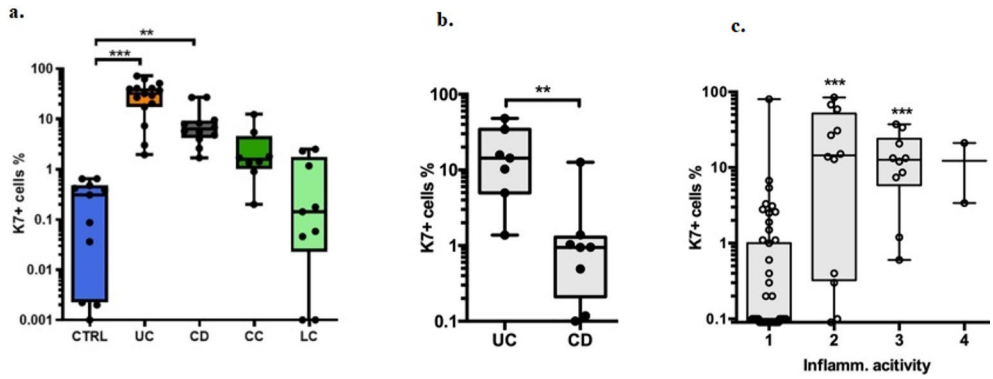

Figure 8. **(a)** The percentage of K7-positive cells in colectomy specimen of patients suffering from non-drug-responsive ulcerative colitis (UC) or Crohn's disease (CD), and in biopsies of the control group (cohort A). **(b)** The percentage of K7-positive cells in colonic epithelium in the biopsies of patients with drug-responsive UC or CD (cohort B). Statistical difference is based on Dunn's multiple comparison test. **P<0.01, ***P<0.001. **(c)** The percentage of K7-positive cells in biopsies vs. inflammatory activity classes in the cohort of drug-responsive patients. 1= no inflammatory activity, 2 = mild activity, 3 = moderate activity, and 4 = severe activity. The percentage of K7-positive cells in biopsies with active inflammation differs statistically significantly from the biopsies with no inflammation. The inflammatory activity does not affect the expression of K7 in epithelial cells in inflamed areas. Modified from the original publication II with permission from Springer Nature.

When Cohort B results were compared to controls from Cohort A, both UC and CD patients in Cohort B had significantly increased percentage of K7-positive epithelial cells (18.4% for UC and 2.2% for CD vs 0.27% for control). These values were generally lower than mean values measured in Cohort A for colectomy patients (32.2% for UC and 9.2% for CD) (**Figure 8a**).

The highest focal K7-positive cell concentrations were found in samples with most severe epithelial changes like crypt loss, atrophy, granulomas, erosion, and ulcers. Crypt length correlated positively with the number of K7-positive cells in CD, but not in UC samples. There was no correlation between the number of

neutrophils and the percentage K7-positive cells, and in concordance with that, the amount of fecal calprotectin had no correlation to K7-positivity. The percentage of K7-positive cells did not correlate with either patient age, gender, BMI, or the time from disease onset.

The regulation of K7 expression was also investigated by using a publicly available transcriptome database (human gene atlas, E-GEOD-14580 and E-GEOD-14580) (Arijs et al. 2009; Galamb et al. 2008; Su et al. 2004). According to the analysis of the provided genomic data, K7 mRNA is not expressed at detectable levels in samples of healthy intestine, but K7 mRNA expression is significantly increased in the colon of IBD patients.

5.3 Keratin-7 expression varies based on the anatomical location in colon and it does not predict the prognosis of IBD after three years follow-up (Study III)

Six patients out of sixteen experienced UC, nine patients had CD, and one had intermediate colitis. For grading of the severity of IBD, histology was used as the reference standard as in previous studies. In total, 67 biopsies of bowel were included in this analysis. The relationship between the percentage of the K7-positive epithelial cells and the independent variables (inflammatory activity and the region of the biopsy) was studied with non-parametric Friedman test. The analysis showed that the inflammatory activity had statistically significant association to K7-expression in ascending ($p=0.004$) and descending colon ($p=0.0004$), but inflammatory activity did not affect K7-expression in ileum, transverse colon, or rectum **(Table 13).** The IBD-related K7 expression was not associated to specific cell subtypes, and it did not have statistically significant impact on the K20 expression in epithelial cells ($p=0.07$).

Table 13. The association between inflammation and K7 expression in distinct sites of the lower gut. The inflammatory activity, number of biopsies in each class, median percentage of K7(+) cells in sites of biopsy from ileum to rectum, and statistical significance of the difference (p-values) in K7 expression between normal and inflamed epithelial cells in terminal ileum and distinct sites of the lower gut. * P<0.05 was considered statistically significant. From the original publication III.

Biopsy site	Represented inflammatory activity	Number of biopsies	K7 positive epithelial cells median%	p-value
ileum	1	11	0.1	
	2	4	0.2	0.87
ascending colon	1	8	0.6	
	2	3	30	0.004*
	3	4	17	
transverse colon	1	8	1.1	
	2	2	13	0.14
	3	1	8.6	
	4	1	21	
descending colon	1	8	0.9	
	2	2	63	0.0004*
	3	5	7.4	
rectum	1	8	0.5	
	2	1	13	0.23
	4	1	3.4	

The prognostic value of K7 in the course of IBD was investigated by determining the K7 expression level (percentage) at the time of enrollment and by following the course of the disease for three years. The patients were divided into two groups based on the severity of the disease. Seven patients had mild or moderate disease, and seven patients had severe disease. One patient did not meet the criteria for follow-up because of poor commitment to treatment and follow-up, and the other one had had colectomy before enrollment and biopsies from colon were not available.

The severity of the disease was compared to the expression of K7 in ascending colon, descending colon, and in that colonic segment where the expression percentage was greatest in each patient. As a result, the severity of the disease during the following three years was not related to expression of K7 at the time of enrollment (colon ascendens p=0.291, colon descendens p=0.589, the colonic segment of the greatest K7 expression p=0.618).

The results of the Studies I–III:

I: The inflammation activity in the mucosa ($p = 0.008$) and the region of biopsy ($p = 0.015$) have significant effect on the SUV obtained from the bowel wall. SUV's that were obtained from areas of severe inflammation activity emerged significantly from the background ($p = 0.006$).

II: K7 was expressed in the colonic epithelium of patients suffering from UC and CD, but not in healthy colon. The expression was the most pronounced in samples with drug-resistant colitis.

III: The inflammatory activity had statistically significant association to K7-expression in ascending ($p=0.004$) and descending colon ($p=0.0004$), but it did not affect K7-expression in ileum, transverse colon, or rectum. The severity of IBD during the three-year follow-up was not related to the expression of K7 at the time of enrollment.

6 Discussion

The study I was the first prospective study using ^{18}FDG-PET/MRI where the correlation of histological inflammation and SUV_{max} of the bowel wall was investigated on patients suffering from IBD, and it suggested that PET/MRI is a promising method to noninvasively detect especially severe inflammatory bowel lesions. The study II was the first study to show that IBD induces neo-expression of K7 in the epithelial cells in colonic mucosa, and that the increase of K7 in epithelial cytoplasm indicated epithelial damage and was associated with poor drug-response. The study III pointed out that K7 expression is not similar in all parts of colon, and it suggested that the function of colonic mucosa in not homogenous. K7 did not predict the disease severity after three years of the analysis.

6.1 The performance of combined PET/MRI provides useful information for the diagnostics and follow-up of IBD

In the Study I, PET/MRI pointed out severe inflammatory lesions in bowel wall as significantly elevated SUV_{max}'s. Moderate inflammation raised standard uptake values, but the raise was not statistically significant when compared with values of normal bowel wall. Mild inflammation did not produce signal different from that of normal bowel wall.

The results are in concordance with studies that have demonstrated that PET/MRI parameters are useful in pointing out both moderate and severe inflammatory lesions in colonic mucosa (Li et al., 2018; Shih et al., 2018). An early study has also shown by PET/CT that ^{18}F-FDG uptake correlates with epithelial damage in both mouse and human colitis (Bettenworth et al., 2013). Also, in upper GI tract, in patients with GER, the severity of esophagitis has been shown to correlate positively with SUV_{max}. (Wu et al., 2014). Consequently, the present study indicates that in IBD the inflammatory process in the wall of colon is characterized by increased uptake of glucose, and that PET/MRI is a useful non-invasive and reproducible method in detecting especially severely inflamed intestinal segments.

Endoscopy and histology are the golden standards in the diagnostics of IBD, but endoscopy estimates only the endoluminal status of the intestine and biopsies

represent only a small part of the gut and foci of the disease. The benefit of imaging is simultaneous detection of mural and extramural disease manifestations like strictures, abscesses, enlarged lymph nodes and engorgement of peri enteric blood vessels. PET/MRI provides all this data and according to this study, also estimates the severity of active inflammation in bowel wall. PET/MR imaging does not require cleansing of bowel, and it is not painful to a patient. An abdominal ^{18}F-FDG PET/MRI scan causes on average 5.3 mSv exposure to radiation. An effective dose of abdominal CT scan without PET varies across countries being somewhere between 7.0–25 mSv depending on local choices of technical parameters (Smith-Bindman et al., 2019). MRI alone does not cause radiation exposure. Repeated imaging during disease follow-up using PET/MRI causes an accumulative dose of radiation. Consequently, PET/MRI is valuable if extramural manifestations are suspected, and it provides an optional investigation method to those patients that are known to have a technically difficult and painful endoscopy. It can also be helpful in differential diagnosis of UC and CD since it can detect transmural inflammation typical for CD. The reliable detection of moderate and mild inflammation is necessary concerning the diagnosis and follow-up of IBD and by increasing the number of subjects in future studies, the wide 95% CI in SUV_{max} in moderate inflammation of the Study I could probably be reduced, and a statistically significant difference in SUV_{max} compared to normal or mildly inflamed bowel might be achieved. The performance of this 3 T PET/MRI equipment and the used protocol could not differentiate between the normal bowel wall and the mildly inflamed bowel wall.

In addition to previous, the region of the biopsy had a statistically significant effect on SUV_{max}. This suggests that the uptake of ^{18}F-FDG is different in distinct regions of bowel even though the inflammation activity is the same. This finding is value of interest and requires further investigation.

The study I was prospective, and an advantage was also a well-documented reference standard. In addition, the study included patients suffering from UC, CD, or intermediate colitis whereas previous studies using PET/MRI focused on assessment of malignancies and non-specified inflammatory state of bowel or just the diagnostics of Crohn's disease of small bowel (Beiderwellen et al., 2016; Russo et al., 2016).

6.2 Ulcerative colitis and Crohn's disease induce Keratin 7 expression in human colonic epithelial cells

In the Study II, UC and CD were shown to induce neo-expression of K7 in the epithelial cells in colonic mucosa. The increase of K7 in epithelial cytoplasm

indicated epithelial damage and was associated with poor drug-response. The percentage of K7 positive cells was the highest in the areas of severe pathological alterations of the epithelium and in the colectomy resects of the patients with drug-resistant UC (32.2% for UC and 9.2% for CD). The percentage of K7 positive cells was generally lower in the group of patients who had drug responsive disease (18.4% for UC and 2.2% for CD vs 0.27% for control). As the number of K7 positive cells is proportional to the degree of epithelial damage it suggests that expression of K7 is linked to the severity of the disease. The amount of K7 mRNA also arouse in colonic epithelial cells in IBD patients indicating transcriptional activation of K7 expression in IBD. Helenius et al. (2016) have previously demonstrated a strong upregulation of K7 in dextran sulphate sodium -induced colitis in murine model. Our study is the first study, where a significant K7 expression is shown in human patients suffering from IBD. In future, K7 could be considered as a prognostic marker for the severity of IBD, and since its upregulation is measurable on both protein and mRNA levels, both protein and nucleic acid assays are applicable.

The inducing factor of K7 expression in inflamed epithelium remains unclear. In this study, the number of neutrophils in epithelium and lamina propria as well as level of faecal calprotectin did not correlate with the percentage of K7 positive cells in colonic epithelium. In LC, which is characterized by excess of lymphocytes within the epithelium, K7 expression was not increased. Age, gender, or BMI of the patient did not affect the expression of K7 indicating that hormonal factors related to sex steroids or white adipose tissue are not related to K7 expression. Instead, many IBD samples with particularly high K7 expression had histiocyte formed granulomas close to epithelium. The role of histiocytes affecting keratin synthesis in epithelial cells is unclear, and this result needs further studies.

Keratins are cytoprotective proteins which are upregulated in stress and regenerative conditions (Nair et al., 2021; Toivola et al., 2010) and K7 neo-expression in the colonic epithelium of IBD patients is consistent with that statement. Keratins, including K7, have been shown to be upregulated or neo-expressed both in human and in murine disease models, for example in kidneys after renal epithelial cell injury (Djudjaj et al., 2016), in mouse pancreatic β-cells after experimental diabetes induction (Alam et al., 2021), and in hepatocytes of patients with cholestatic diseases (Sakellariou et al., 2021). Also, patients with PSC had high K7 hepatocellular expression even with intact bile ducts (Sakellariou et al., 2021). Keratins have been upregulated also in mouse models of colonic stress and inflammation (Helenius et al., 2016). In breast and colorectal cancers, simple epithelial keratin over-expression is associated with poor prognosis (Kabir et al., 2014; Zhang et al., 2019). A sporadic CRC is usually K7 negative, and K7 positivity found in metastasis is often used as a rationale against colorectal origin (Ramaekers et al., 1990; Varadhachary et al., 2008). Interestingly, K7 has been found to be

expressed in 45–70 percent of colonic epithelial cells in colitis-induced CRC (Stenling et al., 2007; Tatsumi et al., 2006). Because IBD is a risk factor for CRC, a possible link between colonic epithelial K7 expression and colitis induced carcinogenesis requires further investigation.

6.3 Keratin 7 (K7) expression in colonic epithelial cells does not predict the disease outcome in three years follow-up

Study III showed that K7 expression in mucosal epithelium had statistically significant association with inflammatory activity in intestinal mucosa in ascending and descending colon, but inflammatory activity did not affect the K7-expression in distal ileum, transverse colon, or rectum. Accordingly, K7 expression varies based on the anatomical location in colon. Helenius et al. (2016) have shown in murine model that K7 expression in ascending colon is different to expression in descending colon in elderly animals. We have previously learned in Study I that basic glucose uptake in colonic mucosa depends on anatomic location. In addition, it has also been reported that total amount of immune cells decreases from cecum to rectum (Boyer et al., 2018). These findings together imply that the function of mucosa is not homogenous throughout the colon but epithelial cells and submucosa act differently in distinct parts of the colon.

Further, in the follow-up study, the severity of the disease for three years was compared with the expression of K7 in ascending and descending colon, and in that colonic segment where the expression percentage was the greatest in each patient. As a result, the severity of the disease during the following three years was not related to expression of K7 at the time of enrolment.

Considering a great variation in severity of IBD and response to medication, a personalized treatment offers a possibility to improve the overall disease outcome and to minimize harmful side-effects of medication. Neutrophil derived F-Calpro depicts short-term inflammatory activity in the intestinal mucosa, but there is no reliable marker that would predict the course of IBD (Zilbauer and Heuschkel, 2022). Keratin 7 is produced in epithelial cells and thus might reflect the epithelial status better than temporal neutrophil activity. Study II stated that high K7 expression in colonic epithelial cells is related to poor response to conservative treatment. This three-year lasting follow up may be too short to see the difference between mild and severe disease, and consequently the monitoring of the patients will be continued. Further, K7 is neo-expressed in inflamed mucosa but the level of expression during and after healing of the mucosa is unknown and should be considered when estimating the potential of K7 as biomarker in UC and/or CD. For example, intestinal barrier maintaining protein keratin has been shown to decrease during inflammation

and increase during the healing process (Wu et al., 2021). Consequently, monitoring of changes in K7 expression over the years could produce a profile that might predict the disease outcome.

6.4 Limitations of the study

Study I: The first limitation in this study was small number of patients with diagnoses of UC, CD, and intermediate colitis. Consequently, a significant difference between normal bowel wall and moderate inflammation, or between mild and moderate inflammation, could not be proved. Also, the site of the biopsy was not the same spot where the SUV_{max} was calculated for that region, but it was calculated at a proximity of the area where the biopsy was taken. This was intentional because taking biopsy causes local inflammation which may artificially raise SUV. In addition, there may be variation in severity of inflammation within one segment (Magro et al., 2013b), and presumably that was the reason why the grade of inflammation and the calculated SUV did not correlate in some sample pairs. In addition to that, inaccuracy in the detection of PET-positive lesions in sequential PET/MRI imaging was caused by bowel peristalsis and the spatial resolution of PET imaging, which is 5–6 mm (Zaidi et al., 2011), while the thickness of inflamed mucosa in UC is less than five millimetres (Ordás et al., 2013). Finally, when considering the order of investigations, eight patients out of ten had ileocolonoscopy before PET/MRI-imaging and two had PET/MRI before ileocolonoscopy. Ideally, the order of the investigations should have been same all the time, preferably the imaging study at first so as not to cause local alternation in SUV by inflammation in biopsied sites of mucosa. The investigations should have been performed temporally as close to each other as possible. Unfortunately, the optimal order and timing were not possible due to the investigation timetable allowed by the PET centre. Even though the time gap between the ileocolonoscopy and the imaging study did not seem to have statistically significant effect on SUV, further studies with greater number of patients are needed to either confirm or falsify this result.

Study II: The study included two different cohorts and the other one (A) was retrospectively analysed. Also, the number of samples and patient were low, since the cohort A included 15 samples from UC patients and 11 samples from CD patients, and the cohort B included seven UC and eight CD patients. The sample site in cohort A patients' colon remained also unclear since the sample was taken from an undefined part of colon after colectomy.

Study III: The number of intestinal biopsies was too small to show statistically significant difference of K7 expression in transverse colon and rectum. The follow

up time of three years was likely to be too short to make a clear distribution between mild and severe forms of IBD, as compared to Study II, where the disease history of the group that ended up in colectomy varied between 0 and 29 years and the median was six years for both UC and CD. Also, the K7 expression level was analysed only from the biopsies at time of enrollment. The expression level during the disease history should be analysed to find out changes in expression. An effective biomarker would be accurate, reproducible, non-invasive, and convenient for sampling (Alghoul et al., 2022). If K7 would be considered as a biomarker of the severity of IBD, an endoscopy should be performed every time to obtain the specimens for histological evaluation. If K7 could be tested from stool, it would offer an easy way to get the sample. However, the stool sample provides a mixture of epithelial products throughout the whole intestine, and it does not differentiate the part of the colon where the K7 is produced.

7 Conclusion

The conclusions of this study were as follows:

I: ^{18}F-FDG PET/MRI is a promising method to noninvasively detect especially severe inflammatory bowel lesions. More data is required to define its sensitivity and specificity, and to create standardized protocol suitable for follow-up of IBD.

II: K7 could be utilized as a specific diagnostic marker for severe IBD, indicating especially disease with poor drug-response and major pathological changes in epithelium. K7 is also accessible biomarker as its immunohistochemistry-based detection methods are already available in pathology laboratories and its upregulation is measurable both on protein and mRNA level.

III: K7 expression varies based on the anatomical location in colon. K7 is not expressed in inflamed terminal ileum. K7 expression did not predict the severity of IBD after three years follow-up. More prospective studies are needed to find out the value of K7 in estimation of the severity of the disease and in choosing the best treatment.

Acknowledgements

The studies related to this thesis were conducted at Division of Medicine, Turku University Hospital; Department of Pathology, Turku University Hospital; Turku PET Centre, University of Turku and Turku University Hospital; Cell Biology and Biosciences, Åbo Akademi University, and Department of Surgery, University of Turku, starting from year 2014.

I wish to express my gratitude to my supervisors, first and foremost to my primary supervisor, professor Markku Voutilainen for excellent guidance, literally endless patience, and highly respected wisdom in puzzling situations. I'm thankful to docent Saila Kauhanen for devoted enthusiasm towards demanding research questions and for introducing me the possibilities of metabolic research in PET centre and just for being there whenever I needed support.

This thesis was made better by expertise and constructive criticism of the official reviewers, docent Markku Heikkinen and docent Irina Rinta-Kiikka. Their feedback was invaluable and inspiring. I wish to express my sincere acknowledgement to both of them as well as to professor Perttu Arkkila for agreeing to be my opponent.

I thank all my co-authors for expert counsel and support during the writing process of the original publications. I especially want to thank professor Diana M. Toivola for sharing her invaluable knowledge in the field of cell biology and docent Harry Kujari for providing expertise in pathogenesis of inflammatory bowel diseases. I sincerely want to acknowledge colleagues Johanna Kallio MD, PhD and Jukka Koffert MD, PhD for their devoted help in the PET study procedures.

Special thanks to Lauri Polari PhD for the invaluable friendship and intelligent approach to upcoming dilemmas that we went through. I really love to work with you.

I'm grateful to my parents Kalervo and Eeva-Kaarina Tenhami for providing me with an excellent example for how to deal with various alternatives in life and for supporting me in every possible way. I thank my beloved daughter Meri-Tuuli and son Valtteri for bringing meaning, happiness, and excitement to my life, and special thanks to my aunt Sari Tenhami for supportive and educational conversations throughout these years.

Finally, I thank my spouse Matti Hujala for all his love and support, and for the practical advice in treatment of IBD patients.

Turku, January 2024
Mervi Tenhami

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TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

Painosalama, Turku, Finland 2024

ISBN 978-951-29-9591-2 (PRINT)
ISBN 978-951-29-9592-9 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)