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Helsinki, Finland

INTESTINAL INFLAMMATION AND OUTCOME OF TREATMENT IN PEDIATRIC INFLAMMATORY BOWEL DISEASE PATIENTS UNDERGOING SURGERY

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine
of the University of Helsinki, for public examination in Biomedicum Helsinki,
in lecture hall 3 on March 22, 2013, at 12 noon.

Helsinki 2013

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ISBN 978-952-10-8654-0 (paperback)
ISBN 978-952-10-8655-7 (PDF)

http://ethesis.helsinki.fi/

Unigrafia
Helsinki 2013

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LIST OF ORIGINAL PUBLICATIONS

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

I Piekkala M, Pakarinen M, Ashorn M, Rintala R, Kolho K-L. Long-term outcomes after surgery on pediatric Crohn's disease patients. J Pediatr Gastroenterol Nutr. 2012; Epub ahead of print

II Piekkala M, Kalajoki-Helmiö R, Martelius L, Pakarinen M, Rintala R, Kolho K-L. Magnetic resonance enterography guiding treatment in children with Crohn's jejunoileitis. Acta Paediatr. 2012;101:631-636

III Mäkitalo L, Piekkala M, Ashorn M, Pakarinen M, Koivusalo A, Karikoski R, Natunen J, Saarialho-Kere U, Kolho K-L. Matrix metalloproteinases in the restorative proctocolectomy pouch of pediatric ulcerative colitis.World J Gastroenterol. 2012;18:4028-4036

IV Piekkala M, Hagström J, Tanskanen M, Rintala R, Haglund C, Kolho K-L. Low trypsinogen-1 expression in pediatric ulcerative colitis patients who undergo surgery. (submitted)

Publication III has also been included Laura Mäkitalo's dissertation by permission.

ABBREVIATIONS

Alb	albumin
ASCA	antibody against Saccharomyces cerevisiae
AZA	azathioprine
anti-TNF	anti-tumor necrosis factor
BAE	balloon-assisted entoroscopy
CD	Crohn's disease
CDAI	Crohn's disease activity index
CRP	C-reactive protein
CT	computed tomography
ECCO	European Crohn's and Colitis Organization
ESPHGAN	European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
ESR	erythrocyte sedimentation rate
FAP	familial adenomatous polyposis
GI	gastrointestinal
Hb	hemoglobin
IBD	inflammatory bowel disease
IBDU	inflammatory bowel disease unclassified
IL	interleukin
i.v.	intravenous
IPAA	ileal pouch-anal anastomosis
IRA	ileorectal anastomosis
MMP	matrix metalloproteinase
MRE	magnetic resonance enterography
NASPHGAN	North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition
NSAID	non-steroid anti-inflammatory drug
pANCA	perinuclear anti-nuclear cytoplasmic antibody
PSC	primary sclerosing cholangitis
PUCAI	pediatric ulcerative colitis activity index
PCDAI	pediatric Crohn's disease activity index
RCT	randomized control trial
SIAA	straight ileoanal anastomosis
TATI	tumor-associated trypsin inhibitor
TIMP	tissue inhibitor of matrix metalloproteinase
TNF-α	tumor necrosis factor-alpha

Tryp	trypsinogen
UC	ulcerative colitis
US	ultrasound
QoL	quality of life
WBC	white blood cell count
WCE	wireless capsule endoscopy
5-ASA	5-aminosalicylic acid
6-MP	6-mercaptopurine

TIIVISTELMÄ

TAUSTA JA TARKOITUS

Tulehduksellinen suolistosairaus (IBD) on yhteisnimitys Crohnin taudille (CD) ja ulseratiiviselle koliitille (UC) eli haavaiselle paksusuolen tulehdukselle. Tulehdus rajoittuu UC:ssa paksusuoleen, mutta CD:ssä tulehdusta voi esiintyä koko suoliston alueella suusta peräaukkoon. Kiusallisia taudin oireita ovat ripuli, vatsakipu ja ulosteen karkailu. Lisäksi lapsilla tauti voi häiritä normaalia kasvua ja kehitystä, erityisesti CD:ssä. Hoito on ensisijaisesti lääkkeellisestä, mutta vaikeimmissa tapauksissa myös kirurgista. Leikkauksessa sairas suolenosa poistetaan, mikä tarkoittaa UC:ssa usein koko tulehtuneen paksusuolen poistoa. Nykyisin poistetun suolen tilalle UC:ssa rakennetaan ohutsuolisäiliö eli J-pussi. Potilaat pystyvät leikkauksen jälkeen pidättämään ulostetta, mutta ulostavat kuitenkin useamman kerran päivässä. Leikkaus parantaa UC:n, mutta CD:ssa leikkauksella hoidetaan lähinnä taudin komplikaatioita ja lääkkeille reagoimatonta tautia, sillä CD voi uusia leikkauksen jälkeen. Kuitenkin rauhallinen taudinvaihe usein mahdollistaa CD-potilaille normaalin kasvun ja kehityksen. Ensimmäisen 10 vuoden kuluessa diagnoosista CD:hen lapsena sairastuneista leikataan n. 30 % ja vastaava luku on UC:ssa n. 25 %.

Tutkimus tuo lisää tietoa lasten IBD:n leikkaushoidosta ja sen pitkäaikaisvaikutuksista. Seurantatutkimuksen tulokset leikkauksen vaikutuksesta potilaan elämään ovat ensiarvoisen tärkeitä, kun potilas ja lääkäri pohtivat leikkauksen mahdollisuutta. On tärkeää löytää leikkausta tarvitsevat potilaat ajoissa ja CD:ssä tähän magneetti enterografia (MRE) on hyvä menetelmä. Sen merkitystä lapsipotilaiden hoitoon ei ole aikaisemmin tutkittu. Vaikka leikkaus parantaa UC:n, usein ohutsuolesta paksusuolen tilalle rakennetun J-pussin limakalvo tulehtuu. Arviolta 75 % leikatuista kärsii tästä pussiitiksi kutsutusta tulehduksesta 10-vuoden seuranta-aikana. On tärkeää tietää, onko pussiitti IBD:n aktivaatio vai muu tulehdus, jotta tämän tulehduksen hoitoa voidaan tehostaa ja mahdollisesti tulevaisuudessa ennaltaehkäistä. IBD:n taudinkulku on ennalta-arvaamaton, joten on tärkeää löytää merkkiaineita, joiden avulla voitaisiin erottaa aggressiivista tautia sairastavat heti diagnoosivaiheessa.

POTILAAT JA MENETELMÄT

(I) Seurantatutkimuksen aineistona olivat kaikki vuosina 1985 - 2008 CD:n vuoksi Lastenklinikalla, HYKS:ssä tai Taysissä leikatut potilaat (n=36). Heille lähetettiin kysely kirjeitse keväällä 2010, jolloin jokaisen potilaan leikkauksesta oli kulunut

vähintään kaksi vuotta. Kysymykset käsittelivät leikkauksen toiminnallista tulosta sekä pitkäaikaisselviytymistä. Väestörekisterikeskuksesta oli aikaisempaa tutkimusta varten valittu sattumanvaraisesti verrokkeja ja heille postitettu kysely, jossa kysyttiin mm. elämänlaadusta samalla kyselyllä, jota tässä tutkimuksessa käytettiin. Vastanneista valittiin jokaiselle potilaalle kaksi iältään ja sukupuoleltaan vastaavaa verrokkia elämänlaadun vertailua varten. Potilastiedoista kerättiin kaikkien leikattujen potilaiden leikkaussyyt, leikkaustyypit, komplikaatiot leikkauksista, lääkitys leikkauksen jälkeen, uusintaleikkaukset sekä tähystystiedot mahdollisen taudin uusiutumisen löytämiseksi.

(II) Kaikkien Lastenklinikalla HYKS:ssa MRE-kuvattujen CD-potilaiden (n=45, tammikuu 2009 – heinäkuu 2011), magneettikuvat etsittiin, jotta voitiin selvittää, miten MRE oli vaikuttanut lasten hoitoon. Kaksi radiologia arvioi magneettikuvat itsenäisesti, sokkoutettuna potilastiedoille. MRE tulosta verrattiin leikkauksessa havaittuun suolen ulkonäköön sekä tähystystutkimukseen. Potilaista selvitettiin myös, miten heidän hoitonsa oli muuttunut MRE tutkimuksesta saadun tiedon perusteella.

(III) Tutkimuksessa, joka pyrki vertaamaan ohutsuolisäiliön eli J-pussin mahdollisen tulehduksen laatua IBD:ssä esiintyvään tulehdukseen, olivat aineistona kaikki UC:n vuoksi vuosina 1985 - 2005 Tays:ssä tai Lastenklinikalla HYKS:ssä leikatut 81 alle 16-vuotiasta potilasta. Potilaille lähetettiin kirjeitse kysely, joka käsitteli sen hetkistä diagnoosia, lääkitystä, komplikaatiota ja pussiittia. Heitä pyydettiin myös osallistumaan käynnille, jossa J-pussista otettaisiin koepala. Tälle käynnille osallistui 28 potilasta ja käynnin aikana tutkimushoitaja keskusteli potilaiden kanssa. Jokaisesta koepalasta värjättiin immunohistokemiallisin menetelmin matriksin metalloproteinaaseja (MMP) MMP -3, -7, -8, -9, -12 ja -26 sekä niiden erityisiä estäjiä (TIMP) TIMP -1, -2 ja -3. Osan näistä tutkituista MMP:stä ja TIMP:eistä tiedetään olevan tärkeitä merkkiaineita IBD:ssä, mutta osan merkitystä ei ole aikaisemmin tutkittu.

(IV) Aggressiivisen taudin ennustetekijotä selvittävää tutkimusta varten etsittiin HYKS:n Lastenklinikan IBD-potilasrekisteristä kaikki vuosina 1990 - 2008 UC:n vuoksi Lastenklinikalla HYKS:ssä leikatut alle 16-vuotiaat potilaat. Mukaan valittiin ne leikatut UC potilaat, joille löytyi kontrolliksi konservatiivisesti hoidettu potilas, jolle oli samanikäisenä diagnosoitu UC ja häntä oli seurattu yhtä kauan kuin leikattua potilasta. Leikattujen (n=24) ja kontrollien (n=27) diagnostisista kudosnäytteistä värjättiin immunohistokemiallisin menetelmin sellaisia, ennen IBD:ssä tutkimattomia kudosmerkkiaineita, jotka voisivat liittyä aggressiiviseen taudinkulkuun, kuten trypsinogeeni 1 ja 2 (Tryp-1 ja -2) ja näiden spesifinen estäjä TATI. Lisäksi värjättiin

IBD:n tärkeä merkkiaine MMP-9. Vertailukudosnäytteet etsittiin 20 lapselta, joille oli tehty paksusuolen tähystys, mutta IBD oli poissuljettu. Leikatuista, kontrolleista ja vertailuryhmästä tehtiin samat värjäykset ja verrattiin ryhmien diagnoosihetken kudospalojen kudosmerkkiaine-esiintymistä.

TULOKSET

(I) Keskimäärin 10 vuoden seuranta-aikana CD aktivoitui 94 %:lla ja jo kahden vuoden kuluttua leikkauksesta yli puolella. Uusintaleikkaukseen päätyi 58 %. Lähes kaikilla oli ollut ainakin yksi leikkaukseen liittynyt komplikaatio. Suolentoiminta seurantahetkellä oli kohtalaista, ulostetarpeen ollessa päivisin keskimäärin 3 kertaa (0-23 kertaa), öisin 0 (0-10 kertaa). 30 %:lla oli täysi pidätyskyky. Tulokset viittasivat siihen, että huonompi suolentoiminta liittyi leikkauksessa poistetun suolen kokonaispituuteen. Potilaiden elämänlaatu ei poikennut iältään ja sukupuoleltaan vastaavista kontrolleista. Kuitenkin niillä leikatuilla potilailla, joilla oli ollut koulu- tai työpoissaoloja viimeisten kuukausien aikana suolioireiden vuoksi, oli elämänlaatu merkittävästi alentunut verrattuna muihin leikattuihin potilaisiin.

(II) MRE-kuvaus onnistui 43/45 CD-potilaalla. Tulehdusta ohutsuolen alueella esiintyi 24 potilaalla. MRE oli ainoa kuvausmenetelmä, joka pystyi näyttämään ohutsuolen taudin 12 %:lla potilaista. MRE-kuvauksen jälkeen 26 % potilaista leikattiin ja lopuilla hoito säilyi lääkkeellisenä. Suurella osalla (73 %) leikkauksessa nähty löydös vastasi MRE-löydöstä, mutta osalla MRE liioitteli tulehduksen laajuutta. Potilailla, joita ei leikattu MRE-löydös vastasi tähystys- tai kapselikameralöydöstä tai näytti laajemman taudin (27 %).

(III) Noin 13 vuoden seuranta-aikana kliinisen pussiitin oireita oli ollut 68 %:lla UC-potilaista, joiden pussista otettiin koepala. Kuudella potilaalla oli aktiivinen pussiitti koepalanottohetkellä. 93 %:ssa koepaloista esiintyi kuitenkin histologista tulehdusta. J-pussissa esiintyi yleisesti MMP -3, -7, -12 ja TIMP -2 ja -3. MMP-3 ja MMP-7 esiintyminen liittyi matalaan tulehduksen tasoon. IBD:lle merkittävä MMP-9 ei värjäytynyt pussissa eivätkä MMP-8 ja -26.

(IV) Kaikki merkkiaineet värjäytyivät heikosti tai kohtalaisesti UC-potilaiden diagnostisten kudospalojen paksusuolen epiteelissä ja Tryp-1 ja -2 sekä MMP-9 myös strooman tulehdussoluissa. Positiivisesti paksusuolen epiteelissä värjäytyneiden näytteiden osuus oli samanlainen kaikkien tutkittujen ryhmien välillä. UC potilaiden näytteistä merkitsevästi useampi värjäytyi MMP-9:n suhteen positiivisesti paksusuolen strooman tulehdussoluissa ja näissä näytteissä myös immunopositiivisuuden

taso oli korkeampaa verrattaessa ei-IBD kontrolleihin. MMP-9 immunopositiivisuus tulehdussoluissa liittyi histologisen tulehduksen tasoon. Leikattujen UC-potilaiden kudosnäytteissä esiintyi paksusuolen epiteelissä merkittävästi vähemmän Tryp-1:tä verrattaessa konservatiivisesti hoidettuihin UC potilaisiin ja ei-IBD verrokkeihin. Tryp-2:n ja TATI:n värjäytymisessä ei havaittu eroa tutkittujen ryhmien välillä. Trypsiininen inhibiittori TATI:n esiintyminen ei liittynyt trypsinogeenien esiintymiseen. Aktiivi tulehdus ei vaikuttanut trypsinogeenien ja TATI:n esiintymiseen.

JOHTOPÄÄTÖKSET

CD:n vuoksi lapsena leikatut tarvitsevat hyvän leikkauksen jälkeisen seurannan, sillä taudin uudelleenaktivoituminen on yleistä leikkauksen jälkeen. Kuitenkin osalla potilaista tauti aktivoituu vasta useiden vuosien kuluttua leikkauksesta, joten seurannan tulee olla riittävän pitkä. Sekä toiminnallinen tulos noin 10 vuotta leikkauksesta että elämänlaatu ovat CD:n vuoksi lapsena leikatuilla hyväksyttävällä tasolla. MRE osoittaa hyvin ohutsuolen taudin nuorilla CD-potilailla ja voi ohjata kliinistä päätöksentekoa niin leikkauksen tarvetta arvioitaessa kuin lääkitystä pohdittaessa. Leikatun UC potilaan pussin ja IBD-suolen MMP-esiintyminen muistuttavat toisiaan, mutta pussin tulehdusta ei voida pitää IBD:n aktivoitumisena. Merkittävimpiä eroja pussin ja IBD-suolen välillä olivat MMP-9 esiintymisen puuttuminen pussista sekä MMP-3 matala esiintyminen pussista otetuissa näytteissä, joissa oli tulehdusta. Tulokset osoittavat myös, että aggressiivista UC:ta sairastavien lapsien paksusuoli eroaa jo diagnoosihetkellä lievempää tautia sairastavien lasten suolesta. Tätä tietoa voidaan mahdollisesti tulevaisuudessa hyödyntää jatkotutkimuksissa, sillä olisi tärkeää selvittää voisiko tehostettu hoito heti taudin alkuvaiheessa estää komplikaatioiden kehittymisen. Tulokset myös vahvistavat aiempaa löydöstä siitä, että MMP-9 liittyy IBD-tulehdukseen.

ABSTRACT

BACKGROUND

Inflammatory bowel disease (IBD) is a common name for Crohn's disease (CD) and ulcerative colitis (UC). UC affects the rectum and the colon, and in CD patients, inflammation may occur in the whole gastrointestinal tract. Symptoms of the disease include abdominal pain, urgency, and incontinence, while in children it causes growth retardation, especially in CD patients. The treatment is mostly medical, but sometimes resection of the diseased bowel is necessary.

The presentation of IBD is more extensive in children than in adults. The risk factors for aggressive disease are not well known. For CD patients, diagnosing small bowel disease is challenging. Magnetic resonance enterography (MRE) offers a new radiation-free method for detecting small bowel disease. However, the way in which MRE guides treatment in pediatric IBD has not been studied yet. Approximately 30% of pediatric CD patients undergo surgery within ten years of diagnosis, while the equivalent number for UC is 25%. UC patients usually undergo proctocolectomy with an ileoanal anastomosis, which is accompanied by an ileal reservoir, the J-pouch. Bowel resections are common among CD patients, with the most frequent sites being the distal ileum and the colon. Long-term outcomes after surgery are poorly established, especially for CD patients. Additionally, the etiology of inflammation seen in the pouch, pouchitis, after proctocolectomy is not known. It is important to know whether the pouchitis results from a reactivation of IBD or a novel inflammation so that it can be better treated and even prevented.

PATIENTS AND METHODS

The study populations comprised IBD patients diagnosed in their childhood and treated either at Helsinki Children's Hospital or at the Department of Pediatrics, Tampere University Hospital.

(I) To study the long-term outcomes after surgery for CD patients, a questionnaire and chart review study of CD patients who had undergone surgery in childhood between the years 1985 and 2008 was conducted. The patients were treated either at Helsinki Children's Hospital or at the Department of Pediatrics, Tampere University Hospital. In total, 36 patients were eligible for the study, which entailed

at least a two-year follow-up time. The questions concerned the functional results of the surgery and quality of life (QoL). Based on the patient charts, indications for surgery, surgical complications, relapses, re-operations, and use of biologicals during follow-up were collected. The QoL of the patients was compared to that of age- and gender-matched controls, which had been collected for previous studies (Pakarinen 2009, Turunen 2009).

(II) To estimate the significance of MRE for detecting CD jejunoileitis, MRE images from all 45 CD patients who had undergone an MRE between January 2009 and July 2011 at Helsinki Children's Hospital were reviewed. Two pediatric radiologists evaluated the pictures blinded to the patient data. The MRE pictures were compared to the macroscopic findings at surgery, when applicable, and to the endoscopic findings within three months of the MRE. The MRE images were also related to therapeutic modifications.

(III) To compare the tissue profile of the matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs) between pouch and IBD in UC patients, and to characterize pouch inflammation, all pediatric UC patients who had undergone a proctocolectomy between the years 1985 and 2005 at Helsinki Children's Hospital or at the Department of Pediatrics, Tampere University Hospital were traced (n=81). A questionnaire concerning the diagnosis, medication, complications and pouchitis was sent to the patients. In addition, the patients were asked to take part in a follow-up visit where the pouches were biopsied: 28 patients took part. At the follow-up visit, a research assistant not familiar to the patients discussed with the patients. The biopsies were evaluated by immunohistochemistry for MMPs (MMP -3, -7, -8, -9, -12, and -26) and their specific inhibitors TIMPs (-1, -2, and -3). Some of the MMPs and TIMPs associate with the inflammation seen in IBD, but the role of others has not been studied before.

(IV) To find the risk factors for aggressive disease, the patients from the IBD registry at Helsinki Children's Hospital who had undergone surgery for UC and who had been diagnosed when they were under the age of 16 between the years 1990 and 2008 were traced. The diagnostic colonoscopic tissue samples were collected from 24 UC patients and 27 matched control patients. The controls were conservatively treated UC patients who had a comparable disease history. Tissue samples were stained by immunohistochemisty for trypsinogen-1 and -2 (Tryp-1 and -2), a trypsin inhibitor (TATI), and MMP-9. The role of trypsinogens in IBD remains unknown, but they are known to activate MMP-9, which is an important biomarker of IBD and related to active inflammation. The tissue samples from 20 healthy children who had undergone a colonoscopy to exclude IBD were stained, and they served as

non-IBD controls. The staining results of the patients who had undergone surgery, the IBD controls, and the non-IBD-controls were all compared to one another.

RESULTS

(I) Nearly all (94%) of the surgically treated CD patients had an endoscopically verified and medically or surgically treated disease re-activation during a median follow-up of ten years. Half of the relapses occurred during the first two years postoperatively. Most of the patients had at least one surgical complication and approximately half had undergone re-resection. The bowel function of the patients after a median of one decade after primary surgery was moderate: The median daytime stool frequency was three (range 0-23), the nighttime stool frequency zero (range 0-10), and 30% were totally continent. The long-term QoL of the patients was comparable with that of the non-IBD, population-based controls. However, patients who had experienced school or work absences due to active disease during the last months had a significantly lower QoL compared to other patients who had undergone surgery.

(II) MRE imaging succeeded in 43 of 45 patients and showed inflammation in the small bowel in 56% of patients. In 12% of patients, the MRE was the only imaging method that was capable of visualizing the small bowel disease. The treatment remained conservative after the MRE in 74% of CD patients; however, 26% of patients underwent bowel resection. While the macroscopic findings in the bowel corresponded to the MRE findings in 73% of patients, the MRE overestimated the disease extension in some patients. In 27% of patients, the MRE suggested a more severe disease than was verified at endoscopy or wireless capsule endoscopy.

(III) Of the 28 pediatric UC patients whose pouch was biopsied, 68% of them had had clinical symptoms of pouchitis during a median follow-up of 13 years. At the time of the biopsy, six patients had clinically active pouchitis. However, histological inflammation was seen in 93% of the biopsies. Whereas most pouch samples showed epithelial and stromal MMP-3, -7, and -12 and TIMP-2 and -3 expressions, MMP-8, -9, and -26 expressions were lacking. In the samples with low inflammatory activity, the epithelial MMP-3 and MMP-7 expressions had increased.

(IV) In the diagnostic tissue samples of the pediatric UC patients, all of the studied markers showed mild to moderate immunopositivity in the colon epithelium and Tryp-1 and -2 and MMP-9 as well as in the inflammatory cells of the colon stroma. In all of the studied markers, the proportions of positively stained samples were

comparable between the studied groups in the epithelium of the colon. The UC group had significantly more samples that were MMP-9 positive for inflammatory cells, and the level of immunopositivity was also higher for the samples from the UC group than for those from the non-IBD control group. MMP-9 immunopositivity was associated with the grade of inflammation at diagnosis. The operated UC patients showed lower immunopositivity of Tryp-1 in the epithelium of their colon samples compared to conservatively treated UC patients and the non-IBD controls. No difference in the expression of trypsinogen-2 or the trypsin inhibitor TATI was seen between patients undergoing surgery, the conservatively treated UC patients, and the non-IBD controls. The expression of TATI did not correlate with the expressions of Tryp-1 or -2 and none of these markers were associated with active inflammation at diagnosis.

CONCLUSIONS

CD patients who have undergone surgery in childhood need proper postoperative follow-up because both early and late disease relapses and surgical complications are common. Overall, the long-term bowel function and QoL are acceptable, but disease relapses and re-resections are associated with poor bowel function and QoL. The MRE detects the small bowel disease in young CD patients and can guide treatment, which demonstrates that MRE can be used as a method to evaluate small bowel disease. While the common inflammation of the pouch shares some similarities with IBD, inflammation cannot be classified as reactivation. The major differences in MMP expressions observed for the pouch and IBD gut had to do with a lack of MMP-9 in the pouch and the fact that the expression of MMP-3 was associated with a low inflammation rate in the pouch. At diagnosis, the epithelium of the colon in pediatric UC patients who will undergo surgery showed a lower expression of trypsinogen-1 compared to that of patients whose disease course is more benign. This finding can be utilized in future studies because it is important to find out if aggressive treatment during the early disease phase could prevent disease complications. The results also support the finding that MMP-9 is expressed in IBD-inflammation.

INTRODUCTION

Inflammatory bowel disease (IBD) covers two chronic diseases of the gastrointestinal (GI) tract: Crohn's disease (CD), which occurs in the whole GI tract, and ulcerative colitis (UC), which is a disease of the colorectum. Occasionally, these two diseases cannot be differentiated from one another and a diagnosis of unclassified colitis (IBDU) is made. Symptoms of IBD include diarrhea, abdominal pain, and weight loss. Growth retardation is characteristic of pediatric CD: It might in fact be the only symptom exhibited by the child at diagnosis. However, the activity of the disease can vary. Some patients may only have one episode of active disease during their lifetime, but a significant subgroup of patients end up suffering from a continuously active and aggressive form of the disease and might require surgery. At diagnosis, it is impossible to foresee the future of patients with the disease. However, these diseases present more aggressively in children compared to adults.

The incidence of IBD is especially high in northern Europe and North America and it has been increasing in the last few decades; in particular, the number of CD patients has grown. A wide range of speculation exists as to why this has happened, but the common view is that the reasons are multifactorial. At the same time as these diseases are becoming more common, the pathogenesis remains mostly unknown. The role of GI flora, genetics, environment, and gut immune system, including all possible mediators, has been studied.

The clinical picture of patients raises doubts that IBD might be possible; the next step towards making a diagnosis is ileocolonoscopy and gastroduodenal endoscopy. In addition to endoscopy, small bowel imaging is necessary. In particular, magnetic resonance enterography (MRE) together with wireless capsule endoscopy (WCE) are good methods for performing small bowel imaging. Histologically, granulomas and the presence of small bowel disease are diagnostic to CD. However, diagnostic uncertainty with respect to the presence of CD or UC is common, since many pediatric CD patients only have a colonic disease and granulomas do not always exist. Through endoscopy, MRE, and WCE, the disease can also be evaluated during relapse or an unremitting disease course.

While medical treatment, including steroids, 5-aminosalisylic acid, immunomodulators, and new biological therapies, can relieve the symptoms, they do not cure the disease. One option for managing the disease is surgery. Common indications that surgery might be required are steroid dependency in UC patients and the complications of a stricturing and perforating disease, such as bowel obstruction in CD patients. During the first ten years after diagnosis, approximately 30% of pediatric CD patients and 25% of pediatric UC patients require surgery.

For UC patients, the surgery is curative because the colorectum can be removed by proctocolectomy accompanied by the formation of a J-pouch from the distal ileum that is connected to the anal canal. The pouch takes on the former role of the rectum, but inflammation of the pouch is not uncommon. For CD patients, the surgery is not curative and the surgeon should save as much bowel length as possible. However, surgery often offers the child a disease-free interval that might enable catch-up growth and weaning off steroids.

This dissertation studies the different aspects of surgical treatment in pediatric IBD patients. Since the surgery for CD patients only relieves the symptoms, it is important to learn the long-term effects of surgical treatment. These were studied by conducting a follow-up study of pediatric CD patients operated on during their childhood. A symptomatic CD patient might have a segment of active disease in the small bowel that requires surgery; however, traditional endoscopy is not able to detect this segment of active disease. The role of the MRE was studied as a treatment guiding method in CD children. The third study aimed to discover the actual base of the inflammation seen in the pouch, termed pouchitis, since experts disagree on whether this inflammation is a unique problem or a reactivation of UC or CD. The last study focused on finding a biomarker that at diagnosis, could characterize those UC patients who would end up with early surgery; this might enable more aggressive medical treatment so as to avoid later disease complications and surgery.

REVIEW OF THE LITERATURE

1 PEDIATRIC ONSET INFLAMMATORY BOWEL DISEASE

1.1 OVERVIEW AND CLINICAL PICTURE

Inflammatory bowel disease (IBD) is a common name for ulcerative colitis (UC) and Crohn's disease (CD), which are systemic inflammatory diseases of the gastrointestinal (GI) tract. The disease course of IBD is unpredictable; some patients have only a single episode of the active disease and remain in remission for the rest of their lives, other patients have recurrent periods of active disease followed by remission, while still others suffer from a continuously active disease (Beattie 2006).

Usually inflammation in UC patients starts from the rectum and extends continuously and proximally in varying extensions that affect the mucosa. UC can be divided into left-sided colitis, where inflammation reaches the splenic flexure, or pancolitis, where the whole colon and rectum are inflamed (Kugathasan 2003). The inflammation in CD patients may affect the whole GI tract from the mouth to the anus in a transmural and patchy manner (Shikhare 2010).

CD can be classified as either a stricturing or a perforating disease depending on the site where the disease is located and, additionally, based on the phenotype (Levine 2011). Stricture causes bowel obstruction, which constitutes a major complication for CD patients. Another complication that can determine the clinical picture in CD patients is fistula, which is caused by perforating inflammation. A fistula can be an abnormal tunnel between two organs, such as the bowel and the vagina, the bowel and the urinary vesicle, or the bowel and the bowel, or else it can be connected to the skin. The CD fistula may be associated with abscess formation in the abdominal cavity or perianal region.

The third subclass of IBD is inflammatory bowel disease unclassified (IBDU), where neither UC nor CD can be ruled out. While patients primarily have colonic disease, they may present with features commonly seen in CD, such as growth failure, rectal sparing, and ileal inflammation, all of which make the diagnosis of UC uncertain (Kappelman 2008). Unless histologically specific findings, such as deep granulomas, submucosal inflammation, or small bowel disease, do not exist, it may be impossible to make a differential diagnosis that distinguishes between the subgroups. In CD patients, the colonic involvement is linked to the young age of the patient at diagnosis and the fact that young patients have less isolated involvement of the terminal ileum (Levine 2009; Van Limbergen 2008; Mamula 2003; Kugathasan 2003; Auvin 2005; Heyman 2005; de Bie 2012b). Overall,

the disease is more extensive in pediatric patients than their adult peers (Van Limbergen 2008).

Table 1 shows the frequency of different symptoms seen in IBD. The signs and symptoms of the disease differ according to the disease course, extension, and complications (Beattie 2006; Diefenbach 2006). In CD patients, abdominal pain may not exist and other indicators may determine the clinical picture. Typically, CD patients present with fever, nausea, vomiting, growth retardation, malnutrition, delayed puberty, psychiatric symptoms, arthropathy, and erythema nodosum. An examination of the perineum may reveal skin tags, fistulae, or abscesses (Beattie 2006). UC patients more commonly present with abdominal pain and bloody diarrhea; weight loss and growth retardation are less common than in CD patients (Beattie 2006).

Table 1. The frequency of different symptoms seen in IBD (modified from Shikhare 2010; Markowitz 2008; Diefenbach 2006; Newby 2005; Hildebrand 1994; Sawczenko 2003; ESPHGAN 2005).

Symptom	CD (%)	UC (%)	IBDU (%)
abdominal pain	62-95	33-76	60-75
diarrhea	56-75	70-90	76-78
weight loss	43-92	22-55	35
bloody diarrhea	14-60	52-97	68-85
fever	11-48	4-34	nd
extraintestinal manifestations	15-25	2-16	nd
growth failure	14-40	3–10	7

nd=not determined.

IBD is not only a disease of the GI tract; between 25 and 30% of patients experience extraintestinal manifestations during their lives (Hyams 1994). Arthritis is the most common manifestation. Approximately one quarter of pediatric patients have arthralgias or arthritis (Passo 1986), approximately 1 to 3% have skin involvement in either erythema nodosum or pyoderma gangrenosum (Hyams 1994), approximately 1 to 3% have episcleritis and uveitis (Hyams 1994), and 5% of UC patients and 20% of CD patients have aphtous stomatitis (Hyams 1994). Delayed growth is one of the biggest problems among pediatric IBD patients (Motil 1993). The etiology of growth failure is multifactorial. Possible reasons include nutritional deficits, increased nutrient losses, malabsorption, increased metabolic demands, and certain medications (Shikhare 2010). Up to 40% of adult IBD patients have osteoporosis (Shikhare 2010). Pediatric IBD patients are at risk for primary

sclerosing cholangitis (PSC); this is the most common liver manifestation of IBD and it more often affects UC patients (Rabizadeh 2008).

IBD increases the risk for malignancies. The risk of contracting colonic adenocarcinoma is greatest in UC patients: The cumulative probability is estimated to be 18% after 30 years with the disease (Eaden 2001). The risk of lymphoma is increased in CD patients (Shikhare 2010), and the patients also have an elevated risk for contracting small bowel adenocarcinoma and colon cancer (Diefenbach 2006).

One of the main aims of IBD care is to maintain the quality of life (QoL) of these patients. The symptoms of IBD might have a big effect on a teenager's life. They most likely will have to limit their social life because they will always have to ensure that a toilet is immediately available. It might also be difficult for them to take part in physical activities. The relapsing clinical picture may restrict patients' future plans because of the fear of disease activation. Social life and friends are a big part of every teenager's life, but being different will expose them to bullying and discrimination. A shorter stature, delayed puberty, and diarrhea form a good cocktail of symptoms that will help separate a teenager from others and make a bully's life easier (Greenley 2010).

Greenley et al. (2010) recently reviewed 19 studies (1,167 IBD youth; mean age: 14.33; mean time since diagnosis: 3.23 years), which summarized the psychosocial functioning of young IBD patients. They found that IBD patients have an equal amount of anxiety symptoms, depressive symptoms, and externalizing symptoms as healthy young people and young people with other chronic diseases. However, IBD patients who suffer from an active disease report experiencing more emotional symptoms and having more sleeping problems than patients in remission (Väistö 2010; Pirinen 2010). On the other hand, the parents of IBD patients report that their child experiences significantly more internalizing symptoms (hopelessness, dysthymic mood, feelings of helplessness, and negative disease-related or unrelated causal attributions) and sleeping problems than healthy children (Greenley 2010; Pirinen 2010). Only 5% of patients and their parents answered in the affirmative when they were asked about the presence of psychosocial problems (Pirinen 2011). Overall, the QoL of IBD patients is significantly lower than among the healthy controls and only marginally higher than the QoL of young people with other chronic illnesses, especially if the disease is active (Greenley 2010). While social functioning decreases among IBD patients compared with healthy teenagers, it is comparable to the amount of social functioning of other patients with chronic illnesses (Greenley 2010).

School is the big part of a young person's life. School lays the groundwork for future studies, and a teenager's social life largely takes place at school. Mackner et al. (2012) studied the academic achievement, attendance, and school-related QoL of 50 IBD patients (mean age: 14.69; mean disease duration: 3.53 years; most had

CD) and compared the results with the answers provide by 42 healthy teenagers. Parents were also reviewed. They reported that IBD patients have significantly more full-day absences from school (average two weeks during the school year) compared with the healthy group, but their QoL and grade point averages were comparable. Absences, though, might have an effect on their grade point averages (Mackner 2012), which, for example, in Finland is the scale by which young adults apply for further studies. Absences also isolate children from the social network of the school. Factors that decreased grade point average were low incomes, the number of clinical visits, experiencing several of the eight syndrome measures on the Child Behavioral Checklist, such as depression and social problems, and unmarried parents. It is interesting that disease-related factors, such as disease activity, years since diagnosis, number of clinical visits, number of hospitalizations, or use of steroids, seem to be less significant predictors of their ability to function in school than demographic and psychosocial factors (Mackner 2012).

1.2 EPIDEMIOLOGY

Approximately 20 to 30% of all IBD patients are diagnosed below the age of 18 (Abraham 2012; Beattie 2006). The most common time at diagnosis is early adolescence (Melvin 2008). The patient charts for 604 new pediatric (≤18years) IBD cases from the years 1987 to 2003 were reviewed in two tertiary care hospitals in Finland. The majority of the patients were 12 to 15 years of age (33%). However, 5.1% were under three years of age and 14% were under six years of age. Those who were diagnosed under the age of three most commonly had IBDU. In total, 21% of all IBD patients underwent surgery during a median follow-up time of three years. Patients with UC had surgery more often than patients with CD or IBDU (Turunen 2006). A study from Sweden covering 56.5% of the pediatric population reported that 639 children (≤15 years) were diagnosed with IBD between 1984 and 1995: 7.5% of them were under the age of six at diagnosis, while the majority (62.3%) of them were over 11 years of age at diagnosis (Lindberg 2000).

The pediatric incidence of IBD is high, especially in Scandinavia, northern Europe and North America (Lindberg 2000; Kugathasan 2003; Lehtinen 2011; Henderson 2011), which suggests that geographical variations might influence the development of IBD. A systematic review suggests that the incidence of IBD is increasing internationally; in particular, the incidence of CD has risen significantly in most countries (Benchimol 2011a). Most studies report a stable incidence of pediatric-onset UC, though information from developing countries in Asia, Africa, and South America is lacking (Benchimol 2011a). In Finland, UC is more frequent than CD, but the incidence of CD is increasing more rapidly (Lehtinen 2011).

The incidence of IBD in Finland in 1987 was 5/100,000, whereas in 2003 it was 15/100,000. The average annual increase in the incidence of IBD was 6.5% (95%; CI 5.4%-7.5%) and the numbers were almost identical between 1987 and 1995 and between 1996 and 2003 (Lehtinen 2011).

1.3 ETIOLOGY

The pathogenesis of IBD remains unknown, but a common view is that it is multifactorial. The current theory suggests that in genetically predisposed individuals, environmental factors and maladaptive immune responses to gastrointestinal flora generate a dysregulated inflammatory cascade creating mucosal injury (McGreal 2008). Figure 1 summarizes the risk factors that are associated with the development of IBD.

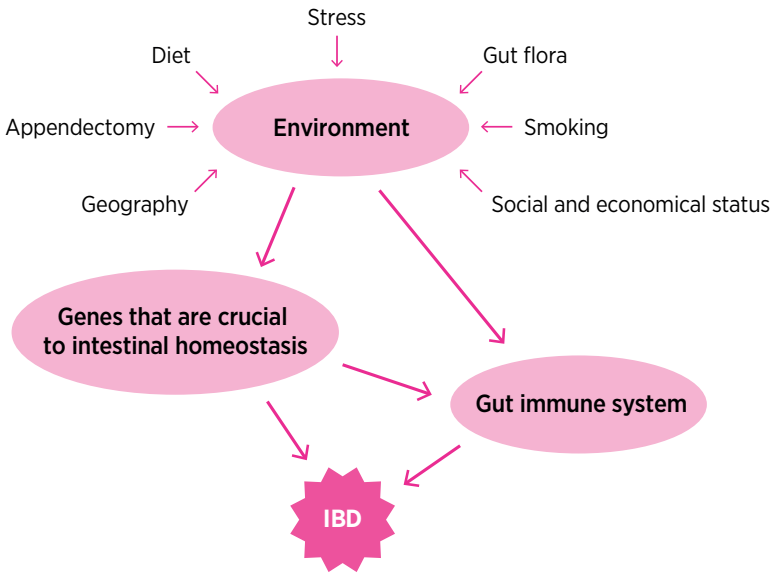


Figure 1. The current view on the etiology of inflammatory bowel diseases and the risk factors associated with them (modified from McGreal et al. 2008).

A positive family history is common in IBD patients; between 11 and 18.5% of pediatric IBD patients have a first- or second-degree relative that has IBD (Kugathasan 2003; Turunen 2009; Jakobsen 2012). The first known mutation associated with CD was noted in the gene NOD2/CARD15, which is in chromosome 16 (McGreal 2008). The gene product of NOD2/CARD15 is a bacteria-sensing cytoplasmic protein. This

suggests that the ability of the immune system to recognize the gut flora in a normal manner might be genetically altered in IBD patients and that the exact mechanism for understanding how these genes actually contribute to the pathogenesis of IBD patients should remain an active area of research (Shikhare 2010). Genome-wide association techniques have made it possible to reveal more genes susceptible to IBD. More than 60 IBD-susceptible gene loci have been found and 60% of them have been replicated in pediatric studies (Henderson 2011). Analyses of the genes and genetic loci implicated in IBD have revealed several pathways that are crucial for intestinal homeostasis, such as barrier function, epithelial restitution, microbial defense, innate immune regulation, reactive oxygen species generation, autophagy, the regulation of both adaptive immunity and endoplasmic reticulum stress, and the metabolic pathways associated with cellular homeostasis (Khor 2011).

Researchers have speculated on the role of a clean environment as a risk factor for IBD. High levels of hygiene might prevent pediatric patients from developing a tolerance to microbes that may present in later life and this might increase their risk for contracting IBD (Ekblom 2004). The role of food in the pathogenesis of IBD has also long been considered. Some researchers have considered immunological mechanisms that link food antigens to gut inflammation, but this logical explanation is not accepted by everyone (Saeed 2008). However, recently it was shown that high sugar intake is related to the manifestation of IBD, whereas the consumption of vegetables and wholegrain bread is protective (Jakobsen 2012). Recent studies also report that CD is positively associated with the number of antibiotic purchases during childhood (Virta 2012a). Patients with IBD are known to have more hospital admissions because of GI infections than the controls (Jakobsen 2012). The relationship of education and social or economic status to the pathogenesis of IBD remains mainly unknown. However, it has been shown that stressful events, such as parents' divorce, are more common in IBD patients than the controls and that there is an increased risk of CD as opposed to UC in patients living in more urban areas (Jakobsen 2012).

The role of proteinases in the pathogenesis of IBD

Matrix metalloproteinases (MMPs) are the family of 24 human zinc-dependent enzymes that are capable of degrading all of the components of the extracellular matrix (Ravi 2007). MMPs present both in healthy and inflamed bowels and they take part in both the physiological barrier function of mucosa and in pathological processes (Ravi 2007). Tissue inhibitors of matrix metalloproteinases (TIMPs) inhibit the function of MMPs. The balance between these two proteinases is crucial for the proper functioning of MMPs (Biancheri 2012). The MMPs that are important

in gut inflammation related to IBD are: MMP -1, -3, -7, -9, -12, and -26 (Baugh 1999; von Lampe 2000; Matsuno 2003; Vaalamo 1998; Bister 2004). Of these, MMP -1, -3, -7, and -9 correlate with the degree of inflammation (Baugh 1999; von Lampe 2000; Matsuno 2003). In IBD patients, the production of MMPs, especially MMP-9, is increased because of the inflammatory reaction and proinflammatory cytokines such as IL-1β and TNF-α (Sternlicht 2001; Gan 2001).

For pediatric IBD patients, MMPs and TIMPs have been studied by immunohistochemistry in colonic tissue samples (Mäkitalo 2010) and in sera (Mäkitalo 2012; Kofla-Dlubacz 2012). It has been shown that MMP-10 and TIMP-3 are highly expressed in the bowel wall stroma of pediatric IBD patients and could serve as histological indicators of IBD (Mäkitalo 2010). Table 2 lists the presentation of MMPs and TIMPs in the IBD and healthy bowel.

Another group of proteases are serine proteases. The group includes plasminogen activators, chymotrypsin, trypsin, and proteolytic enzymes produced by granulocytes and mast cells such as cathepsin G and neutrophil elastase (Biancheri 2012). Like MMPs, they take part in the normal and pathologic extracellular matrix protein turnover and regulate proinflammatory cytokines (Biancheri 2012). Trypsinogens are the most commonly studied enzymes from the serine protease group, but their role in IBD remains mainly unknown.

The pancreas secretes trypsinogens as well as enteropeptidase, which is also known as enterokinase, and it activates trypsinogens to trypsins when they enter the duodenum (Neurath 1976). The activated trypsins can further autoactivate the trypsinogens (Nemoda 2005). Physiologically, the pancreatic trypsins mediate the proteolysis of dietary proteins and they are the key enzymes involved in activating other pancreatic proenzymes (Neurath 1976).

Trypsinogens and trypsins are also expressed in extrapancreatic tissue because they are found in the Paneth cells of the small intestine (Bohe 1986), where they have a wide range of functions. For example, trypsins are capable of activating protease-activated receptors (PAR), especially PAR-2 (Cottrell 2003; Fox 1997), which helps regulate homeostasis, inflammation, and tissue repair (Macfarlane 2001). Both human and cancer-derived trypsins are able to activate promatrix metalloproteinases, especially proMMP-9 (Lauhio 2008; Paju 2001; Sorsa 1997; Lukkonen 2000).

The trypsinogen inhibitor TATI, which is the same molecule as the pancreatic secretory trypsin inhibitor (Huhtala 1982), inhibits the action of trypsin in a 1:1 molar ratio (Marchbank 1998). TATI is secreted together with trypsinogen into the pancreatic juice (Kazal 1948). However, TATI is also expressed in the mucous-producing cells of the small intestine and colon (Bohe 1986; Bohe 1990; Bohe 1997). The physiological role of TATI is to protect the pancreas from destroying inadvertently activated trypsin (Pubols 1974,) but experts also speculate that

extrapancreatic-secreted TATI has other roles as well. TATI is known to prevent the excessive digestion of GI mucous (Playford 1991; Marcbank 1996). The amount of TATI is reduced in IBD as well as in the gastric conditions associated with abnormalities in the internal mucous layer, such as gastric ulceration (Playford 1995). TATI also plays a role in ulcer healing and tissue regeneration (Marchbank 1995). However, the role of TATI in IBD remains mainly unknown.

Table 2. Expression of matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs) in the inflammatory bowel disease, healthy intestine, and inflammation.

MMP/ TIMP	IBD	healthy ileum
MMP-3	found in both children and adults and correlates with inflammation (Bailey 1994; Heushckel 200; Louis 2000; Meijer 2007a; von Lampe 2000; Kirkegaard 2004)	not found (Saarialho-Kere 1996; von Lampe 2000)
MMP-7	elevated in adult IBD patients (Rath 2006) weak expression in epithelium in pediatric UC patients (Mäkitalo 2010)	not determined
MMP-8	found in ulcer bases in neutrophils, but not in epithelium (Arihiro 2001)	sporadically in neutrophils (Arihiro 2001)
MMP-9	generally expressed in both children and adults (Baugh 1999; Kirkegaard 2004; Mäkitalo 2010)	unclear
MMP-12	expressed in the murine model of colitis in mice and adult IBD (Pender 2006; Vaalamo 1998)	not found (Vaalamo 1998; Salmela 2001)
MMP-26	in pediatric and adult CD patients (Mäkitalo 2010; Bister 2004)	found (Bister 2004)
TIMP-1	not found in pediatric IBD colon, but elevated in adult IBD patients (Mäkitalo 2010; Louis 2000)	not determined
TIMP-2	found in IBD patients regardless of inflammation (von Lampe 2000)	not determined
TIMP-3	found in IBD stroma and in pediatric and adult IBD patients and it correlates with inflammation (Vaalamo 1998; Mäkitalo 2010; Mäkitalo 2009)	found (Cesaro 2009)

healthy colon	**generally in inflammation**	**elsewhere in the gut**
not found (Saarialho-Kere 1996; von Lampe 2000)	MMP-3 activates TNF-α (Mohan 2002) TNF-α-antagonist downregulates and decreases activity (Meijer 2007a)	found in necrotizing enterocolitis (Pender 2003)
helps myofibroblasts to grow and function in colitis, resulting in the healing of GI ulcers (Bamba 2006)	MMP-7 activates TNF-α (Mohan 2002)	gastric mucosa, exocrine epithelium, necrotizing enterocolitis (Saarialho-Kere 1995; Honda 1669; Ravi 2007; Bister 2005b)
sporadically in neutrophils (Arihiro 2001)	not determined	not determined
not determined	TNF-α-antagonist downregulates (Meijer 2007; Mäkitalo 2009)	not determined
not found (Vaalamo 1998)	can activate TNF-α (Ravi 2007)	celiac disease, necrotizing enterocolitis, ischemic colitis (Vaalamo 1998; Bister 2005a, 2005b)
not determined	TNF-α-antagonist downregulates (Mäkitalo 2009)	celiac disease, necrotizing enterocolitis (Bister 2005a, 2005b)
not determined	TNF-α-antagonist downregulates (Mäkitalo L 2009)	necrotizing enterocolitis (Pender 2003)
found (Kirkegaard 2004)	not determined	not determined
found (Kirkegaard 2004; Cesaro 2009)	inhibits TNF-α; TNF-α-antagonist downregulates (Amour 1998; Mäkitalo 2009)	graft-versus-host disease (Vaalamo 1998; Salmela 2003)

2 DIAGNOSTICS AND ASSESSMENT OF DISEASE ACTIVITY

As previously presented, the symptoms of IBD are varied. The symptoms may also be linked to other diseases, some of which are more common among the pediatric population and some of which are rarer. Differential diagnoses have to be made between irritable bowel syndrome, bacterial infectious etiologies, and rare causes. In the case of rectal bleeding without diarrhea anal fissure, colorectal polyps, solitary rectal ulcer syndrome, and Meckel diverticulum have to be considered (Shikhare 2010). Clinical suspicion is raised if children have persistent (more than four weeks) or recurrent (more than two episodes in six months) symptoms that are linked to IBD (abdominal pain, diarrhea, rectal bleeding, weight loss) (ESPHGAN 2005). The diagnosis of IBD is based on the clinical picture, blood and fecal tests, ileocolonoscopy, gastroduodenoscopy (including both multiple biopsies), and radiological imaging of the small bowel (ESPHGAN 2005). The same tests can be used when evaluating disease activity during the disease course.

2.1 ENDOSCOPY

Endoscopy is the key investigation that is performed when IBD is suspected or when the disease activity is being evaluated. It makes a morphologic and histological examination of the bowel possible. There are multiple atypical phenotypes that make it difficult to differentiate between CD, UC, and IBDU in children. Many other conditions, such as active infectious colitis (Lamireau 2008), eosinophilic colitis, immunodeficiency disorders, and metabolic diseases such as glycogen storage disease type 1b and Hirschsprung's disease, can cause IBD-like alterations to bowel wall and histology (Russo 2008). The cleansing procedure performed before endoscopy might also induce macroscopic alterations (Kolho 2012). Since a colonoscopy in most cases requires bowel emptying and general anesthesia, it is a stressful procedure for children.

Colonoscopy, including intubation of the terminal ileum, is the gold standard of IBD diagnostics. Multiple biopsies have to be taken from all parts of the lower intestinal tract, even from the bowel wall that macroscopically shows no alterations. Visualizing the ileum is a crucial part of differentiating the CD from the UC because approximately 5 to 10% of children with CD present with isolated small bowel inflammation (de Bie 2012b; Sawcenko 2003). However, many CD patients have pancolitis (Sawcenko 2003). Upper endoscopy is a part of the diagnostics as well, regardless of the upper gastrointestinal symptoms (ESPHGAN 2005).

The macroscopic appearance of normal bowel mucosa is salmon-pink in color with a visible network of branching vessels that can be seen beneath the mucosa.

In histology or an histological examination, the normal mucosal surface is flat with a normal crypt density and architecture, the surface epithelium is intact, and the mucin content is normal and no neutrophil infiltration exists (Jenkins 1997).

Colonoscopy Findings in UC patients

In classic UC, the inflammation extends proximally from the rectum, and in approximately 80% of pediatric UC patients it extends to the splenic flexure or involves the whole colon (Kugathasan 2003; Van Limbergen 2008). The macroscopic features typically seen in UC patients are mucosal erythema, friability and granularity, and ulceration that may result in perforation (Glickman 2005). Pseudopolyps are more common in UC patients than in CD patients. Pseudopolyps have a short stalk and are smooth surfaced compared to adenomas. They are formed from surviving or regenerating mucosa or from granulation tissue (Russo 2008). Macroscopic skip lesions, wall thickening, strictures, fissures, and fistulas are not seen in children (Glickman 2005).

Histological Findings in UC patients

Histologically, the inflammation is limited to the mucosa and superficial submucosa—deeper layers might be involved in the toxic megacolon. The non-specific findings of UC include neutrophilic infiltration in the mucosa, cystic changes, and atrophy. In UC patients, the shape of crypts can change, resulting in an irregularity in the crypt's shape and placement, crypt branching, and crypt shortening, with a separation of the crypt bases from the muscularis mucosae by aggregates of lymphocytes and plasma cells. Cryptitis can also occur and crypt abscesses are possible; when diffuse, they suggest UC. Paneth cell metaplasia caused by an increase in the crypt epithelial turnover is possible and these cells can also occur in the left colon. The epithelium can degenerate and goblet cells may become depleted (Glickman 2005; Russo 2008).

Colonoscopic Findings in CD patients

In CD patients, the inflammation can exist in all parts of the GI tract. The inflammation is often focal and patchy and it is important to remember that negative biopsy findings do not exclude the diagnosis of CD in typical clinical patients. They require a further follow-up and a clinical evaluation of the symptoms to resolve diagnostic uncertainty (Glickman 2005). Isolated ileocecal inflammation occurs

in approximately 25% of pediatric CD patients, with both ileocolonic (60%) and extensive colitis (60%) being more common (Martín-de-Carpi 2012).

Early CD may macroscopically present only as apthous ulcers, which usually occur over a lymphoid follicle and are limited to the mucosa. Apthous ulcers can occur because of many other causes as well, which make them not diagnostic for CD. In CD patients, longitudinal fissures develop later and make the mucosa look like "cobblestone."

Histological findings in CD patients

Histology in CD is comparable to histology in UC, except that the inflammation is transmural. However, in some cases CD never extends deeper than mucosa (Glickman 2005). Transmural lymphoid aggregates are very typical for CD patients, but they are not 100% specific because they can be seen in fulminant colitis and in other transmural inflammatory conditions. Nonetheless, transmural lymphoid aggregates help in making a differential diagnosis between CD and UC (Glickman 2005). Other histological features typical for CD patients are lymphangiectasias, neural hyperplasia, and vascular changes (Russo 2008). Chronic ileal inflammation in CD patients includes villous blunting, crypt branching, irregularity, and atrophy. In the ileum, mucous glands may replace intestinal crypts so that the mucosa resembles that of the gastric antrum (pseudopyloric metaplasia) (Glickman 2005).

CD is one of the inflammatory conditions where granulomas can exist. CD-specific granulomas are well-formed and non-necrotic and they consist of tightly grouped aggregates of epithelioid histiocytes and are located basally away from the inflammation site (Russo 2008). Granulomas can be found in all parts of the GI tract and often in the terminal ileum or upper GI tract, which highlights the importance of gastroduodenoscopy (Glickman 2005). Infectious causes need to be excluded as a cause of granulomatous inflammation (Glickman 2005). Granulomas are only found in approximately half of all patients at diagnosis (de Bie 2012b). A study of adults by Heiman et al. (2008) linked a high prevalence of granulomas to the young age of the patient at diagnosis, the short duration of the disease, and a more extensive disease course.

Gastroduodenoscopy

Unspecific upper GI inflammation (chronic active gastritis or duodenitis) is typical for both CD and UC patients regardless of the symptoms (Scmitz-Moormann 1990; Ruuska 1994b; Tobin 2001; Sharif 2002; Abdullah 2002). Upper GI involvement is

neither sensitive nor specific for IBD patients (Sharif 2002; Xin 2004). Granulomas, duodenal cryptitis, and focal gastritis are more commonly seen in CD patients than are in other causes of upper GI inflammation (Tobin 2001; Sharif 2002). Macroscopic abnormalities considered significant for the diagnosis of CD are ulcers, cobblestoning, and stenosis (de Bie 2012b). However, esophagoduodenoscopy is crucial for the diagnosis of CD: In 7.5% of pediatric patients, the diagnosis of CD relied only upon the presence of granuloma(s) or ulcerations in the upper GI tract, with the ileocolonoscopy being normal (de Bie 2012b). A much wider range, between 2% and 21%, of diagnostic yield has been reported, and this might be because of the fact that the size, site, and number of biopsies taken can vary (Paerregaard 2009).

Exceptions found in colonocscopy that make differential diagnosis difficult

In UC patients, the terminal ileum may show some sign of inflammation known as backwash ileitis, which is linked to pancolitis and the severity of the inflammation. It occurs in approximately 25% of adult UC patients with pancolitis (Glickman 2005; Goldstein 2006; NASPHGAN 2007). The ileal inflammation seems to be a continuum of the inflammation in the proximal colon and is rare in left-sided colitis (Goldstein 2006; NASPHGAN 2007). Backwash ileitis has no strict morphological criteria. It is characterized as short involvement of the ileum, diffuse ileal erythema and granularity, lymphoid nodules, and mild mucosal inflammation. Linear ulcerations, deep fissures, granulomas, or gobblestoning are not related to backwash ileitis. The ileocecal valve is normal. No radiological or endoscopical signs of transmural disease exist (Goldstein 2006; NASPHGAN 2007; Russo 2008). Non-specific inflammation in the ileum in patients with the typical features of UC does not indicate a need to change of diagnosis to CD if other specific CD features do not exist (linear ulcers, cobblestoning, granulomas) (NASPHGAN 2007). Periappendiceal inflammation, also known as "cecal patch," especially in patients with left-sided colitis, is only a morphological variant of UC and does not support a CD diagnosis if extensive and significant cecal inflammation is absent (Glickman 2005; NASPHGAN 2007).

Newly diagnosed and un-treated pediatric UC patients may present with rectal sparing (23% to 30%) (Glickman 2004; Washington 2002; Rajwal 2004). Accordingly, colonic inflammation can be less severe in newly onset pediatric UC compared with adults and it can be patchy (Glickman 2004; Washington 2002). One potential explanation for this patchy inflammation in both the rectum and colon has to do with the shorter disease duration before endoscopy (Washington 2002). Also, the data from adults suggest that medication (topical 5-ASA, sulphasalazine, and steroids) may alter the pathology of the bowel so that inflammation may be patchy and the rectum spared (Kim 1999; Bernstein 1995).

If a differentiation between CD and UC cannot be made, the disease is diagnosed as IBDU. However, the small bowel has to be evaluated before making an IBDU diagnosis (see next chapter). IBDU patients need a good clinical follow-up and repetitive endoscopies to ensure that a specific diagnosis can be made in the majority of cases. The subtype classification of IBD is important because the surgical and medical management procedures are different for UC and CD patients (Glickman 2005).

2.2 SMALL BOWEL IMAGING

Together, ileocolonoscopy and gastroduodenoscopy provide a good means for evaluating the upper and lower part of the GI tract. However, a large part of the small bowel remains unvisualized if only those two methods are used. The exact frequency of small bowel disease in CD patients is unknown; most likely, the presence has been underestimated and it may have an increased frequency among children and adolescents (Cuffari 2005). It is known that in less than 10% of pediatric CD patients, the diagnosis relies only upon small bowel imaging, with terminal ileum being normal at ileocolonoscopy (de Bie 2012b). Typical symptoms in patients who experience small bowel disease are weight loss, linear growth, and pubertal failure, and those patients more commonly suffer from strictures as well (Cuffari 2005).

Radiological evaluation of the small bowel is mandatory for all patients except in the case of making a definite diagnosis of UC. An IBDU diagnosis cannot be made before evaluating the small bowel (ESPHGAN 2005). Table 3 summarizes the advantages and disadvantages of the different methods used to evaluate small bowel disease.

Magnetic resonance enterography (MRE) and computed tomography (CT) offer a way to achieve direct visualization of the bowel wall and mesentery and evaluate the disease extent and extramural complications. Before MRE imaging, the bowel should be distended with an oral contrast agent; it can also be given using a nasojejunal tube. Sufficient distension is crucial because collapsed loops can lead to false-positive findings of wall thickening (Di Nardo 2012). Agents such as n-butyl-ioscine or glucacon and breath-hold acquisition are used to prevent motion artifact. An intravenous contrast medium increases the sensitivity for detecting CD alterations. With both CT and MRE, the mucosa enhances and thickens if it is inflamed (Torkzad 2012; Toma 2007). In those patients with longstanding CD, the mural stratification might be lost. The thickening of the bowel wall might be a mark of irreversible fibrosis, which can be accompanied by prestenotic dilatation, indicating bowel stricture (Toma 2007). Mucosal hyperenhancement and bowel wall thickening have been the most sensitive predictors of active disease in children (Wallihan 2012;

Torkzad 2012). The availability of fast breath-hold sequences, a decreased scanning time, and increased resolution have favored the MRE use in small bowel imaging (Stuart 2011). In fact, MRE is the preferred technique for evaluating the small bowel in a pediatric population (van Assche 2010). The advantages of MRE are that it lacks ionizing radiation, that it has superior soft tissue contrast, which enables a differential diagnosis between edema and fibrosis, that it has multiplanar capability, that it depicts transmural and extramural disease, and that it provides functional and quantitative information about the bowel wall (Toma 2007; Di Nardo 2012). In pediatric studies, the sensitivity to detect active disease in the distal ileum, when compared to the histological findings at ileocolonoscopy or the surgical specimens, has ranged between 81% and 90% (Wallihan 2012; Dillman 2011). However, the sensitivity to detect colonic disease is not as good: It ranges between 32% and 45%. False-negative findings are also common (Wallihan 2012; Dillman 2011).

With wireless capsule endoscopy (WCE), a patient swallows a capsule camera (measures 26x11mm), or else the capsule is inserted into the duodenum by endoscope. The capsule then travels through the bowel and takes two frames per second. Radio signals transmit the collected information from the camera to the recorder, which the patient carries attached to his or her belt. The battery life of the camera is approximately ten hours and the capsule exits the bowel naturally. Afterwards, the telemetrically transmitted images are evaluated using a computer program (Di Nardo 2012). Table 3 lists the problems associated with the WCE. The WCE is a significantly better diagnostic tool in established non-stricturizing CD compared to other tools, such as enteroscopy, small bowel fluoroscopy, CT (Dionisio 2010), MRE, and ultrasound (U.S.) (Di Nardo 2011). On the other hand, the capsule retention rate is increased in CD and that is why MRE is preferred to WCE in the case of the suspicion of stricturizing CD (Atay 2009; Casciani 2011; Cohen 2011). However, the retention rate was 2.4% in large pediatric retrospective WCE and patency capsule (the soluble capsule that is placed similar to the WCE to ensure that there are no strictures in the bowel) study (n=277) that included mostly (86%) suspected or confirmed CD patients (Cohen 2012). For 23 pediatric patients who were at risk of capsule retention, the patency capsule was placed inside the patient before the video capsule. The WCE was cancelled for three patients because of a failed passage of patency capsule. This suggests that the patency capsule might be useful in select patients with a high risk of capsule retention (Cohen 2012). However, the patency capsule does not detect all strictures of the small bowel and false-positive findings occur that might limit the usefulness of it (Yadav 2011).

Balloon-assisted enteroscopes (BAE) have a working length of 200 cm and an outer diameter of 9.4 mm. All of the small bowel can be evaluated in single or multiple procedures (Di Nardo 2012). Enteroscopy is an invasive procedure and a perforation risk exists, especially in the case of active CD with adhesions and

strictures, which might also limit the examination and the evaluation of the entire small bowel. The advantage of enteroscopy is that it makes it possible to dilate the strictures and take biopsies (Pohl 2007). The success rate of enteroscopy is less than that of WCE (Di Nardo 2012). Enteroscopy as a diagnostic tool in pediatric IBD is not broadly used and only a few studies with small patient samples exist. These have shown that enteroscopy is capable of detecting disease lesions that are not seen when using WCE or MRE (Di Nardo 2012, 2011; de Ridder 2012). In addition, enteroscopy can alter the therapy (de Ridder 2012) and allow for successful dilatation of small bowel strictures (Di Nardo 2012, 2011).

Table 3. Methods for evaluating small bowel disease (modified from Cuffari 2005; Di Nardo 2012; Stuart 2010; Turner 2012).

Imaging	Advantages	Limitations
Barium fluoroscopy	• excellent mucosal detail • ability to also detect early mucosal changes	• high radiation dose • less sensitive to detecting transmural inflammation or extraluminal complications, distal ileal inflammation or penetrating complications
Ultrasound	• low cost and widely available • Doppler helps to differentiate the nature of stenosis • extramural complications visualized • able to detect CD complications, such as strictures • high negative predictive value • good screening test	• low pick-up for mild disease • operator-dependent • CT and MRE are better at detecting CD complications, such as strictures
CT	• shows bowel wall thickening and enhancement • the entire colon can be visualized • extramural complications can be visualized • able to detect CD complications, such as strictures	• high radiation dose • unreliable for proximal disease • contrast medium has risks • bowel wall distension can be incomplete • risk of motion artifact
MRE	• no exposure to ionizing radiation • multiplanar imaging; able to detect bowel wall thickening and enhancement • excellent soft tissue contrast • extramural complications can be visualized • able to detect CD complications • enables differentiation of edema and fibrosis	• bowel distension can be incomplete • risk of motion artifact • need sedation in young • contrast medium has risks • bowel preparation acquired
WCE	• the entire colon can be visualized • easy for patient • high negative predictive value • good evaluating tool in known CD patients	• movement of capsule cannot be controlled • inflammation can be missed • difficult to localize disease • transmural or extraintestinal alterations cannot be seen • risk of capsule retention, especially in the case of CD • poor specificity
Enteroscopy	• therapeutic procedures possible • possibility to take biopsies	• invasive procedure • risk of bowel perforation • transmural or extraintestinal alterations cannot be seen • difficult to localize disease • lacks systematic study

CT = computed tomography
MRE = magnetic resonance enterography WCE wireless capsule endoscopy
WCE = wireless capsule endoscopy

2.3 LABORATORY AND FECAL MARKERS

Table 4 shows the frequency of abnormal laboratory values found in both CD and UC patients at diagnosis. Laboratory and fecal markers help differentiate IBD from other causes of abdominal pain and other IBD-like symptoms. However, no single laboratory marker is diagnostic; a recent review of 526 newly diagnosed IBD patients revealed that all blood tests (hemoglobin level, platelet count, albumin level, erythrocyte sedimentation rate) were completely normal in 54% of children with mild UC and in 21% of children with mild CD at the time of diagnosis. However, only 3.8% of children with CD and 4.3% of children with UC who had moderate or severe forms of the disease at endoscopy had normal laboratory markers. ESR is the marker that is least likely to be normal at diagnosis (table 4), (Mack 2007).

Table 4. Frequency (%) of abnormal laboratory markers at diagnosis in 526 newly diagnosed IBD patients (data adopted and modified from Mack 2007).

	Laboratory marker			
	ESR	Hb	platelet count	Alb
CD				
mild	65	49	42	18
moderate	84	76	57	49
severe	86	80	66	69
UC				
mild	26	38	5	13
moderate	72	69	51	24
severe	85	95	50	90

Laboratory screening can be combined with several tests of serologic markers. ASCA (antibody against Saccharomyces cerevisiae) is found more often in CD patients (40-70%) than in UC patients (0-15%) or in healthy controls and is linked to the ileal and cecal disease (NASPHGAN 2007; Ruemmelle 1998; Hoffenberg 1999; Bartůnková 2002; Zholudev 2004). pANCA (perinuclear anti-nuclear cytoplasmic antibody) is more common in UC patients (60-70%) than in CD patients (10-25%) (Ruemmelle 1998; Bartůnková 2002; Zholudev 2004). However, CD patients who have colitis are often pANCA positive, which makes a differential diagnosis difficult (Ruemmele 1998; NASPHGAN 2007). Serological markers are not specific for IBD and can be found in a diversity of other diseases, such as celiac disease (Ashorn 2008), chronic granulomatous disease (Yu 2011), and PSC (Muratori 2003). The

tests are also expensive and they either do not measure disease activity or they cannot determine the site or extent of the disease; thus, they are not widely used in IBD diagnostics. The use of serologic markers is questionable in younger patients because it is less likely that the markers are positive (Mamula 2002).

In addition to blood tests, fecal tests are used to evaluate IBD. While the infectious causes of enteritis (Escherichia coli, Salmonella, Shigella, Yersinia, Campylobacter, Clostridium difficile toxin, and parasites) should be excluded by fecal cultures, a positive culture finding does not exclude IBD (ESPHGAN 2005). Fecal calprotectin can be found in the cytosol of inflammatory cells, it can remain in stool samples for up to seven days, and it can be increased in many intestinal pathologies but not in moderate gastrointestinal bleeding (Fagerhol 2000). This marker increases with colonic inflammation and correlates with IBD disease activity (Bunn 2001a, 2001b; Kolho 2006). Ileal disease as well increases the amount of calprotectin (Shaoul 2012). In a meta-analysis that included 371 children with confirmed IBD, the sensitivity of the calprotectin test to detect pediatric IBD was 0.92 (CI 0.84 to 0.96) and the specificity was 0.76 (CI 0.62 to 0.86) (van Rheenen 2010). Thus, normal levels of fecal markers do not exclude the need for endoscopy in patients with typical IBD symptoms. However, for patients who undergo endoscopy for suspected IBD, the calprotectin levels are significantly higher in those with IBD than in children without IBD (Henderson 2012).

3 TREATMENT

The goals of treatment for pediatric patients should include induction of remission, preventing disease relapses, optimizing growth and development, improving QoL, and limiting disease complications. While the definition of remission varies between studies, steroid-free remission is still a clinically relevant goal, especially for children. The initial treatment depends on the severity and distribution of the disease. These are determined by endoscopic, radiologic, and laboratory investigations together with the clinical picture. Table 5 shows the Paris classification used to evaluate the severity and distribution of the disease in pediatric CD and UC patients (Levine 2011).

Table 5. Paris classification of pediatric Crohn's disease and ulcerative colitis (modified from Levine et al. 2011).

Crohn's disease		
Age at diagnosis	A1a	0 - <10 years
	A1b	10 - <17 years
	A2	17 - 40 years
	A3	>40 years
Location	L1	distal 1/3 ileum ± limited cecal disease
	L2	colonic
	L3	ileocolonic
	L4a	upper disease proximal to ligament of Treitz
	L4b	upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum
Behavior	B1	non-stricturing, non-penetrating
	B2	stricturing; occurrence of constant luminal narrowing with prestenotic dilatation and/or obstructive signs or symptoms
	B3	penetrating; occurrence of bowel perforation, intra-abdominal fistulas, inflammatory masses and/or abscesses at any time during the course of the disease (excludes isolated perianal or rectovaginal fistulas)
	B2B3	both penetrating and stricturing, either at the same time or different times
	p	perianal disease modifier
Growth	G0	no evidence of growth delay
	G1	growth delay
Ulcerative Colitis		
Extent	E1	ulcerative proctitis
	E2	left-sided UC (distal to splenic flexure)
	E3	extensive (hepatic flexure distally)
	E4	pancolitis (proximal to hepatic flexure)
Severity	S0	never severe
	S1	always severe
L4a/L4b may coexist with L1, L2, and L3, respectively Severity is defined by Pediatric Ulcerative Colitis Activity Index (PUCAI) ≥65		

For UC patients, the disease activity can be evaluated using the Pediatric Ulcerative Colitis Activity Index (PUCAI) (Turner 2007), whereas for patients suffering from Crohn's disease, it can be evaluated using the Pediatric Crohn's Disease Activity Index (PCDAI) (Hyams 1991). The PUCAI is useful in daily practice

because the index can be determined without invasive or radiological investigations compared to PCDAI, which requires laboratory investigations (table 6).

Table 6. Clinical indexes used to evaluate disease activity in pediatric inflammatory bowel disease.

PEDIATRIC ULCERATIVE COLITIS ACTIVITY INDEX, PUCAI	
daily average during the last two days, or, if clinical status is changing rapidly, during the most recent 24-hour period	
Abdominal pain	
none	0
pain can be ignored	5
pain cannot be ignored	10
Rectal bleeding	
none	0
small amount in less than 50% of stools	10
small amount in most stools	20
large amount, >50% of the stool content	30
Stool consistency	
formed	0
partially formed	5
completely unformed	10
number of stools per 24-hour period	
0-2	0
3-5	5
6-8	10
>8	15
nocturnal stools (any episode that caused the patient to wake up)	
no	0
yes	10
activity level	
no limitation	0
occasional limitation	5
severe restricted activity	10
Total score: <10 in remission; 10-34 mild disease; 35-64 moderate disease; >65 severe disease Treatment response is achieved if the PUCAI drops by at least 20 points (Turner 2012).	

PEDIATRIC CROHN'S DISEASE ACTIVITY INDEX, PCDAI	
daily average during the last week	
Abdominal pain	
none	0
mild and temporal; does not interfere with activities	5
moderate/severe; daily and nocturnal, affects activities	10
Stools per day	
formed stools or up to 1 liquid stool, no blood	0
up to 2 semi-formed stools with a small amount of blood, or 2-5 liquid stools without blood	5
more than 5 liquid stools, nocturnal diarrhea, rectal bleeding	10
General well-being	
well, no limitation of activities	0
moderate, occasional difficulty in maintaining appropriate activities	5
very poor, frequent limitation of activity	10
Weight	
stable weight gain	0
weight loss 1-9%	5
weight loss >10%	10
Height at diagnosis	
<1 channel decrease	0
channel decrease between 1 and 2 channels	5
channel decrease >2	10
Height during follow-up	
height velocity ≥ -1SD	0
height velocity between -1SD and -2SD	5
height velocity ≤ 2SD	10
Abdomen	
no tenderness, no mass	0
tenderness, or mass without tenderness	5
tenderness, involuntary guarding, definite mass	10
Perirectal disease	
none, asymptomatic tags	0
inflamed tags or 1-2 indolent fistula(e) or fissure(s), scant drainage, no tenderness	5
active fistula, drainage, tenderness, or abscess	10

Extraintestinal manifestations (fever ≥38.4ºC for 3 days during the past week, definite arthritis, uveitis, erythema nodosum, pyoderma gangrenosum	
none	0
1	5
≥2	10
Hematocrit	
children under 10 years of age	
>33	0
28-32	2.5
<28	5
girls 11-19 years of age	
>33	0
29-33	2.5
<29	5
boys 11-14 years of age	
>34	0
30-34	2.5
<30	5
boys 15-19 years of age	
>36	0
32-36	2.5
<31	5
ESR	
<20	0
20-50	2.5
>50	5
Albumin	
>35	0
31-34	5
<30	10
Total score: <10 disease in remission; 11-30 mild disease; >30 moderate or severe disease	

The first treatment is usually medical, but in certain cases surgery is also necessary during the early disease phase (see section 3.2). Recently, new guidelines for treating pediatric UC were published (Turner 2012). However, there is still a lack of good treatment guidelines for pediatric CD patients and most of the knowledge

about the treatment is based on adult trials. Because of differences in physiology, pharmacokinetics, pharmacodynamics, and the aims of the treatment, the data from adult studies alone are insufficient to guide pediatric treatment. Pediatric patients often need nutritional support to ensure adequate caloric intake because of the fact that during the active disease phase, the patient's caloric intake decreases and the symptoms cause a loss of appetite. A proper nutritional status is required for normal development and growth. In children, and especially in teens, the disease also has a big effect on their mental well-being and many patients benefit from psychiatric care.

3.1 MEDICATION

Table 7 lists the treatments used for pediatric CD and UC patients. For mild to moderately active UC, the combined therapy of administering oral medication and rectal enemas of 5-aminosalisylic acid (5-ASA) is more effective than oral therapy alone (Marteau 2005), and rectal enemas are recommended if the patient can tolerate them. For CD patients, the alternative to primary steroid treatment is exclusive enteral nutrition (EEN). With EEN, the patient ingests enteral formulas either orally or via a nasogastric tube for 12-14 hours for eight to six weeks. The caloric intake is calculated using the recommended daily allowance, as well as levels of activity, fever, and growth. During the treatment period, patients do not eat any other food: Only additional water is allowed. After six to eight weeks, the feeding is gradually (during the course of three weeks) replaced by unrestricted normal food. During treatment, the antigen load to the gut is markedly reduced and fiber is eliminated compared to unrestricted food intake (Ruuska 1994a). EEN has been reported to be as effective as traditional steroid treatments (Ruuska 1994a; Heuschel 2000; Dziechciarz 2007). The major benefit of EEN is that it improves growth and development without the side effects of steroid treatment (Heuschel 2000). However, according to the Cochraine review (Zachos 2007), steroids are more effective in inducing remission (table 8).

Table 7. Induction and maintenance therapy in pediatric ulcerative colitis and Crohn's disease. Steroid dependency is defined: whereas remission is achieved via steroids, symptoms recur within 3 months of complete taper or when the dose is lowered or if the use of steroids lasts longer than 14-16 weeks (Turner 2012).

1) PRIMARY TREATMENT; AFTER ENDOSCOPY AND EVALUATION OF THE CLINICAL PICTURE	
Ulcerative colitis	
mild inflammation; diarrhea during the daytime or soiling	5-ASA (oral and possibly rectal enema)
moderate inflammation, bloody diarrhea during the daytime	oral prednisolone+5-ASA
severe inflammation, more than 5 bloody diarrhea stools during the daytime and diarrhea at night	i.v. prednisolone
no response to i.v steroid	i.v. siclosporine
no response to i.v siclosporine	i.v. infliximab
fulminant colitis not reacting to treatment; toxic megacolon	colectomy and later proctocolectomy and j-pouch
Crohn's disease	
mild ileocolonic inflammation, no delay in puberty	5-ASA
moderate inflammation	oral prednisolone+AZA/ exclusive enteral nutrition
severe inflammation or no response to oral treatment	i.v. prednisolone
no response to steroids and AZA	methotrexate
disease resistant to other treatments or extensive small bowel disease and delay in puberty in acute phase	i.v. infliximab
no response to proper medical treatment or stricture in the bowel	bowel resection

2) MAINTENANCE THERAPY; GOAL OF TAPERING THE STEROID DOSE	
Ulcerative colitis	
remission achieved by steroids and maintained when tapering	5-ASA
steroid dependency, introduction of AZA	AZA+oral prednisolone
no response to AZA, steroid dependency or remission achieved by i.v. infliximab	i.v. infliximab
treatment-resistant disease or dysplasia in histology	proctocolectomy+j-pouch
Crohn's disease	
if remission is achieved by steroids	AZA ± 5-ASA
if remission is achieved by infliximab or disease is dependent on steroids	i.v. infliximab, bridging to AZA
delay in puberty and localized disease, steroid dependency and resistant/intolerant to other treatments or stricture in the bowel	bowel resection

5-aminosalisylic acid (5-ASA), azathioprine (AZA)

For CD patients, azathioprine (AZA) or 6-mercaptopurine (6-MP) are usually started together with induction therapy because the healing effect of these agents begins only after 3-6 months of use. If a patient cannot tolerate thiopurine therapy, methotrexate, another immunosuppressant, can be used as an alternative. If this therapy fails, or if the patient is still dependent on steroids, then a TNF-α-antagonist, infliximab, or surgery need to be considered. Intravenous infliximab was the first biological agent to be approved (1999) for pediatric patients with moderate to severe CD (Sandhu 2010), and recently it was approved for patients with moderately or severely active pediatric UC (Hyams 2012). It is a chimeric tumor necrosis factor-α (TNF-α) antibody that blocks the action of TNF-α (a pro-inflammatory cytokine that is involved in systemic inflammation and acute phasereaction) (Hanauer 2006; van Deventer 1997). If remission is induced with infliximab, then this indicates the need for maintenance therapy (Sandhu 2010).

The use of oral medical therapy during the first year after diagnosis was studied recently in Finland using two national registers: The Drug Reimbursement Register and the Drug Purchase Register. Three out of four pediatric IBD patients used corticosteroid during the three first months after diagnosis, and the use of steroids was stable during the time period 1999-2009. Nearly all of the IBD patients used 5-ASA after diagnosis, reflecting the presence of colonic disease also in CD patients. The use of AZA was more common among CD patients than UC patients; nonetheless, the use of AZA has become more frequent. The combined therapy of administering 5-ASA/steroid and AZA has become more widespread among CD patients as an initial treatment, reflecting the more aggressive therapeutic strategy being used for CD (Virta 2012b). The management of patients with a medically stable disease varies. According to a recent American study of 121 pediatric CD patients, 10.7% of patients were on antibiotics after a median of 46.4 months from diagnosis, while 8.3% of them were on steroids, 46.3% of them were on 5-ASA, 47.1% of them were on immunomodulators, and 36.3% of them were on biologicals. In a UC/IC cohort (n=43, follow-up median 47.3 months), 62.7% of patients were on 5-ASA, 4.7% of them were on steroids, 30.2% of them were on immunomodulators, and 9.3% of them were on biologicals (Rosen 2011). This reflects the fact that CD patients need more aggressive treatment to remain in remission compared to UC patients.

3.1.2 Efficacy and side-effects of different medicines

Table 7 presents the patients' response rates to different therapies used in pediatric IBD. In most of the studies, remission was determined as clinically non-active disease without steroid treatment.

Table 8. Short- and long-term responses to conservative treatments used in pediatric inflammatory bowel disease.

Medicine	Short-term response			Long-term response			Ref
	%	time		%	time		
steroids	63%	1 month	CD	nd	nd	nd	Jakobsen 2011b
	64%	1 month	UC	nd	nd	nd	
5-ASA	nd	nd	nd	40%	1 year	UC	Zeisler 2012
EEN	71%	8 weeks	CD	41%*	1 year	CD	de Bie 2012a
thiopurines	50%	4 months	CD/UC	40%	1 year	CD	Turner 2011 Wilson 2010 Hyams 2011 Riello 2011
	nd	nd	nd	49%	1 year	UC	
methotrexate	37%	6 months	CD	25%	1 year	CD	Willot 2011
	25%	6 months	UC	13%	1 year	UC	
infliximab	90%	8 weeks	CD	50-80%	1 year	CD	Wilson 2010 Assa 2012 de Bie 2012c Hyams 2012
	73%	8 weeks	UC	29%	1 year	UC	

* Most of the patients had maintenance therapy involving azathioprine.
EEN=exclusive enteral nutrition.
nd=not determined

Overall, 60% of pediatric patients are in steroid and surgery free remission after 2 to 3 years of medication (Hyams 2009; Assa 2012). Infliximab treatment has been reported to improve linear growth, especially in prepubertal patients (Malik 2011; Walters 2007). However, controversial data also exist that report no significant growth improvement after infliximab therapy (Sinitsky 2010; Diamanti 2009; Pfefferkorn 2009; Wewer 2006).

Still, approximately 10% of CD patients and one-third of UC patients initially do not respond to infliximab, whereas 5 to 13% of CD patients discontinue therapy because of the side effects (Schnitzler 2009; Rutgeerts 2005; Assa 2012). Some patients also lose their initial therapeutic response at a median of eight months from the first dose (de Bie 2012c; Assa 2012). The reason for this variation remains unknown, but it is likely caused by multiple factors such as the disease and immune phenotype as well as genetic background (de Bie 2012c). In adult CD patients, the annual risk of loss of response to infliximab therapy is 13% per patient per year (Gisbert 2009). The loss of response to infliximab therapy may be managed by increasing the dose (Rutgeerts 2004) or by shortening the infusion interval (Schnitzler 2009; Assa 2012). Still, 47% of early CD respondents require surgery within one and half years of the follow-up (Afzal 2007). Corresponding findings come

from adult data, where 17% of CD patients and 36% of UC patients treated using infliximab therapy underwent abdominal surgery after a mean of 2.6 infusions of infliximab (Ljung 2004), reflecting the fact that biological treatment is not always curative.

The challenge in pediatric care is the side effects of the medications. Table 9 shows a side-effect profile for the most commonly used medicines. The disease is steroid-dependent in approximately 30 to 40% of pediatric patients, but the definition of dependency varies (Sidoroff 2012). Oral budesonide has fewer side effects compared to oral prednisolone because the major part of the released medicine is absorbed in the distal ileum or colon. However, the use is limited to mild/moderate forms of ileocecal Crohn's disease.

Adverse effects cause a withdrawal of thiopurines in approximately 9% of users (Prefontaine 2010), while the corresponding number for methotrexate users is 14% (Willot 2011). There is heterogeneity in the drug-metabolizing enzyme of thiopurines. It may be useful to determine the genotype because slow metabolizers are more prone to side effects (Shikhare 2010).

Taking infliximab can cause many different kinds of side effects. The symptoms of acute infusion reactions include shortness of breath, flushing, nausea, headaches, hypoxemia, thachycardia, or even anaphylaxis. Fifteen percent of all infliximab-treated pediatric patients experience such side effects. Most of these reactions are mild and the patients react well to stopping of treatment and/or reducing the flow rate of the infusion. Premedication (antihistamines, antipyretics, azathioprine, or steroids) have not been effective in preventing these reactions (de Bie 2012c; Kolho 2007; Hämäläinen 2012). Infliximab can activate latent mycobacterium tuberculosis. Thus, the latency has to be tested for prior to starting the medication by taking a chest X-ray of the patient (de Bie 2012c; Shikare 2010). Hepatosplenic T-cell lymphoma is the feared side effect because this fatal form of lymphoma has been observed in young IBD patients exposed to immunomodulators while taking biologicals, and the concomitant therapy should not be used for such pediatric patients (Kotlyar 2011; Diak 2010). Another problem, however, is that we are not aware of the long-term side effects of biologicals because they have only been used since 1999.

Table 9. Side effects associated with the medicines used to treat pediatric inflammatory bowel disease.

Medicine	Side effect	Reference
Steroids	moon face, acne, weight gain, hirsutism, mood swings and psychoses, osteopenia, permanent skin striae, cataracts, growth retardation	Sidoroff 2012
Aminosalicylates	headache, diarrhea, abdominal pain, nausea, dyspepsia, rash, intestinal nephritis	Moyer 2008
Thipurines	pancreatitis, fever, arthralgias, malaise, nausea, diarrhea, rash, myelosupression, opportunistic infections, hepatitis	Sandborn 1996
Methotrexate	nausea, vomiting, rash, mucositis, headache, hepatitis, cirrhosis, myelosuppression, pneumonitis, serious infections	Sandborn 1996, Willot 2011
Infliximab	infusion reactions, opportunistic infections, reactivation of latent infection, liver enzyme elevations, delayed hypersensitivity reactions, including joint pain, and swelling associated with fever and/or rash rarely: systemic lupus erythematosus-like syndrome, vasculitis, hepatosplenic T-cell lymphoma, cardiac symptoms, psoriasis, skin eruptions	de Bie 2012c, Shikare 2010

3.2 SURGERY

According to recent reports, approximately 20 to 30% of pediatric CD patients undergo surgery during the first decade after diagnosis (Pacilli 2011; Turunen 2009). The corresponding figure for UC patients is 25% (Turunen 2009; von Allmen 1995; Coran AG 1985). Most UC patients undergo their first surgery within the three first years after the diagnosis (Turunen 2006). Compared with adult data, the median time to operation is shorter for pediatric UC patients (Van Limbergen 2008) and children undergo a colectomy more often; only 10% of adult patients require a colectomy within the first ten years after diagnosis (Hoie 2007). This may not be the case for pediatric CD patients because the amount of time to the first resection was longer for the pediatric population than for adults (Van Limbergen 2008). Bowel resection during the first years after diagnosis is more common in adult population: 30% of adults undergo an operation within the first five years after diagnosis (Ramadas 2010).

The rate of surgery among pediatric CD patients has been decreasing over time. According to a recent Canadian report, the surgical rates within three years of diagnosis decreased from 18.8% to 13.6% between the years 1994 and 2007. No change was seen in the UC surgery rate (Benchimol 2011b). Comparable results have been reported in Denmark (Jakobsen 2011a). The reasons for the decrease in surgical rates are numerous and the impact of immunomodulative medication remains unclear; after infliximab treatment, 25-35% of pediatric CD patients and

29-39% of pediatric UC patients still need surgery (de Bie 2012b). For adult CD patients during the years 1993 to 2004, the surgery rates for small bowel surgery and right colon resection have remained stable, but the rate of surgeries for left colon resection, other colon resection, and rectal resection have declined moderately, even though the surgical repair of small bowel fistulas has increased (Jones 2010).

3.2.1 Indications

For both CD and UC patients, one of the leading indications for surgery is the presence of an active disease despite intensive medical therapy and steroid dependency. The patient may also end up needing acute surgery because of a toxic megacolon, intractable hemorrhage, or bowel obstruction (Barrena 2010; Ba'ath 2007). For UC patients, the surgery, usually a total proctocolectomy, can cure the disease (von Allmen 1995). For CD patients, the decision to operate is more complicated because the disease can appear in the whole GI tract and resection of one inflamed bowel segment does not remove the risk of a recurrent disease and further resections (Pacilli 2011; Baldassano 2001; Besnard 1998). For CD patients, however, bowel resection can be the only alternative in certain cases because medical therapy may fail to treat the complications of transmural inflammation (strictures, fistulas, perforation, and severe perianal disease) (Wiese 2012; Pacilli 2011). Surgery is one way to manage the growth failure, which is a major problem among pediatric CD patients (Motil 1993). Resecting an inflamed bowel segment before the end of puberty often offers a disease-free interval for normal growth and development to occur (Ranger 2006; Newby 2005; Besnard 1998).

The big question for IBD patients is, when is the right time to operate? Currently, there are potent medications. But is surgery safe when a patient is receiving high doses of steroids and other medications? It is known that high preoperative steroid doses increase the risk of complications (Uchida 2010; Markel 2008). In general, it is recommended that surgery should be performed, especially for UC patients, electively in a calm disease phase and, if possible, with only a minimal dose of steroids.

3.2.2 Characteristics of the patients who undergo surgery

The clinical picture of IBD varies radically and it is difficult to anticipate a patient's disease course. As many as 37% of pediatric CD patients suffer from aggressive disease, defined as the development of complex perianal disease, colonic resection, more than one small bowel resection, or definite stoma, a median of five years after

diagnosis (Savoye 2012). Currently, we are unable to determine which patients will eventually undergo surgery. This would be important because those patients would possibly benefit from aggressive treatment already at the time of diagnosis (Jakobsen 2011a; Vernier-Massouille 2008; Gupta 2006). However, aggressive treatment strategy is not beneficial for all patients because of the known side effects and high costs of the treatment (table 7). Table 10 presents the known surgical risk factors for pediatric IBD patients. However, many of these clinical risk factors are actually complications of the disease and a proper model that shows how to prevent them from occurring is lacking.

Table 10. Clinical signs that predict the need for surgery among pediatric inflammatory bowel disease patients.

Factors that predict surgery for pediatric Crohn' disease patients	**Reference**
less than 14 years of age at diagnosis	Savoye 2012
growth delay at diagnosis and during follow-up	Savoye 2012; Vernier-Massouille 2008; Gupta 2006; Schaefer 2010
stricturing and penetrating disease behavior at diagnosis and during follow-up	Vernier-Massouille 2008; Gupta 2006
complications during follow-up	Vernier-Massouille 2008
use of corticosteroids	Vernier-Massouille 2008
positive ASCA serology ± negative pANCA serology	Amre 2006; Gupta 2006
increased immune reactivity	Dubinsky 2008
leukocytosis at diagnosis	Gupta 2006
hypoalbuminea at diagnosis	Gupta 2006
female gender	Gupta 2006
primary diagnosis UC	Gupta 2006
NOD2/CARD15 risk alleles	Russel 2005; Kugathasan 2004
Factors that predict surgery for pediatric ulcerative colitis patients	
steroid dependency	Falcone 2000
pancolitis	Falcone 2000; Gower-Rousseau 2009
extraintestinal manifestations at diagnosis	Gower-Rousseau 2009
disease extension during follow-up	Gower-Rousseau 2009
severe disease (>5 stools/day and daily gross blood) at diagnosis	Hyams 1996
risk score calculated from white blood cell count and hematocrit at diagnosis	Moore 2011

3.2.3 Resection types

Table 11 presents the different surgical options for pediatric IBD patients.

Ulcerative colitis

Nowadays, a proctocolectomy that includes resection of both the colon and rectum is the preferred elective surgery for UC patients. A permanent stoma is avoided by either performing a straight ileoanal anastomosis (SIAA) or by constructing an ileal reservoir and ileal pouch anal anastomosis (IPAA) (figure 2). The pouch procedure was initially developed by Parks and Nichols in the 1970s (Parks 1978), and nowadays it is primarily used for UC patients (Bach 2007) and has displaced the SIAA. For an IPAA procedure, a pouch is formed from the terminal ileum joining the antimesentric borders of the ileal limbs and the end of the pouch is connected to the analcanal (Nandivada 2012).

The anastomosis can be performed in two alternative ways, either by performing a rectal mucosectomy and hand-sewn ileoanal anastomosis or by using an end-to-end circular stapler without separating the mucosal sleeve (figure 3) (Lillehei 2007). With the latter procedure, 2-4 cm of rectal mucosa is preserved; this may later be associated with a risk for cancer and recurrent disease, so-called cuffitis (Remzi 2003). There are many pouch models, but a J-pouch is preferred because of its limited use of the bowel and because it can reliably be emptied and is easy to construct (Utsunomiya 1980).

A pouch procedure might be performed in one, two, or three stages. For a procedure involving only one stage, the proctocolectomy and IPAA are performed without temporarily diverting the ileostomy. This is an alternative for patients who have a good nutritional status and no risk factors for pelvic sepsis, such as a high-dose steroid treatment (Ryan 2011; Weston-Petrides 2008; Tjandra 1993). A two-stage procedure is most commonly used. With such a procedure, the pouch is created at the same time as the proctocolectomy, but with a temporary ileostomy that allows the IPAA to heal properly. The stoma is closed later after it has been confirmed that the ileoanal anastomosis will heal (Rintala 2002). During an acute phase, usually because of fulminant colitis, or in the case of an uncertain diagnosis, a subtotal colectomy with an end ileostomy is performed and the rectal stump is closed. Electively, after the diagnosis has been confirmed, the rectum is removed and the IPAA is formed with or without a protective ileostomy (Ryan 2011). The rectum might be preserved in young females because surgery can reduce fertility (Waljee 2006), but life-long follow-up visits to check the rectum stump are required and the disease often relapses. The IPAA is contraindicated

in cases of pelvic floor dysfunction, decreased anal sphincter muscle tone, or known CD (Shen 2005).

Table 11. Surgical options for pediatric inflammatory bowel disease patients, details see text.

SURGICAL OPTIONS FOR ULCERATIVE COLITIS PATIENTS	
proctocolectomy	IPAA
	total removal of the rectum or the mucosectomy of the rectal mucosa
	model of pouch: W, S, J, which is recommended
	anastomosis: either hand-sewed or done using an end-to-end circular stapler
	one-, two-, or three-phase procedure, see text
	contraindications: pelvic floor dysfunction, decreased anal sphincter muscle tone, known CD
subtotal colectomy	
IRA	an option when the rectum has to be preserved: in young females because pelvic surgery can reduce fertility
temporal ileostomy	if surgery is done during an acute phase, and the diagnosis of UC is uncertain; extended to proctocolectomy during a calm disease phase or when the diagnosis has been confirmed
SURGICAL OPTIONS FOR CROHN'S DISEASE PATIENTS	
bowel resections	
	possible in all parts of the bowel
	should preserve as much bowel length as possible because of the high risk of a relapse
stricturoplasty	
	alternative when stricture is shorter that 20 cm or when the bowel length is too short for further resection
subtotal colectomy	IRA
proctocolectomy	permanent ileostomy
segmental colon resection	
temporary ostomy	in the case of severe perianal disease
fistula operations	Seton strings, closing the fistula whole or resection of the fistula

IPAA= ileal pouch-anal anastomosis

IRA= ileorectal anastomosis

Crohn's disease

For CD patients, the resection has to be as short as possible. A resection is possible for all diseased bowel segments, if indicated. To preserve as much bowel length as possible, stricturoplasty is an alternative. It is feasible in diseased segments up to 20 cm in length (von Allmen 2008; Lee 1982). Strictureplasty does not remove the diseased segment, but it does widen the bowel lumen.

In the case of CD-associated pancolitis, a colectomy is also an option. For CD patients, the rectum can often be spared the effects of the active disease, which makes ileorectal anastomosis feasible. If the rectum has to be resected because of severe inflammation, a permanent ileostomy is performed. A pouch procedure is not recommended for CD patients because of the high risk of pouch complications (Alexander 2003). If the colon is only segmentally inflamed, a segmental colon resection may be considered; however, with a segmental resection, there is a significant risk that the disease will recur in both adult and pediatric populations (Tekkis 2006; Griffths 1991).

In the case of a severe perianal disease, which is complicated by abscesses or fistulas, a temporary ostomy might be an alternative. The perineum might heal after the feces are diverted, and the ostomy can be closed after the perineum has completely healed. The fistulas connected to the skin can also be cured using a non-cutting seton string, which helps the fistula drain. For more complex fistulas, corrective surgery or even resection might be needed (Wiese 2012).

Laparoscopic surgery

From adult studies, it is known that laparoscopic surgery enables a shorter hospital stay and a quicker return of proper bowel functioning. The number of early complications are either equal to those for open surgery or they have decreased (Kessler 2011; Umanskiy 2010; Fichera 2009). Also, smaller studies of a pediatric population show that laparoscopic surgery is safe and an effective alternative for both UC and CD patients (von Allmen 2003; Mattioli 2011; Potter 2012; Laituri 2011). A postoperative hospital stay, the duration of the pain killers being used, and the time it took patients to return to a full diet were shorter for the laparoscopic surgery group, and the patients were satisfied with the cosmetic result (von Allmen 2003; Mattioli 2011). However, laparoscopic surgery is not always possible because of intense inflammation, enteric fistulas, or multiple areas of disease involvement. Laparoscopic surgery is contraindicated during an acute phase and in critically ill patients (Parray 2011).

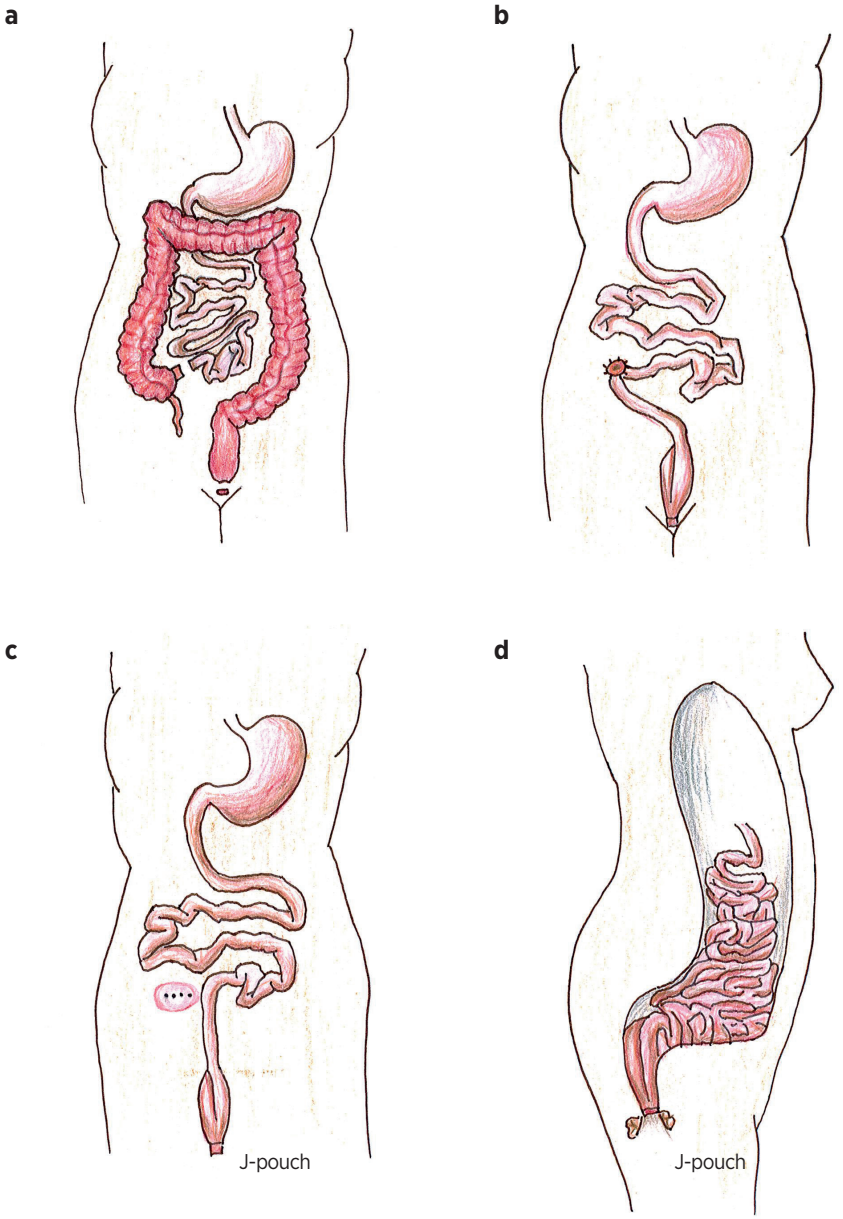


Figure 2. Two-stage J-pouch procedure (IPAA).
a) Proctocolectomy
b) First stage with temporary ileostomy
c) Second stage, ileostomy closed and J-pouch reconstructed
d) Side view of second stage

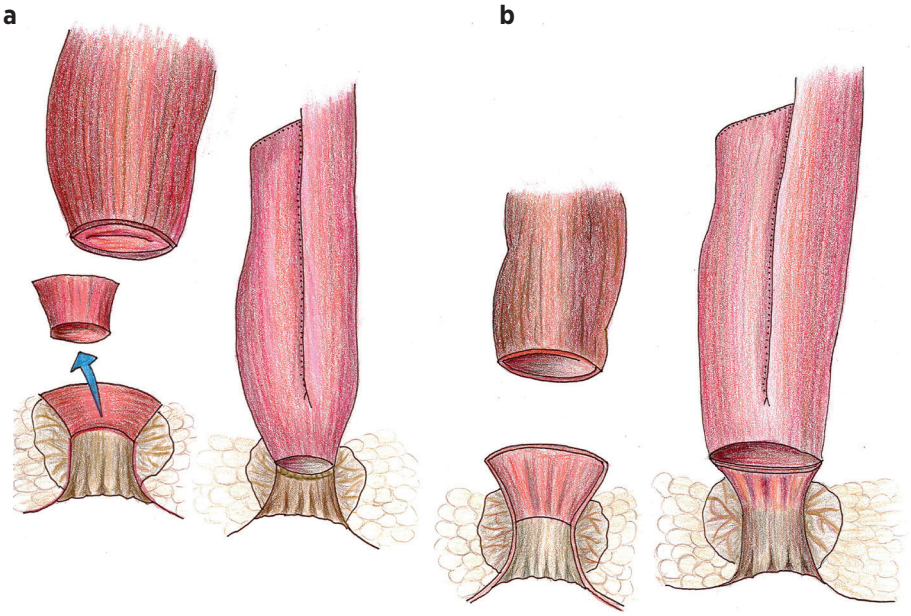


Figure 3. Two alternative ways of performing an ileorectal anastomosis:
a) Total removal of the rectum and mucosectomy;
b) stapled anastomosis with a 2 to 4 cm mucosal remnant.

3.2.4 Long-term outcomes of surgery

Knowing about the general outcomes of surgery is important when a patient is considering surgery as an alternative to medical treatment. Surgery is always risky and the possibility of complications needs to be considered. Table 12 summarizes the results of postoperative pediatric UC studies on the complication rates in terms of bowel function. A higher stool frequency is associated with an SIAA procedure compared with an IPAA procedure. Soiling and surgical complications, especially bowel obstructions, are common after both procedures. Pouch failure in pediatric patients is associated with the duration of the disease before surgery, an expression of perianal disease prior to surgery, the presence of late complications (pelvic infection, small bowel obstruction), anal stricture, and a postoperative diagnosis of CD (Alexander 2003). The preoperative use of immunomodulators or infliximab is not connected to a higher rate of postoperative complications in a pediatric series (Schaufler 2012; Markel 2008); however, a preoperative high-dose use of steroids has been linked to higher complication rates (Uchida 2010; Markel 2008).

Of pediatric and young patients who were misdiagnosed with UC, approximately 13 to 16% got their disease reclassified as CD between two and seven years postoperatively (Mortellaro 2011; Turunen 2009; Alexander 2003). Of those patients who were diagnosed with CD after a restorative proctocolectomy, up to 87% suffered from chronic pouchitis-like symptoms; similar data also exist for adult patients (Brown 2005). This indicates that CD can be one cause of the chronic pouch problems in pediatric patients. The characteristics of CD-like inflammation in the pouch include perianal abscesses, anal fissures, the inflammation of the ileal limb proximal to the pouch, strictures, and fistulas (NASPHGAN 2007).

Postoperative long-term follow-up studies on the pediatric CD population are sparse, and the existing studies primarily focus on relapse and reoperation rates. A study by Ba'ath et al. (2007) of 26 resected pediatric CD patients showed that 38% of them experienced complications during a follow-up of 1.7 years. The re-resection rate as a result of disease complications and disease reactivation vary between 8% and 27% during the first two years postoperatively (Boualit 2012; Pacilli 2011; Ba'ath 2007). There are no studies on long-term postoperative bowel function. However, it has been well established that performing surgery on CD patients may provide a disease-free interval for normal growth and development if the surgery is done before late puberty and if the disease remains in remission postoperatively (Boualit 2012; Newby 2009; Besnard 1998; Ranger 2006). After surgery, nearly all children experience catch-up growth (Pacilli 2011; Besnard 1998; Barrena 2011; El-Baba 1996; Puntis 1984; Langholz 1997).

Table 12. Studies pertaining to postoperative bowel function, complications and pouchitis rates for pediatric UC patients.

	Follow-up (years)	Study period	n	n= IPAA	n= SIAA	Stools during day (IPAA)	Stools during day (SIAA)	Stools at night (IPAA)	Stools at night (SIAA)	Soiling (IPAA)	Soiling (SIAA)	Complications	Pouchitis
Patton 2010	4.5 ± 4.5	1980-2005	31	27	nd	nd	nd	nd	nd	nd	nd	55%	39%
Barrena 2010	12	1992-2009	29	nd	nd	nd	nd	nd	nd	nd	nd	nd	33%
Seetharamaiah 2009	2	1977-2005	168	91	112	nd	nd	nd	nd	5%	8%	nd	49% (IPAA) 24% (SIAA)
Pakarinen 2009	10	1985-2005	52	41	9	5.6 ± 2.4	7.4 ± 3.1	1.3 ±1.7	1.8± 1.3	54%	67%	75%	73%
Koivusalo 2007	10 (1-21)	1985-2004	47	37	10	4 (2-10)	5 (4-10)	0 (0-3)	1 (0-4)	during the day 11%, at night 11%	during the day 10%, at night 30%	40%	49%
Wewer 2005	3.7 (0.3-9.2)	1992-2002	27	30	nd	6 (3-10)	nd	1(0-2)	nd	56%		38%	48%
Mattioli 2005	5.3 (1.2-9.6)	1994-2002	16	nd	16	nd	5.3 ± 2.8	nd	nd		during the day 12.5%	nd	94% had endoscopic cuffitis
Ceriati 2004	6.5 (1.7-13)	1988-2001	21	3	25	nd	nd	nd	nd	77% for both groups		47%	nd
Stavlo 2002	3.7	nd	18	26	nd	nd	nd	nd	nd	0%		31%	nd
Rintala 2002	4 (0.5-6)	1991-1999	29	40	nd	nd	nd	7%	nd	0%		38%	31%
Durno 1998	5.8 ± 3.3	1980-1995	73	54	19	nd	nd	nd	nd	during the day, 53-67% for both groups		50%	44%
Romanos 1995	3.7	1984-1995	12	12	nd	nd	nd	nd	nd	nd		40%	42%

nd=not determined.

Fertility

A meta-analysis has shown that a person's infertility rate increases threefold after an IPAA procedure compared with medically treated UC patients (Waljee 2006). However, patients who undergo surgery have a more severe form of the disease, which can already impair fertility. Women should, however, be aware that a risk of infertility is associated with restorative proctocolectomy. Infertility may largely be due to scarring of the fallopian tubes, which is caused by abdominal surgery (Asztély 1991). However, the chance of having a child later in life is 80% (Lepistö 2007).

Quality of life

One of the main aims of either a medical or surgical treatment of IBD is to preserve a patient's QoL. A pediatric UC study by Pakarinen et al. (2009) demonstrated that a patient's QoL is fairly normal one decade after surgery compared with their healthy peers. Most patients are satisfied with the surgical outcome (Wewer 2005; Rintala 2002; Durno 1998) and can attend school or work normally after the surgery (Barrena 2010; Durno 1998). However, an increased frequency of bowel movements, especially at night (Stavlo 2002; Pakarinen 2009), is associated with a decreased QoL. QoL also decreased in patients who had surgical complication or who missed school (Pakarinen 2009). The results from the pediatric studies are comparable to the results from the adult data (Hahnloser 2004; Berndtsson 2007). Only two small studies exist on postoperative QoL in pediatric CD patients. These report that regardless of the number of disease relapses, QoL is normal or better than it was presurgically when assessed approximately five to six years postoperatively (Barrena 2011; El-Baba 1996). However, adult studies have shown that the relapsing nature of CD can be linked to an impaired QoL (Scarpa 2006; Casellas 2000; Thaler 2005). The adult data also show that QoL improves after surgery and that the results are durable (Thirlby 2001).

3.2.5 Postoperative care and recurrence

Recurrent disease is a problem for CD patients, unlike for UC patients, where the disease can be cured by a proctocolectomy. For CD patients, the disease can occur in any part of the GI tract, and a resection of the diseased segment cannot prevent this from occurring. The relapses can be divided into an endoscopic relapse and a clinical relapse. It is known that an endoscopic relapse precedes a clinical relapse (Rutgeerts 2005). For adults, ileocolonoscopy is the first line method, which is also

recommended by the European Crohn's and Colitis Organization (ECCO), used to ascertain disease recurrence. WCE and MRE are alternative tools (Van Assche 2010). There is mounting evidence that the fecal biomarkers calprotectin and lactoferrin are predictive of postoperative recurrence (Lamb 2009; Gisbert 2009), but the use of these markers in clinical practice needs to be studied more systematically. The most commonly used score for depicting clinical relapse among adults is Crohn's disease activity index (CDAI) (Yoshida 2011; Regueiro 2009; Rutgeerts 2005). Rutgeerts endoscopic score has been developed for adults to provide prognostic information on the risk of clinical relapse (table 13) (Rutgeerts 1990).

Table 13. Rutgeerts endoscopic score.

Score	Definition	Risk of clinical relapse
i0	no lesions seen in endoscopy	less than 10% during 10 years of follow-up
i1	< 5 aphtous lesions indicate remission	less than 10% during 10 years of follow-up
i2	> 5 aphtous lesions with normal mucosa between the lesions or skip areas of larger lesions or the lesions confined to ileocolonic anastomosis	20% during 5 years of follow-up
i3	diffuse aphtous ileitis with diffusely inflamed mucosa	50-100% during 5 years of follow-up
i4	diffuse inflammation with already larger ulcers, nodules, and/or narrowing indicate a recurrence, and the higher the number the more severe the disease	high

The definition of relapse varies between pediatric reports and they all rely on clinical indices. For example, Pacilli et al. (2011) assessed the Harvey-Bradshaw index Besnard et al. (1998) and Baldassano et al. (2001) assessed the PCDAI (table 5). The reported relapse rates were approximately 50% within 2 to 3 years in these reports. If only relapses in previously intact bowel segment are considered, then scholars found that there was a 22% relapse rate within 4.7 years (El-Baba 1996). Alternatively, if relapse is defined as the first dose of immunosuppressant or biological agent given postoperatively, then scholars found that the relapse rate at two years was 18% (Boualit 2012). Compared to adult data, in which clinical recurrence rates at five years vary from 17 to 55% (Yamato 2005; Buisson 2012b), pediatric patients experience slightly more relapses, although the endoscopic relapse rate seems to be comparable between children and adults (Buisson 2012b).

Table 14 lists the known risk factors for an early recurrent disease in a pediatric population. According to the ECCO, the risk for postoperative recurrence after ileocolonic resection increases in adults if a patient is an active smoker or has had

prior bowel surgery, a penetrating disease, a perianal disease, or extensive small bowel disease (Van Assche 2010). The risk profile of the patient affects the choice of postoperative treatment.

Table 14. The known risk factors for early recurrent disease in pediatric Crohn's disease patients after bowel resection.

Clinical sign	Reference
diffuse ileocolonic disease	Pacilli 2011; Griffths 1991
upper gastrointestinal involvement or perianal disease	Besnard 1998
colon resection	Griffths 1991
failure of medical treatment as the only indication for surgery	Pacilli 2011; Baldassano 2001
preoperative use of 6-mercaptopurine	Baldassano 2001
more than one year of active disease before operation	Baldassano 2001; Griffths 1991

Postoperative treatment

There are no medication guidelines for pediatric patients that would prevent the chances of relapses. Similar to the overall treatment strategies for pediatric IBD patients, the guidelines also rely on adult data. The ECCO recommends using a thiopurine treatment for adult patients with CD who have risk factors for an early relapse in order to prevent both an endoscopic and a clinical relapse (Van Assche 2010). Prophylaxis should be started two weeks after surgery and the duration should be at least two years. For patients with isolated ileal resection, or for patients who have no risk factors, a high dose of mesalazine is an alternative. Infliximab is an alternative for patients who have more than one risk factor, for patients who have been on anti-TNFs for more than six months prior to surgery, or for patients who failed the thiopurine treatment (Buisson 2012a).

Many drugs have been evaluated for their ability to prevent recurrent postoperative CD. The effects of the antibiotics ornidazole and metronidazole have been studied in placebo-controlled studies of adults (Rutgeerts 2005; Rutgeerts 1995). Using ornidazole for one year and using metronidazole for three months significantly diminished the first year clinical and endoscopic relapse rates. However, the one-year use of ornidazole was not able to prevent further relapses (Rutgeerts 2005). Side effects were common with both therapies and led to the therapy being discontinued (Rutgeerts 2005; Rutgeerts 1995).

The use of 5-ASA compounds to prevent relapses has resulted in various outcomes. According to a recent review and meta-analysis report that included 11 randomized control trials (RCTs), the relapse of CD (definition varies) at one year

occurred in 43.3% of those treated with 5-ASA compared to the placebo group or those receiving no therapy (50.5%). While 5-ASA significantly reduced the risk of recurrence, this benefit was limited to mesalamine only (number needed to treat=10). The number of adverse events was not associated with the use of mesalamine (Ford 2010).

In terms of both the overall analysis and sensitivity analysis, the meta-analysis report showed that purine analogs, azathioprine, and 6-MP, are more effective for preventing both clinical and endoscopic recurrence at one year than either a placebo or mesalamine. However, they are not able to prevent severe (i3-i4) endoscopic relapses. Adverse events leading to the discontinuation of the drug are more common in purine analog users. After the purine analog treatment, between 21% and 55% of patients still had severe endoscopic relapse and between 5% and 36% of patients experienced a clinical recurrence compared to patients treated with a placebo or mesalamine: For the latter two groups, the recurrence rates were between 23% and 78% and 13% and 57.5%, respectively (Peyrin-Biroulet 2009).

In two RCTs, Regueiro et al. (2009) studied the postoperative use of infliximab compared to a placebo group and Yoshida et al. (2011) studied the postoperative use of infliximab compared to mesalamine users. Both studies used infliximab 5mg/kg. Reguiero et al. administered infliximab starting two to four weeks postoperatively during weeks 0, 2, and 6 and after that drug interval was prolonged to 8 weeks. They allowed immunomudulators or mesalamine to be used with the treatment group if the dose had been stable 12 weeks before surgery. Youshida et al. administered infliximab every eight weeks starting four weeks postoperatively and all patients received oral mesalamine postoperatively. In these studies, between 9.1% and 21.4% of the patients had an endoscopic relapse after one year of taking infliximab compared to the control group, where between 81.3% and 84.6% of patients had a relapse after one year. Of the patients who took infliximab, a significantly higher portion of them were in remission (Rutgeerts score <i1) compared to the controls ($p=0.004$) (Yoshida 2011). At end points of 12 or 36 months, the CDAI score diminished more for the infliximab group than for the controls, but the difference was statistically insignificant (Regueiro 2009; Yoshida 2011). A higher complication rate has not been linked to the postoperative use of infliximab (Regueiro 2011).

3.2.6 Pouchitis

The constructed ileal reservoir, pouch, or the end of the ileum in an SIAA (Perrault 1997) can be inflamed after proctocolectomy. This inflammation is idiophatic (Hoda 2008; Seethremaih 2009) and it occurs in between 31% and 73% of patients after an IPAA or SIAA (table 12). Pouchitis is the most common complication related to

proctocolectomy and the first episode of pouchitis usually occurs six months after the proctocolectomy (Carter-Kent 2008). The etiology of inflammation remains unknown. Table 15 lists the known risk factors. In one pediatric study, 48% of patients had an acute simple presentation of pouchitis, 7% suffered from chronic pouchitis, and 9% experienced pouch failure (Alexander 2003).

Table 15. Risk factors associated with the expression of pouchitis.

Risk factor	Reference
overgrowth of commensal bacteria in the pouch	Landy 2012; Shen 2005
fecal stasis	Sandborn 1994a; Scmidt 1998
nutritional deficiencies	Sandborn 1994a; Scmidt 1998
ischemia	Sandborn 1994a; Scmidt 1998
extraintestinal manifestations , especially PSC	Lohmuller 1990; Penna 1996
expression of pANCA	Kuisma 2004; Fleshner 2001
NSAID use	Achkar 2005
backwash ileitis	Scmidt 1998
altered short-chain fatty acid metabolism	Landy 2012
differences in innate immune cells and receptors compared to nonpouchitis patients and FAP patients	Landy 2012
extensive colonic disease preoperatively	Scmidt 1998
young age at proctocolectomy	Coffey 2009

FAP=familial adenomatous polyposis; NSAID=non-steroid anti-inflammatory drug; PSC=primary sclerosing cholangitis; pANCA=perinuclear anti-nuclear cytoplasmic antibody.

Diagnosing pouchitis is not always a straightforward process, and it should be based on clinical symptoms, an endoscopic relapse, and acute histologic findings (Sandborn 1994b) (table 16). Not all patients who suffer postoperatively from diarrhea and abdominal pain have pouchitis: Irritable pouch syndrome, cuffitis, stenosis of the pouch, CD, celiac disease, and infection can cause similar symptoms (Carter-Kent 2008). Endoscopy helps when making a differential diagnosis because pouchitis has typical histological and macroscopic findings (table 16). The new environment that ileum encounters causes changes to the mucosa of the pouch, and when the mucosa becomes colon-like, it might be susceptible to conditions that primarily affect the colon. All of this raises a question: Is pouchitis a novel presentation of IBD? This hypothesis might be supported by the fact that the incidence of pouchitis is higher in UC than in familial adenomatous polyposis (FAP) (Lohmuller 1990; Perrault 1997; Lovegrove 2006).

Table 16. Symptoms and endoscopic findings related to pouchitis in pediatric inflammatory bowel disease patients (Bath 2011; Sandborn 1994b; Stallmach 1999; Sarigol 1999; NASPHGAN 2007).

Symptoms	
	increased stool frequency
	urgency
	rectal bleeding
	abdomino-pelvic pain
	weight loss
	fever
Macroscopic findings	
mild case	mucosal hyperemia and edema
	diminished vascular pattern
	contact friability
more severe case	mucosal hemorraghe
	aphtous and larger ulcers
	pseudomembrane formation
Histologic findings	
	villous atrophy
	slight crypt hyperplasia
	increase in infiltration of both acute and chronic inflammatory cells
	ulceration
	the ileal mucosa may become colon-like

A recent Cochraine review suggests that metronidazole and ciprofloxacin are both effective in treating acute pouchitis, with ciprofloxacin being better (Holubar 2010). Steroid enemas are also alternatives to antibiotics and are equally effective as metronidazole (Holubar 2010). For those patients who have chronic pouchitis induced during remission as a result of antibiotics, the probiotic VSL#3 (it includes strains of lactobacilli, bifidobacteria, and Streptococcus salivarius subsp. thermophilus) reduces the rate of recurrences (Holubar 2010). However, pouchitis can be antibiotic-refractory. This condition may be managed using a combination of antibiotics, 5-ASA, or anti-TNFs (Barreiro-de Acosta 2012; Shen 2012). In such cases, the secondary causes of pouchitis (for example, ischemia, cytomegalovirus or Clostridium difficile infection, autoimmunity diseases, CD, cuffitis, or fistulas) should be carefully considered (Shen 2012). If the pouchitis does not respond to the treatment, this indicates the need for ileostomy, with or without a pouch excision.

AIMS OF THE STUDY

The aim of this dissertation was to study different aspects of surgical treatment in pediatric IBD patients.

The specific aims were as follows:

I To study the long-term prognosis of CD patients who had undergone primary surgery during childhood.

II To study the effects of MRE on the treatment choices for pediatric CD patients.

III To examine the MMP and TIMP profiles in the pouches of proctocolectomized pediatric UC patients and to compare these profiles to the known MMP and TIMP profiles of IBD patients, with the aim of finding similarities or differences between pouchitis and IBD.

IV To find a tissue marker that could aid at diagnosis in predicting which patients will suffer from an aggressive disease course. Recognizing such markers would enable future studies on the possibility of whether or not an aggressive therapy at an early disease phase would prevent disease complications and surgery.

PATIENTS AND METHODS

1 PATIENTS AND CONTROLS

The study populations consisted of IBD patients diagnosed during childhood and treated at either Helsinki Children's Hospital or the Department of Pediatrics, Tampere University Hospital.

(I) Long-term outcomes of Crohn´s disease

To study the long-term outcomes of pediatric CD after surgery, a questionnaire was sent to patients (see methods) and chart review study was conducted on those patients who had undergone surgery in childhood between 1985 and 2008 in Helsinki and Tampere. In total, 36 patients had at least a two-year follow-up and were included to the study. The controls were age- and gender-matched healthy persons; their names had been collected from the Populations Register Centre to serve as controls for a long-term follow-up study on pediatric UC patients who had been colectomized during their childhood (Pakarinen 2009). There were two controls for every CD patient.

(II) MRE

To estimate the impact of the MRE on pediatric Crohn's jejunoileitis, all 45 CD patients who had undergone an MRE between January 2009 and July 2011 at Helsinki Children's Hospital were traced.

(III) Pouchitis

To study the nature of pouchitis, all UC patients who had been colectomized under the age of 16 at Helsinki Children's Hospital or Tampere University Hospital between the years 1985 and 2005 were traced (n=81). The postal addresses for sending an invitation letter to a follow-up visit and a questionnaire (see methods) were traced for 79 patients. Of these, 35 participated in the follow-up visit, but seven were re-diagnosed with CD. Thus, 28 were eligible to take part in the study (Pakarinen 2009).

(IV) Predictive factors for aggressive disease

All of the patients from the IBD patient registry for Helsinki Children's Hospital who had undergone surgery because of UC and who had been diagnosed with the disease under the age of 16 between the years 1990 and 2008 were traced. From this population, 24 patients were identified who could be matched with a control patient (n=27). The disease controls were conservatively treated, they had a comparable primary diagnosis, and they had been diagnosed at the same age as the operated patients. The control patients were followed for at least the time that it took between the time of diagnosis to when surgery was performed in operated patients. Twenty children who had undergone an endoscopy to exclude IBD or because of colorectal bleeding, pancreatic insufficiency, or a stomach ache served as non-IBD controls.

Table 17 presents the background data, endoscopic findings according to the Paris classification (Levine 2011), and laboratory markers at the time of diagnostic endoscopy for patients undergoing early surgery, their matched control patients, and the non-IBD controls. The distribution of disease at the diagnostic endoscopy was comparable between the patients undergoing surgery and the disease controls. The use of a TNF-α-antagonist was similar between patients undergoing surgery and conservatively treated controls during the follow-up and before primary surgery.

Table 17. Background data, endoscopic findings according to the Paris classification, and laboratory markers at the time of diagnostic colonoscopy in pediatric onset ulcerative colitis (UC) patients and controls. Operated and non-operated patients were matched according to the year of diagnosis and follow-up on the operated patients. It was confirmed that the controls did not have inflammatory bowel disease.

		Early surgery	Conservatively treated	Non-IBD Controls
No. of patients (male)		24(11)	27(15)	20(10)
Age at diagnosis, years (median; range)		13.1(2.1-16.0)	12.1(2.8-16.6)	13.5(2.7-16.8)
Time from diagnosis to surgery (median; range)		2.1(0.1-6.6)	-	-
Follow-up, years (median; range)		8(2-20)	6(3-11)	1(0-4)
Paris classification				
E1-2 S0	ulcerative proctitis or left-sided UC (distal to splenic flexure); non-severe disease	0	4	-
E1-2 S1	ulcerative proctitis or left-sided UC (distal to splenic flexure); severe disease	3	3	-
E3-4 S0	extensive disease (hepatic flexure distally) or pancolitis; non-severe disease	5	9	-
E3-4 S1	extensive disease (hepatic flexure distally) or pancolitis; severe disease	16	11	-
Histological inflammation				
no inflammation		-	-	18
mild		6	10	1
moderate to severe		18	17	-
Laboratory markers (median; range)				
Hb; g/l		112(86-135)	116(86-140)	131(93-153)
ESR; mm/h		25(7-61)	22(4-63)	8(2-23)
CRP; mg/l		20(<3-92)	9(<5-36)	<3(<3-<5)
WBC; E9/l		9.6(6.7-26.3)	7.5(4.1-15.9)	5.1(3.0-16.3)
Alb; g/l		34.6 (28.0-40.0)	36.1 (23.6-42.6)	42.3 (39.8-47.2)

2 METHODS

(I) Long-term outcomes of Crohn's disease

The patient charts were reviewed to summarize the following: medication before surgery, indications of the need for surgery, indications and number of re-resections, all endoscopies after surgery, and surgical complications. The questions mailed to the patients in spring 2010 concerned the following: their current health status, QoL, bowel function (stool frequency, soiling, stool consistency, urgency, discrimination of flatus, evacuation, medications to control stool frequency), occurrence and treatment of pouchitis (proctocolectomized patients with IPAA), satisfaction with surgery, and restrictions in their daily lives as a result of the surgery. The controls were asked only about their QoL. QoL was measured using a visual analogue scale (scores 1-7). Four questions assessed three dimensions of QoL (physical, emotional, and social functioning) and the patients' overall QoL (Pakarinen 2009; Turunen 2009).

(II) MRE

Two pediatric radiologists blinded to the patient data re-evaluted the MRE pictures for the purpose of the study. The T1 pictures were used to evaluate the enhancement and bowel wall thickness in the ileum and jejunum as well as any polyps, ulcers, fistulas, and extramural findings. The MRE pictures were compared to the macroscopic findings for possible intestinal resection and to the endoscopy findings within three months of the MRE and related to the therapeutic modifications.

The MRE was carried out using a 1.5 T MR scanner (Achieva, Philips). On the afternoon prior to imaging, the patients took a bisacodyl to empty the bowel and fasted until after the imaging was completed. During the imaging day and the day preceding it, they were not allowed to eat meat, vegetables, fruit, porridge, whole grain bread, or butter or drink milk. To maximize luminal distension, the patients drank a combination of 70% sorbitol (amount depending on age) and 200 ml of water in a 15-minute period, and after that, they drank 400-600 ml of water in a 30-minute period. Before imaging, the patients were administrated 0.5 ml of hyoscine butylbromide (20 mg/ml) to prevent motion artifact. An intravenous administration of gadolinium (0.2 ml per kg body weight) was used as a contrast medium in the dynamic study. The total time of imaging was approximately 45 minutes.

(III) Pouchitis

The questions mailed to the patients concerned their current diagnosis, complications, medical therapy, pouchitis, and its treatment. During the follow-up visit, a biopsy of the pouch and blood and fecal samples were obtained. Fecal calprotectin (<100ug/g) was used as a mark of active inflammation. A research assistant asked the patients about any inflammatory symptoms and their use of antibiotics or painkillers during the previous month. They discussed the answers to the postal questionnaire together.

The MMPs -3, -7, -8, -9, and -12 and TIMPs -1, -2, and -3 from the tissue samples were immunohistochemically stained (protocol described in detail in article III). The histological grade of inflammation in the samples was assessed according to the scoring system for pathological changes in the ileal reservoir mucosa. Polymorphic infiltration, ulceration, chronic inflammatory cell infiltrate, and villous atrophy were evaluated (score 0-3; 0 indicating no alterations and 3 severe alterations) (Shepherd 1987; Pakarinen 2010; Mäkitalo 2010). Two different investigators independently evaluated in a semi-quantitative fashion the expression of MMPs and TIMPs in specimens under a light-field microscope at x 100 magnification. The following scale was used to mark the staining intensity: 0=less than 20 positive cells; 1=20-50 positive cells; 2=50-200 positive cells; and 3=more than 200 positive cells (Mäkitalo 2009; Mäkitalo 2010).

(IV) Predictive factors for aggressive disease

Using the diagnostic ileocolonoscopy biopsies (n=69 for the colon and n=49 for the ileum), the tissue markers trypsinogen-1 and -2, TATI, and MMP-9 were immunohistochemically stained (protocol described in detail in the article IV).

The tissue profiles of the IBD patients undergoing surgery, the conservatively treated patients, and the non-IBD controls were compared. Two different investigators independently evaluated the samples in a semi-quantitative fashion under a light-field microscope at x 100 magnification. The following scale was used to mark the staining intensity: 0=no positive cells; 1=low intensity; 2=medium intensity; 3=high intensity (Böckelman 2011). The staining in the epithelium of both the ileum and colon samples and the staining in the inflammatory cells was evaluated in all of the samples.

The overall level of histological inflammation was collected from primary diagnostic reports and was scored from 0 to 2 according to the presence of ulceration, the number of acute and chronic inflammatory cells, crypt distortion, and goblet cell depletion (modified from Beattie 1996). The macroscopic distribution of the

disease at endoscopy was scored from 0 to 3: 0=non-inflammatory appearance; 1=proctitis; 2=left-sided colitis; and 3=pancolitis. The presence of backwash ileitis was also evaluated.

3 STATISTICAL ANALYSIS

Non-parametric Mann-Whitney's and Kruskal-Wallis tests were performed to investigate the significance of the continuous variables for each group (I,III,IV). A Spearmann correlation covariation test was used to study the correlations (III,IV). Fisher's exact test was used to compare the frequencies at which the correlations occurred within the different groups due to the small number of cases (I,III,IV). Kaplan-Meier curves were used to analyze survival (I). A p value of <0.05 was considered significant (I, III, IV).

4 ETHICAL CONSIDERATIONS

The Ethics Committee for the University of Helsinki Children's Hospital approved the study protocol (I, III). The National Supervisory Authority for Welfare and Health approved the use of tissue samples (IV).

RESULTS

(I) LONG-TERM OUTCOMES AFTER SURGERY ON PEDIATRIC CROHN'S DISEASE PATIENTS

The median age for bowel surgery was 14 years (range 6-18), the disease duration before operation was three years (range 0-10), and the median follow-up time after primary surgery was ten years (range 2-21). The indications of primary bowel resections were as follows: disease activity and symptoms despite optimal medical therapy (56%), bowel stricture (25%), fistula (6%), and emergency surgery (23%). Figure 4 summarizes the characteristics of the primary resections, disease reactivation, re-resections, and biological treatment. The median time to the first infusion of a TNF-α-antagonist after the primary operation was eight years (range 0.2-15.7). The re-resection rate was similar between the different types of operations ($p \geq 0.05$).

Surgical complications after primary resection occurred in 77% of patients (median 2; range 0-12). The most common complications were adhesive bowel obstructions: In total, 12 patients (32%) had 24 episodes. Anastomotic strictures were also frequent: Nine patients (25%) had a total of 53 strictures. Complications indicated the need for 16 re-resections and five fistulectomies for a total of 11 patients. One patient developed short bowel syndrome after nine resections. All of the proctocolectomised patients who had a pouch had suffered from pouchitis-like symptoms and one experienced pouch failure. For these patients, the disease was diagnosed as UC prior to primary surgery, but a diagnosis of CD was histologically confirmed a median of 1.6 (0.1-11.0) years after primary surgery.

Of 36 patients, 25 (67%) filled out the questionnaire. Of these, five had small bowel or ileocecal resection, 13 had both ileal and colon resections, and six had a colon resection, a colectomy, or a proctocolectomy. The different dimensions and overall bowel function were similar between the different types of operations ($p \geq 0.05$; table 19). Patients who had only ileum resections, however, had lower daytime and maximal stool frequencies compared to patients who also had colon resections. Tables 18 to 20 show the characteristics of the different dimensions of bowel function.

Of the patients who responded to the questionnaire, 30% reported absences from school or work because of bowel problems during the previous month, 20% had been absent more than once per month and disease had caused a delay in education for 46% of the patients. Overall, the patients' satisfaction with their surgery was high (96%): Only two patients (4%) were unsatisfied with the outcome of the surgery.

The different dimensions and overall QoL were statistically equal among the patients and their matched controls (two controls for every CD patient) and were not affected by the type of surgery or the re-resections. Patients with school or work absences had a significantly lower overall physical and social QoL compared to the other patients (details in figure 5).

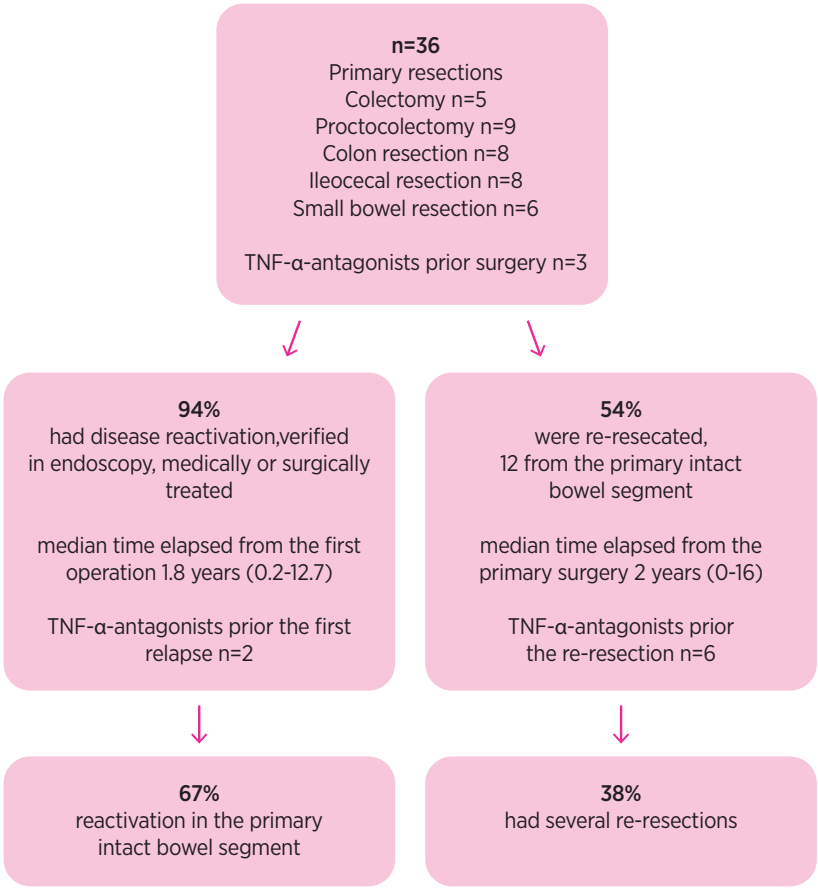


Figure 4. Characteristics of disease re-activation and re-resections in 36 pediatric Crohn's disease patients who underwent surgery during childhood.

Table 18. Stool consistency and defecation a median follow-up time of 10 years after surgery in 18 patients with pediatric onset Crohn's disease.

Stool consistency (n=16)	Watery	Loose	Normal
%	11	56	22
Urgency period, minutes (n=16)	None	5	≥15
%	0	17	63
Discrimination of flatus (n=16)	Never	Partly	Always
%	6	22	73
Complete evacuation (n=17)	Never	Sometimes	Always
%	17	28	39

Table 19. Stool frequency and fecal continence a median follow-up time of 10 years after surgery in 18 pediatric Crohn's disease patients.

	colon resection/ colectomy/ proctocolectomy n=6 median (range)	**small bowel resection** n=5 median (range)	**colon and ileal resection** n=7 median (range)	**all** n=18 median (range)
Stool frequency				
Daytime	3 (1-23)	2 (0-6)	6(3-15)	3 (0-23)
Nighttime	0(0-10)	0 (0-3)	1 (0-2)	0(0-10)
Maximum/24h	5 (2-30)	3 (0-9)	8(5-15)	5(0-30)
Minimum/24h	2 (1-5)	1 (0-3)	5(0-15)	1(0-15)
Continence				
Daytime soiling, %	33	20	57	39
Nighttime soiling, %	50	0	43	33
Totally continent, %	50	80	14	33
Medication for stool control, %	33	0	57	33

Table 20. Stool frequency and fecal continence related to re-resections in pediatric Crohn's disease patients operated on in childhood.

	no re-resection n=7	**one re-resection** n=5	**several re-resections** n=8
Stool frequency			
Daytime	2(0-23)	3(2-5)	6(3-15)
Nighttime	0(0-10)	1(0-2)	1(0-2)
Maximum/24h	4(0-30)	5(3-6)	10(5-15)
Minimum/24h	2(0-5)	1(0-3)	4(0-15)
Continence			
Daytime soiling, %	25	25	44
Nighttime soiling, %	38	25	50
Totally continent, %	63	50	33
Medication for stool control, %	29	20	42

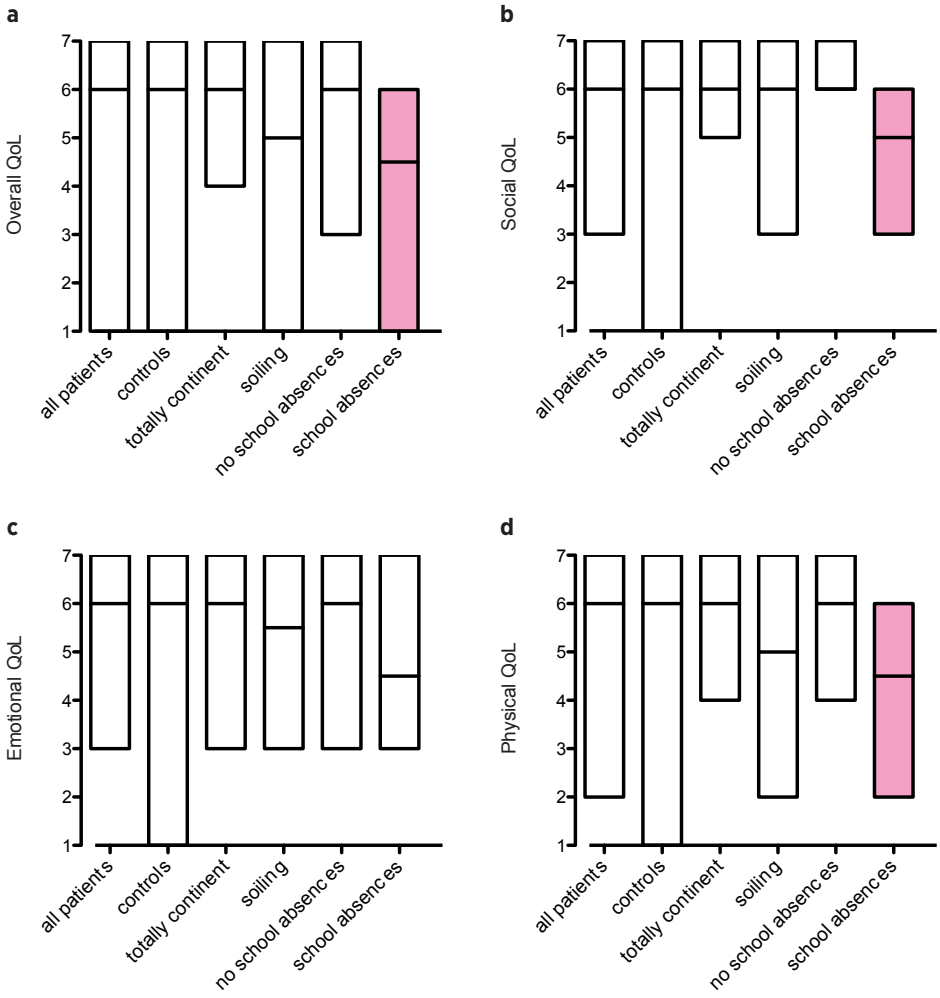


Figure 5. Overall (a) and different dimension (b: social, c: emotional, d: physical) of quality of life (QoL) (score 1-7; line at median, higher number indicating better QoL) for pediatric Crohn's disease patients who underwent surgery during childhood and a subgroup of patients a median of ten years after primary surgery compared to age- and gender-matched, population-based controls. Patients with school absences had significantly lower QoL compared to patients without school absences when the bar is marked in pink.

(II) MAGNETIC RESONANCE ENTEROGRAPHY GUIDING TREATMENT IN CHILDREN WITH CROHN'S JEJUNOILEITIS

Between January 2009 and June 2011, 45 (31 male) pediatric CD patients had undergone MRE. The median age at the time of the imaging was 15 years (range 8-18) and the median disease duration was 2.3 years (range 0-13.2). The visualization of the jejunum and ileum was sufficient for the purposes of the study in 43 patients. The imaging failed for two patients because of vomiting and an inability of drink enough sorbitol fluid. Ten patients (23%) had problems during the imaging session: Either their fluid intake was inadequate or the patients could not hold their breath long enough, and in some cases the contrast medium was already in the lower bowel segments. Regardless of these problems, the pictures could be evaluated.

The MRE showed an active disease segment in the small bowel of 24 patients and in the colon of two patients. For 17 patients, the MRE showed no marks of inflammation. Figure 6 shows the therapeutic modifications related to the MRE findings and macroscopic findings of the possible operation. All operations (n=9) were performed within ten months of the MRE (median 3.4 months), and the macroscopic finding corresponded to the MRE in 73% of patients; in two cases (22%), the MRE overestimated the inflammation. An endoscopy was performed on 13 patients within three months of the MRE, and the findings were comparable for eight patients (62%); for five patients (38%), the MRE showed more extensive disease in the small bowel.

In five cases (12%), only the MRE was able to visualize an active disease segment in the distal ileum, and two of the patients also had an active disease segment in their jejunum. Three of these patients had started TNF-α-antagonist therapy, one patient went for an ileocecal resection within two months of the imaging, and one patient continued with their current treatment. The WCE was performed on two patients. One patient had no disease confirmation during either of the procedures, whereas the WCE revealed an active disease segment in the other patient.

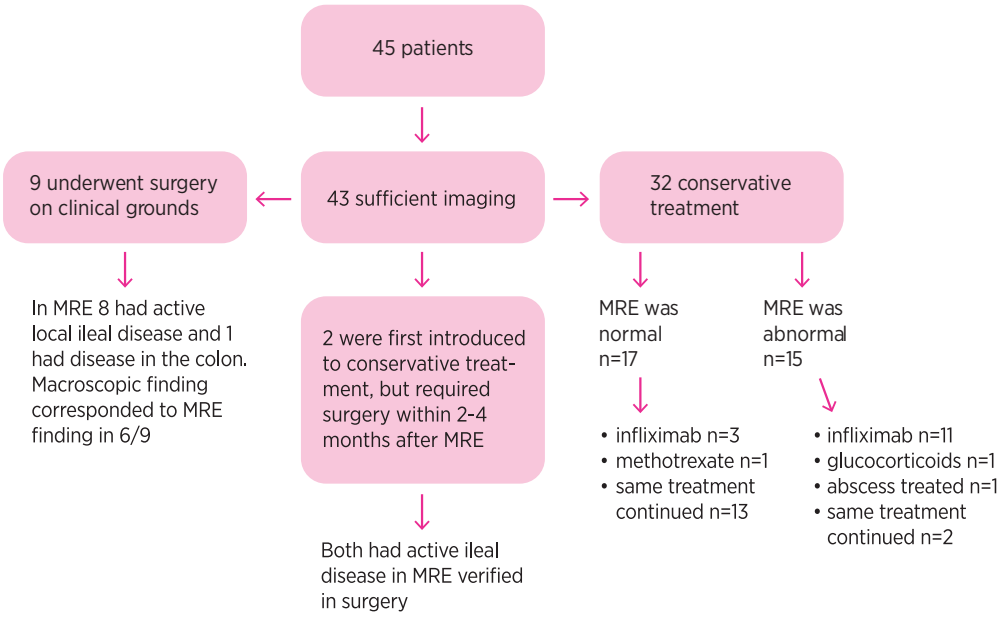


Figure 6. Therapeutic modifications after magnetic resonance enterography related to possible macroscopic findings during operation for pediatric Crohn's disease patients.

(III) MATRIX METALLOPROTEINASES IN THE RESTORATIVE PROCTOCOLECTOMY POUCH OF PEDIATRIC ULCERATIVE COLITIS

Of the 28 (9 male) UC patients who took part in the study, 21 had IPAA and seven had SIAA. Both procedures included a transanal mucosectomy and hand-sewn anastomosis. The median age of the patients at the time of the study was 25 years (range 8-41), and the median follow-up time after colon surgery was 13 years (range 4-22). Pouchitis had occurred in 19 patients (68%), with 13 (46%) patients reporting a single episode and six (21%) patients recurrent (≥4 episodes) episodes of pouchitis. During the follow-up, 18 patients had received antibiotics and seven patients had also received steroids to treat the pouchitis. Six patients had active pouchitis at the time of the study. All of these patients were on metronidazole medication and one was also on 5-ASA. For the other 12 patients, the last episode of pouchitis had occurred more than one month prior, usually several months earlier, and they were not taking any antibiotics . The type of operation (IPAA vs. SIAA) had no effect on the frequency of pouchitis.

Histological inflammation was detected in 26 of 28 samples (93%). The inflammation in the biopsies of patients who had experienced pouchitis during the follow-up was mild for seven patients, moderate for three patients, and severe for seven patients. For those patients who had active pouchitis, the inflammation varied from mild to severe. Two of the samples were too small to determine the grade of inflammation.

Table 21 shows the staining profile and intensity of the UC pouches. The number of positive samples both in the epithelium and in the stroma was high in MMPs -3, -7, and -12 and in TIMPs -2 and -3 (with a median of 23 positive samples; range 20-28). The MMPs -8, -9, and -26 showed only a minor number of positive samples (with a median of 0 positive samples; range 0-9). The MMP and TIMP profiles were similar for the patients who had experienced single or several episodes of pouchitis and those who had not. Also, the frequency of pouchitis or the type of operation (IPAA vs. SIAA) had no effect on the MMP or TIMP profile. The inflammation markers, fecal calprotectin, ESR, or CRP, showed no correlation with the MMPs or TIMPs. The expression of MMP-7 in the epithelium was higher for those patients with no active inflammation than for those patients with active inflammation ($p=0.02$). The histological grade of inflammation in the samples correlated negatively with the epithelial expression of MMP-3 and MMP-7 (corr. coeff. -0.614, $p=0.002$ and corr. coeff. -0.472, $p=0.027$, respectively).

Table 21. Expression profiles and staining intensity (scored from 0-3) of MMPs and TIMPs in tissue samples of pediatric ulcerative colitis patients with IPAA or SIAA.

		number of positive samples	mean, intensity score	SEM
MMP-3	e	22	0.93	0.114
	s*	20	1.25	0.183
MMP-7	e	28	1.36	0.092
	s*	27	1.89	0.149
MMP-8	e	0	0.00	0.000
	s**	1	0.04	0.036
MMP-9	e	0	0.00	0.000
	s***	9	0.32	0.090
MMP-12	e	20	0.75	0.098
	s***	24	1.46	0.141
MMP-26	e	0	0.00	0.000
	s****	3	0.11	0.060
TIMP-1	e	0	0.00	0.000
	s	0	0.00	0.000
TIMP-2	e	23	0.89	0.094
	s*	23	1.75	0.203
TIMP-3	e	23	1.04	0.120
	s*****	28	2.39	0.130

e=epithelium; s=stroma
* plasma cells, macrophages, eosinophils
** neutrophils
*** plasma cells, macrophages, eosinophils, intraepithelial neutrophils
**** plasma cells, neutrophils
***** plasma cells, marcrophages

(IV) LOW TRYPSINOGEN-1 EXPRESSION IN PEDIATRIC ULCERATIVE COLITIS PATIENTS WHO UNDERGO SURGERY

All of the studied markers (Tryp-1 and -2, TATI, MMP-9) were stained in the epithelium of the colon and in Tryp-1 and Tryp-2 and MMP-9 in the inflammatory cells of the colon stroma. Overall, the staining of the studied markers was low. Figure 7 presents the proportions of positively stained samples, and figure 8 shows the immunopositivity levels. The number of samples was comparable between the different groups (surgery, conservative treatment, non-IBD controls) for all of the studied markers when positivity was detected in the colon epithelium.

UC patients undergoing surgery showed a significantly lower level of Tryp-1 immunopositivity in their colonic epithelium compared to both the conservatively treated (p=0.03) and non-IBD controls (p=0.04) with a comparable staining status (figure 8). The immunopositivities of Tryp-2 and TATI were comparable for the studied groups (figure 8). No association was found between the staining of Tryp-1 and Tryp-2 or the staining of TATI with trypsinogens.

UC patients, regardless of the disease outcome, had more positive MMP-9 inflammatory cell samples in the colon stroma than the non-IBD controls (p=0.0006). The immunopositivity of MMP-9 in the inflammatory cells was significantly higher in both the surgery group (p=0.003) and the conservatively treated group (p=0.004) than in the non-IBD patients. Surgically and conservatively treated patients had comparable MMP-9 staining in their inflammatory cells. The staining level of MMP-9 was not associated with trypsinogens.

MMP-9 intensity in the inflammatory cells was positively associated with the grade of inflammation at diagnosis (95% confidence interval, 0.22-0.62; p=0.0002) but not with the other markers of active disease. No association was observed between the other studied markers and aggressive disease onset, the histological grade of inflammation, laboratory markers, or the macroscopic distribution of the disease. The presence of backwash ileitis did not relate to the expression profile of the markers.

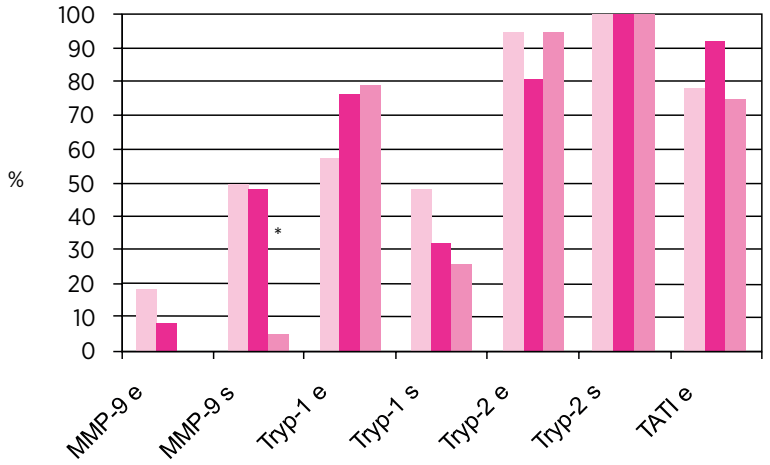
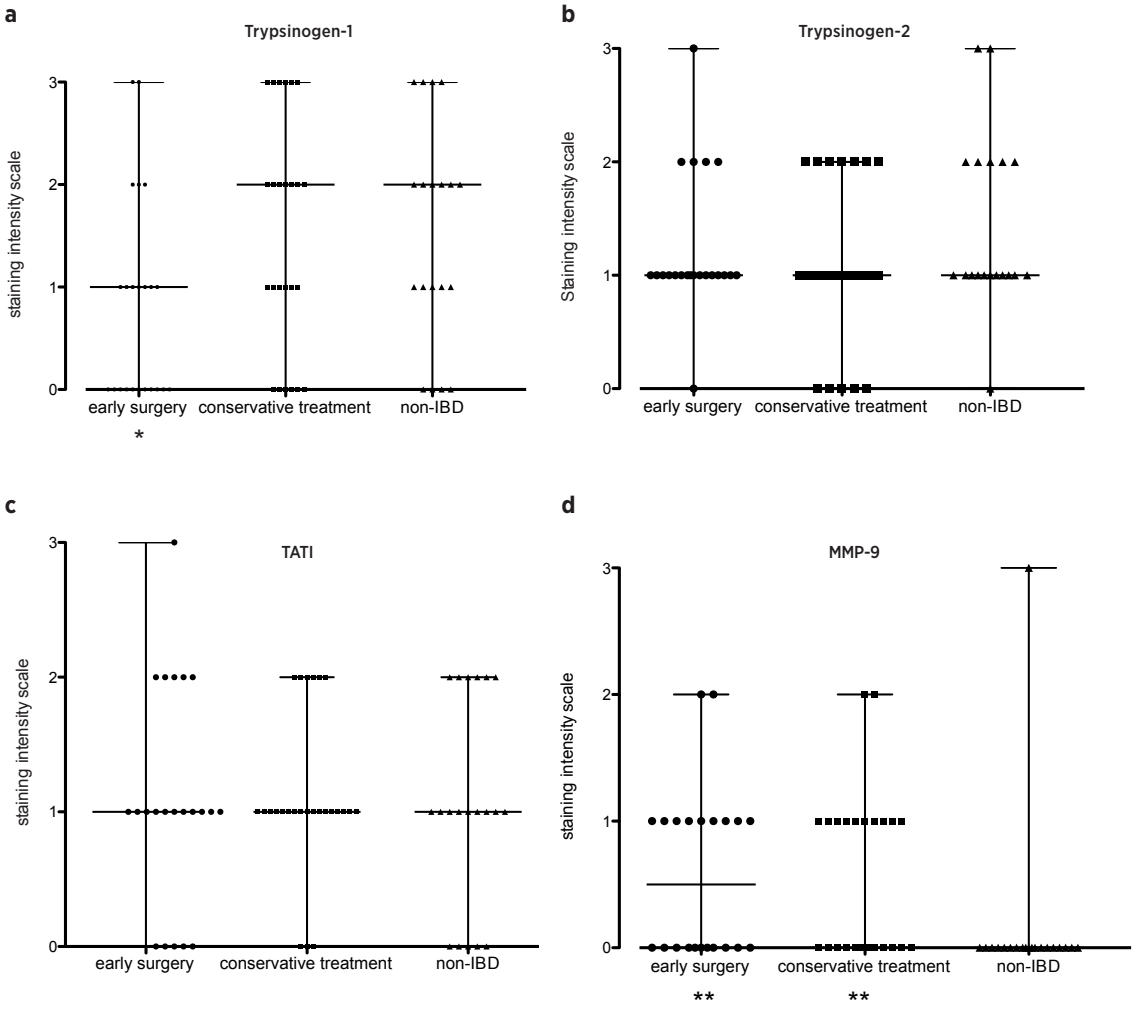


Figure 7. Proportion of positive samples (%) of trypsinogen-1 and trypsinogen-2, the trypsin inhibitor (TATI), and matrix metalloproteinase 9 (MMP-9) in the diagnostic colonic biopsies of pediatric ulcerative colitis (UC) patients who had undergone an operation during the disease course (light pink) compared to conservatively treated UC patients (dark pink) and non-IBD controls (pink). For the index case, the age at diagnosis and the follow-up time until surgery were matched in the IBD patient group.

e; epithelium, s; inflammatory cells of the colon stroma

* UC patients had significantly more MMP-9 positive samples when positivity was detected in the inflammatory cells of the colon stroma.



* immunopositivity significantly lower.

** immunopositivity significantly higher.

Figure 8. **a)** The level of immunopositivity in trypsinogen-1 (Tryp-1), **b)** trypsinogen-2 (Tryp-2), **c)** the trypsin inhibitor (TATI), **d)** and matrix metalloproteinase 9 (MMP-9) (scored from 0 to 3; line in median) in the epithelium of the colon (Tryp-1, Tryp -2, TATI) or in the stromal inflammatory cells (MMP-9) in the diagnostic tissue samples of pediatric ulcerative colitis patients who had undergone surgery or were conservatively treated and control children for whom inflammatory bowel disease had been excluded.

DISCUSSION

This dissertation evaluated the surgical treatment for IBD patients. Results on postoperative bowel function and QoL for pediatric CD patients are sparse. Study I successfully established that most IBD patients have a QoL similar to that of the controls and an acceptable bowel function. Disease relapses, re-resections, and complications during postoperative follow-up were also common among surgically treated patients. CD can exist throughout the GI tract, and it is especially challenging to visualize small bowel disease. Study II was able to show that a novel MRE imaging method can depict small bowel disease and thus guide both medical and surgical treatment. Pouchitis is a common complication related to the ileal pouch anal anastomosis (IPAA), which is a part of restorative proctocolectomy in UC patients. While the study found no confirmation that this inflammation was a reactivation of IBD in UC patients, some similarities were still found. Study IV for the first time evaluated the presence of trypsinogens and their inhibitor, TATI, in pediatric UC patients. The study found that already at diagnosis, the epithelium of the colon differs in pediatric UC patients who will undergo surgery during childhood/adolescence compared to conservatively treated patients and healthy children. By studying the outcomes of the patients who have undergone surgery, this dissertation introduces new information that can be used to further develop current treatment strategies.

LONG-TERM RESULTS OF SURGICAL TREATMENT

Since the role of surgery in treating CD patients is palliative, it is extremely important to know how patients will benefit from the surgery. The reports on the long-term outcomes of surgery are sparse for pediatric CD patients. One reason for this is that operated CD patients form a heterogeneous group. The operations range from short ileal resection to proctocolectomy and/or ileostomy, making generalizations about the postoperative functional results difficult. However, having an adequate amount of knowledge about the long-term results is essential for patients and their families when they are considering surgery. Likewise, CD patients require knowledge about their condition when the need for an operation is acute and there is no alternative to surgery. For example, this may be the case when a patient has a stricture in the bowel and is suffering from obstructive symptoms. A more complicated situation occurs when a patient has an active disease segment in the bowel but no obstruction can be seen. A patient may experience a delay in growth, but may otherwise be doing fairly well with the current medication, which may include a TNF-α-antagonist,

and in some cases, steroids. Determining whether a patient should wait until there are signs of obstruction or whether the diseased segment should be resected right away is the challenge.

Study I focused on reviewing the outcome of surgery at a median of ten years after resection in pediatric CD patients. According to the results, the resection, regardless of the type, only relieves the disease activity for a short period of time: Nearly half of the patients had both an endoscopic and a clinical relapse during the two years after the first resection. More than half of the patients also required further resections during the follow-up. Comparable results have been reported in other pediatric studies (Besnard 1998; Baldassano 2001; Pacilli 2011; Patel 1997). However, Boualit et al. (2012) recently reported a relapse rate of 47% and a re-operation rate of 29% during the first ten postoperative years. These figures are significantly lower than those found here. Boualit et al. defined relapse as the first dose of an immunosuppressant or TNF-α-antagonist postoperatively for patients who were naïve to these medicines before the operation. The differences between the relapse figures reported in study I and those reported by Boualit et al. can be explained by the fact that there were less patients in our study, that the patients were diagnosed at a younger age, that the time from diagnosis to surgery was longer, and that we defined active disease relapse differently. Compared to published adult data, the relapse figures in the present study were slightly higher. In adults clinical relapse occurs in 17% to 55% during the first five years and 11% to 32% of them require a second operation (Yamato 2005; Buisson 2012b). Overall, these figures are high and raise a question: Do the patients really benefit from surgery? In the case of a growth-retarded child, two years is a long time and it is known that even a short period of time when the disease is in remission may enable the child to experience catch-up growth, especially if surgery is performed before late puberty (Newby 2005; Besnard 1998; Ranger 2006). By resecting the diseased segment, it is usually possible to taper the steroid doses and the patient may have less side effects.

Half of the patients in our study were operated on for acute reasons, including strictures, bleeding, and fistulas. The rest were challenging patients who received maximal medical therapy, but who still experienced severe symptoms. The study could not find any difference in the relapse rates of the disease between these two groups, reflecting the fact that the actual indications of the need for an operation do not alter the result. However, the small number of patients may confound these results.

While TNF-α-antagonists have improved the effect of medical therapy, it has not been shown that they diminish the overall surgery rates for pediatric CD patients (de Bie 2012b). The use of a TNF-α-antagonist in this study population was rare: Only three patients had had TNF-α-antagonists prior to their first resection and only two before the first disease reactivation. However, nearly half of the

patients had been on a TNF-α-antagonist at some point during their disease course and one-third of the patients required future surgery after the initial dose, though in most cases the time interval between the first dose and surgery was a matter of years. Due to the high relapse rate, nowadays it is recommended that patients use postoperative treatment to diminish the number of relapses, especially if the patients have risk factors for an early relapse (Van Assche 2010). Usually, thiopurines or mesalazine are used, but infliximab is the first choice for those patients who are at high risk or who have used TNF-α-antagonists more than six months prior surgery (Van Assche 2010; Buisson 2012a). There is evidence that all of these medications are effective in preventing relapses in adult patients (van Loo 2012). The use of scheduled postoperative treatment in the present population was rare, and this can partly explain the high relapse rate. No treatment guidelines for postoperative treatment exist for pediatric patients. Adult studies have, however, shown that some kind of postoperative medication is probably beneficial, including within the pediatric age group, to improve the outcome of surgery. At Helsinki Children´s Hospital, the postoperative follow-up routinely includes measuring the fecal calprotectin levels. If high levels of fecal calprotectin are detected, then the patients are scheduled for a colonoscopy to detect possible disease reactivation, since it is known that an endoscopic relapse precedes a clinical relapse of the disease (Rutgeerts 2005).

Just as postoperative disease reactivation was common, so too were surgery-related complications. During the long-term follow-up, 77% of patients had suffered from at least one surgical complication. Most of the complications were managed via a conservative treatment, but 11 patients required further resections. There are no studies that have assessed the complication rate resulting from pediatric CD surgery. Twenty-five percent of the complications were anastomotic strictures, which are usually caused by disease activation postoperatively and not related to the surgical technique or decision-making process; thus, they do not constitute a complication according to the tradition definition. Pediatric UC patients who have undergone a proctocolectomy show a comparable rate of complications (Pakarinen 2009). Here, preoperative steroid use was not associated with the higher complication rate, contrary to the data reported before (Uchida 2010; Markel 2008). IPAA was erroneously constructed to nine patients whose diagnosis at the time of the primary surgery was UC. All of these patients suffered from pouch problems: This is in line with other studies that report the presence of IPAA-related problems among CD patients (Alexander 2003). This is, however, a problem that is difficult to foresee, since pancolitis is common in pediatric CD patients; for this reason, it is challenging to make a differential diagnosis for CD and UC patients (Mamula 2003; Kugathasan 2003; Auvin 2005; Heyman 2005). However, if the diagnosis is uncertain, a colectomy should initially be performed, while pouch construction

should be done later, when the diagnosis has been confirmed (Ryan 2011). However, it cannot be said with certainty that CD has been the only reason for the pouch problems among these patients; there are many other risk factors as well (see table 15 on page 61).

The fact that most CD patients will have a period of disease reactivation after surgery underlines the importance of demonstrating the actual effect of primary bowel resection on the everyday life and bowel habits of the patients. It should be remembered that even without resection, patients would often suffer from disease relapses. Here, patients had on average two to three bowel movements per day and were continent at night; minor soiling was reported by approximately one-third of the patients. When compared to healthy Finnish individuals of an equal age (18-26 years), the bowel function was poorer among CD patients (Kyrklund 2012). However, 40% of healthy individuals reported soiling of some kind; but the frequency was lower than among CD patients, who, on average, reported soiling one to three times per week. The number of stools per day was higher among CD patients: Most of the healthy individuals had a stool frequency ranging from once every other day to twice a day (Kyrklund 2012). We noted that the tendency of having impaired bowel function was related to the total amount of resections: Those patients who had undergone several resections had a higher stool frequency, more stools per day and their soiling was more frequent, although the differences were non-significant and the study groups small (n=5-8). However, those who had both ileal and colonic resections had statistically more bowel movements per day compared with patients who had an ileal resection only. Surprisingly, there are no data on the overall bowel function of pediatric CD patients (either resected or unresected) in the existing literature. A common view is that CD patients have altered bowel function, especially during an active disease phase. Taken together, one short resection does not significantly alter the bowel function, but those patients who have undergone a re-resection suffer from altered bowel function, especially if both the ileum and colon are resected.

A good overall QoL is one of the goals of IBD treatment. Study I found that operated CD patients, as well as UC patients (Pakarinen 2009), have an equal overall, social, emotional, and physical QoL, regardless of the number of disease relapses, as age- and gender-matched controls with no IBD. A previous study using the same QoL questionnaire for a similar age group reported that the overall QoL was significantly lower in pediatric onset IBD patients compared with that of the age- and gender-matched controls (Turunen 2009). The patient population in the present study was smaller, but the results indicate that an operation might have a positive effect on a patient's QoL. Other pediatric (Barrena 2011; El-Baba 1996) and adult (Thilby 2001) studies report similar findings. However, the present study found that if the patients suffer from disease relapses that disturb school

attendance or work, then the QoL is significantly lower than it is for other operated patients. Patients who suffered from soiling also had a lower QoL, but the results were statistically non- significant.

THE CHALLENGE OF DIAGNOSING SMALL BOWEL DISEASE IN PEDIATRIC CROHN'S DISEASE

Surgery remains a second-line therapy for IBD patients because many patients go into remission as a result of medical therapy (Bie 2012; Hyams 2012; Jakobsen 2011a; Turner 2011; Wilson 2010; Hyams 2011; Riello 2011; Hyams 2009). However, a subgroup of patients suffers from side effects, mostly as a result of steroids (see table 9 on page 47). A longstanding active disease can cause complications for CD patients, including strictures, fistulas, and bowel perforation, and most of these complications require surgical treatment (Wiese 2012; Pacilli 2011). The best way to manage these complications is to detect active inflammation during the early disease phase and start administering medication. However, identifying active inflammation is not as straightforward a process for CD patients as it is for UC patients. Traditional endoscopy is not able to evaluate the presence of small bowel disease and an ileocecal angle cannot always even be reached (Mamula 2005). CD patients require a radiological imaging of their small bowel to detect inflammation. At diagnosis, approximately 5 to 10% of CD patients have an isolated upper GI tract disease and up to one-fifth of them have jejunal or proximal ileal disease only or along with ileocecal disease (de Bie 2012b. Sawczenko 2003). The incidence of small bowel disease during the disease course is unknown and possibly underestimated.

Barium fluoroscopy has long been available to evaluate the presence of small bowel disease in CD patients; however, it is inaccurate and predisposes patients to a high dose of radiation (Di Nardo 2012). CT exposes them to a high dose of radiation as well. Thus, experts began using an MRE. An MRE procedure detects small bowel disease in pediatric CD patients and also visualizes the extramural complications (Magnano 2003; Pozza 2011; Zappa 2011; Dillman 2011; Wallihan 2012; Torkzad 2012) without exposing the patient to ionizing radiation. The present results show that the MRE findings are in most cases comparable with the macroscopic findings seen at the time of surgery or an ileocolonoscopy. Other researchers have confirmed this finding (Toma 2007; Di Nardo 2012; Dillman 2011; Wallihan 2012; Sauer 2012). However, while an MRE slightly overestimates the severity of the small bowel disease, it makes it possible to localize the disease and plan the surgical approach. For colonic disease, an MRE may underestimate the disease activity and its degree of sensitivity for detecting colonic disease may be low (Horsthuis 2010; Dillman 2011; Wallihan 2012).

A WCE procedure is another modern way to evaluate pediatric small bowel in CD patients (Moy 2007; Guilhon De Araujo Sant'anna 2005; de'Angelis 2007; Jensen 2010; Mehdizabeh 2010). However, compared to the MRE procedure, a WCE procedure visualizes only the mucosal changes and it may make it difficult to interpret the localization of the disease. While a WCE procedure serves as a good tool for diagnosing CD, the risk of capsule retention is high. This may favor the use of an MRE (Atay 2009; Cheifetz 2006; Cohen 2011). With both procedures, patients must alter their diet one day prior to the procedure. For an MRE procedure, the patient has to drink large amounts of sorbitol, polyethylene glycol (Magnano 2003; Laghi 2003), mannitol (Borthe 2006), or psyllium (Alexopoulou 2009), which can cause diarrhea, and in the case of small bowel stricture, also vomiting. Thus, an MRE procedure may cause more discomfort for the patient than a WCE procedure. Both procedures are feasible for patients approximately six years of age and older (Torkzad 2012; de'Angelis 2007; Jensen 2010). For younger patients, a WCE procedure may be introduced in the general anesthesia, but an MRE procedure needs the co-operation of the child.

The overall success rate of the MRE procedure was high in the present study. Vomiting caused imaging failures and it was the most common side effect of the procedure (Magnano 2003; Borthe 2006). Administering sorbitol fluid with a nasojejunal tube (enteroclysis) may improve the success rate. However, the place of the tube has to be confirmed by X-ray, which then predisposes the child to a radiation dose that should be avoided, especially in pediatric patients. An MRE procedure requires administering gadolinium intravenously for contrast enhancement in order to visualize the bowel wall. The use of gadolinium is associated with a small risk of nephrogenic systemic fibrosis (Toma 2007). To minimize this risk, a safer contrast medium, gadorate meglumine, was used in the present study.

The results here show that, in addition to making good visualization of small bowel disease possible, an MRE procedure also helps clinicians with decision-making. An MRE procedure contributed to the decision to perform surgery on or change the medication of approximately one-third of the patients studied. For those patients whose MRE was negative, the treatment usually remained the same as it was before, but the imaging offered valuable information on the situation in the small bowel. Recently, Sanka et al. (2012) studied 34 pediatric patients using an MRE procedure and concluded that the procedure guides treatment and helps with the diagnosis, which is in line with the present results. In this study, an MRE procedure was the only method that could show the active disease segment in the distal ileum or jejunum in approximately 10% of the studied cases. Another study found that an MRE procedure could detect distal ileitis in 7 out of 24 patients (29%) whose distal ileum was not reached at ileocolonoscopy (Wallihan 2012). Dillman et al. (2011) detected previously unknown jejunal involvement in 13% of the studied

patients (n=32) (Dillman 2011). These results further emphasize the fact that an MRE procedure should be used as the first-line method to evaluate possible small bowel disease in children with CD (van Assche 2010).

PROTEINASES ASSOCIATED WITH AN AGGRESSIVE DISEASE COURSE AND POUCHITIS

Two studies (III and IV) assessed the expression of proteinases in the bowel wall to better understand both the pathogenesis of aggressive pediatric IBD and the possibility of finding a marker/markers that could help in optimizing the treatment.

Study III focused on characterizing the observed inflammation in the ileal pouch after proctocolectomy in pediatric UC patients by studying MMPs and TIMPs. Earlier studies report that these proteinases are related to the inflammation seen in pediatric IBD patients (see pages 23-27). The symptoms of pouchitis (see table 16 on page 62) are quite similar to IBD reactivation, but this inflammation often reacts to antibiotic treatment (Holubar 2010), unlike the UC-related inflammation. Overall, in a cohort of 28 proctocolectomized UC patients, pouchitis occurred in 68% of patients, which is in line with previous reports with a comparable follow-up period (see table 12 on page 56).

While some patients had active pouchitis at the time of the biopsy, others had no symptoms, and one-third of the patients had never experienced pouchitis. However, inflammation was seen in nearly all of the samples, even though the patient had never experienced clinical pouchitis. The MMP and TIMP profiles were comparable for all of the patient groups. This result indicates that clinical pouchitis has little effect on the overall MMP and TIMP profile of the pouch.

The MMP and TIMP profile of the pouch shares some similarities with the profile seen in IBD patients, but some abundant dissimilarities were also detected (see table 2 on pages 26 and 27). The wide expression of MMP-3, TIMP-3, and TIMP-2 was similar to that of IBD. However, the MMP-3 expression correlated negatively with the inflammation markers, which is contrary to that of IBD (see table 2 on pages 26 and 27). TIMP-3 is also expressed in a healthy ileum and colon, while TIMP-2 is expressed in a healthy colon and is not specific to IBD. Thus, the expression of MMPs and TIMPs in the pouch can resemble their expression in a healthy colon, since pouch mucosa may become colon-like (see table 16 on page 62). The total absence of MMP-9 in the pouch mucosa was the most striking difference between the pouch and IBD mucosa, since MMP-9 is widely expressed in IBD (see table 2 on pages 26 and 27 as well as study IV). The expression of both MMP-8 and MMP-7 was also different from that seen in IBD. The high expression of MMP-7 in the pouch could be related to the regenerative process of the pouch, since a low

expression of MMP-7 is seen during active inflammation and the expression has been linked to the healing process of GI ulcers. The expression of MMP-26 and TIMP-1 did not support or rule out the IBD etiology of pouchitis.

Based on these results, the subclinical pouch inflammation seems to be different from that seen in IBD, although some similarities were detected. This might be supported by the fact that they responded differently to the medical treatment, although this was not studied here. MMPs -3, -7, and -12 and TIMPs -2 and -3 were detected in the pediatric UC-associated pouch and their expression was not related to the symptoms of pouchitis. However, since the follow-up for patients was long, patients had had various treatments during their disease course that might have altered the MMP and TIMP expressions seen in the bowel.

It is known that not all pouch-related problems are caused by pouchitis. A subgroup of patients may ultimately have CD, with the diagnosis only being confirmed after a restorative proctocolectomy (study I; Mortellaro 2011; Alexander 2003). There are no existing studies that have determined the MMP profile of the ileal pouches of patients whose final diagnosis changed from UC to CD. This kind of study would be valuable if a specific marker of the ileal pouch in UC (or CD) patients could be confirmed. It is known that pouch problems caused by CD are chronic and can even result in pouch failure (study I; Alexander 2003). By finding a biomarker, these patients could have more effective treatment. However, it must be remembered that CD is only one factor causing chronic pouchitis (Alexander 2003).

The focus of study IV was to find markers that could distinguish pediatric UC patients with an aggressive disease course at diagnosis from patients with a more benign disease. If this would be possible, a more active approach to devising therapeutic strategies could be considered. For example, an aggressive therapy, including immunosuppressive treatment, could be started before the disease causes complications or the patient has side effects from, for example, high doses of steroids. Complications and steroid dependency are known to be the leading reasons for surgery (Barrena 2010; Ba'ath 2007). Knowledge about the effectiveness of an early aggressive treatment supports this finding (Jakobsen 2011a; Vernier-Massouille 2008; Gupta 2006). Tryp-1, Tryp-2, and TATI were stained by immunohistochemistry because they are associated with the inflammation, even though their specific role in pediatric IBD remains unknown (see details on pages 23 to 27). MMP-9 was also studied because it is known that trypsins activate proMMP-9, one of the main markers of active IBD (Baugh 1999; Lauhio 2008; Paju 2001; Sorsa 1997; Lukkonen 2000).

The low trypsinogen level detected in the inflamed samples is an interesting finding because trypisinogens and other serine proteases degrade extracellular matrix proteins and the pro-forms of acute-phase reaction proteins (Biancheri 2012) and help regulate homeostasis, inflammation, pain, and tissue repair by activating

protease-activated receptors (PAR) (Cottrell 2003; Fox 1997; Macfarlane 2001). However, serine proteases are also capable of eliciting a sustained increase in the transepithelial resistance of both ion and larger molecules by activating protein kinase C in a cell model of intestinal epithelial cells (Swystun 2009; Buzza 2010; Netzel-Arnet 2011). Thus, we can speculate that the level of trypsin in UC patients undergoing surgery is too low to sustain this physiological role of trypsin. This is supported by the findings on epithelial barrier dysfunction related to ion permeability (Prasad 2005; Zeissig 2007) and larger molecule permeability (Pearson 1982), which has been associated with the development of IBD in adult patients (Swystun 2009).

Trypsinogens and trypsins have also been found in Paneth cells in a healthy small bowel and in CD patients (Bohe 1986; Cottrell 2003). The role of trypsin in these cells is associated with the activation of human alpha defensin 5 (HDA5) (Ghos 2002). Defensins are antimicrobial peptides produced by a wide range of epithelial cells (Ramasundara 2008). In the intestine, they maintain the balance between protection from pathogens and a tolerance for normal flora. The amount of HDA5 has been reduced in the terminal ileum of CD patients (Wehkamp 2005). This reduction, however, was not confirmed in children (Zilbauer 2011). Nonetheless, the levels of HDA5 in UC patients were significantly higher compared to the levels in CD patients and controls (Zilbauer 2011). Since the Tryp-1 expression in the epithelium of the colon was lower in patients undergoing surgery than in the controls, we can speculate that patients who suffer from an aggressive disease also have a lower expression of HDA5 in their colon and are more prone to the effects of bacteria during an early disease course. Unfortunately, there was no possibility to determine the levels of defensins in this study.

Since the expression of the trypsin inhibitor TATI was low, it cannot explain the mild expression of trypsinogens in the inflamed UC mucosa. However, the low expression of TATI is in line with previous data, which have shown that the amount of TATI is low in IBD and gastric ulceration (Playford 1995). Based on these results, the trypsinogen and TATI levels in pediatric UC patients seem to be low; Tryp-1 levels are especially low in patients who have an aggressive disease course. In the future, the low level of Tryp-1 might be used as an indicator of aggressive disease, regardless of the level of inflammation. This would be beneficial because subgroup of patients who might need surgery may only have mild inflammation during the early disease phase. Further studies of larger patient groups are needed to confirm these findings.

The expression of MMP-9 was studied because it is related to active IBD (Baugh 1999; Mäkitalo 2009) and trypsinogens are known to activate pro-MMP-9 (Lauhio 2008; Paju 2001; Sorsa 1997; Lukkonen 2000). In this study, no correlation between the expression of MMP-9 and studied trypsinogens was found; however, this link needs to be studied further. The increased immunohistochemical expression of

MMP-9 in the inflammatory cells of the colon was associated with pediatric UC patients, which other studies have also confirmed (Mäkitalo 2010), and was found also to correlate with the degree of inflammation (Baugh 1999). In this study, MMP-9 was also weakly expressed in the epithelium of the colon in pediatric UC patients, but not in the non-IBD controls, reflecting a wider expression of MMP-9, as reported before. However, since the number of studied tissue samples was small, this finding needs to be studied further before it can be confirmed.

Colonic disease is common in pediatric CD patients (Mamula 2003; Kugathasan 2003; Auvin 2005; Heyman 2005). According to unpublished results, the low expression of Tryp-1 in the epithelium of the colon could help differentiate pediatric UC patients from CD patients at diagnosis, especially those who have an aggressive form of the disease, since Tryp-1 levels were higher in CD patients at diagnosis than in UC patients (Piekkala et al., unpublished data). This finding, together with the different expressions of MMP-7 in the pancolitis of UC and CD patients (Mäkitalo 2010), indicates that the colon inflammation might be different in these diseases. The finding may be informative because some pediatric CD patients who have a colonic disease may be misdiagnosed as having UC and undergo a proctocolectomy accompanied by an ileal pouch anal anastomosis (IPAA), which is not recommended for CD patients because of the high rate of pouch problems (Alexander 2003).

STRENGTHS AND LIMITATIONS OF THE STUDY

A follow-up period of ten years in studies I, III, and IV made it possible to assess the long-term results of the disease. However, a long follow-up time can also be challenging. During the last decade, the treatment of young IBD patients has evolved and, for example, therapy involving TNF-alpha antagonists has only recently been introduced. Most treatment strategies, however, have remained the same for a long time. As in study I, the indications of the need to operate were similar during the follow-up period. In addition, none of the patients had systematic postoperative medical therapy during the follow-up period; however, postoperative treatment diminishes the relapse rates in adults and this should also be studied in children. Study I also includes both patients who underwent their first surgery before the era of biological therapy and those who underwent surgery after it had been introduced. However, biological therapy has not been shown to decrease the overall surgery rates (de Bie 2012b). Therefore, in terms of management, there were no major differences between those patients who underwent surgery recently and those who were operated on at an earlier point in time. The strength of study IV is that it compares patients who underwent an early surgery with patients who were diagnosed at the same age as the operated patients and followed at least during

the time that had lapsed between the diagnosis and the operation. This matching makes the compared groups as homogenous as possible and diminishes the possible bias caused by a long follow-up.

The use of a prospective questionnaire was the strength of studies I and III. Bowel function was evaluated using a previously validated Bowel Function Score (BFS) questionnaire (Rintala 1995; Rintala 1997). The QoL questionnaire used in study I had previously been used in a similar age group (Pakarinen 2009; Turunen 2009). The QoL and its four different dimensions were studied with an easy-to-use visual scale. Younger patients are often hesitant to fill in long questionnaires. While validated IBD-related QoL questionnaires (IBDQ-32, IMPACT questionnaire) exist for both adults (Guyatt 1989) and children (Otley 2002), they include up to 33 questions and it takes between 10 and 15 minutes to answer them (Otley 2002), making the whole questionnaire long and laborious to complete. It must be said that the validation has not been translated into Finnish. With the present questionnaire, the number of missing answers was low in questions concerning bowel function, suggesting that patients had understood the questions. Another strength of study I was that age- and gender-matched population-based controls were used to compare the QoL among patients. To date, no data exist on the overall bowel function in pediatric CD patients (either conservatively treated or operated patients). Study I for the first time reports on postoperative bowel function in pediatric CD patients. In study III, the patients were asked about the occurrence of pouchitis or infection and the use of medication at the same time that a biopsy was taken. The person who helped with the questionnaire was not familiar to the patient and not a member of the management group. Thus, the answers were less likely to be biased by the patient-doctor relationship. Study I and part of study III were postal questionnaires and patients answered the questions by themselves at home. Since the age of those responding to the postal questionnaire was approximately 22 years (study I; Pakarinen 2009), it is unlikely that the parents had impact on the answers.

The strength of study II was that two pediatric radiologists blinded to patient outcomes evaluated the pictures for the study by filling in a standardized evaluation form. This study was among the first to show that MRE is able to guide treatment. The main limitation, however, was the long time gap between the MRE and endoscopy or surgery (three months). However, the ultimate aim was to show the importance of MRE in guiding treatment and not to compare the MRE finding with macroscopic or histological findings, since others have already reported those findings (Toma 2007; Di Nardo 2012; Dillman 2011; Wallihan 2012; Sauer 2012).

Studies III and IV share the same limitation: The expression of proteinases was detected using only a single method—immunohistochemistry. To strengthen the results, the biopsies should have been analyzed using different methods, such as Western blot and PCR. However, this was not possible in these studies because the

biopsies were old and small and the methodology for doing it was not available. The strength of study IV is that the normal expression of proteinases in the gut wall was studied by staining the biopsies from healthy children. Study IV is also unique because it analyzes the stained diagnostic tissue samples from patients whose disease course was known. The strength of study III is that it was the first study to report the expression of MMPs -3, -7, -12 and TIMP-2 and -3 in the ileal pouches of pediatric onset UC. In study IV, the expression of trypsinogen and TATI was for the first time determined in the diagnostic tissue samples of pediatric IBD patients.

All patient data, such as medication, disease activity, relapses, and re-resections was retrospectively collected. Retrospective studies are prone to selection bias and it is impossible to properly evaluate disease activity. In retrospective studies, the data are not systematically registered, which limits the comparisons. For all of the studies discussed here, the small number of patients has to be considered, with number of studied patients ranging from 28 to 43. However, these numbers are in line with most pediatric studies from this field. A small patient series is a common limitation of pediatric studies. In study I especially, the small number of studied patients restricts the interpretation of the results when considering postoperative relapses, complication rates, and functional outcomes. Regardless of the limitations, most of the results are novel and offer a basis for broader studies.

CONCLUSIONS

The treatment for IBD is controversial, since both conservative and surgical methods exist. However, while surgery can cure UC patients, there is no curative treatment for CD patients. Both methods have their place in the treatment of IBD, but choosing between surgery and medical therapy for most patients is often a challenge.

According to the existing literature, performing surgery on CD patients enables growth, especially if it is done before late puberty. On the other hand, relapses and re-operations are common and surgery should be reserved for patients that require it mostly for acute reasons. This dissertation introduces new information on the long-term outcomes in CD patients who underwent surgery in childhood and who were followed up for a median period of a decade. The patients had a comparable QoL to that of the controls. However, surgery remains a second-line therapy because patients who had more surgeries and a longer total length of resection had more problems with their bowel function. Nonetheless, the patients who ended up having surgeries originally had a more aggressive disease. Also, those patients who were absent from school or work because of bowel problems had a diminished QoL. The conclusion is that surgery has it place, but postoperatively everything has to be done to protect patients from relapses and the need for additional surgery.

Evaluating small bowel disease is a part of CD diagnostics. An MRE shows the presence of small bowel disease and extramural complications quite well. The advantages of an MRE are that it is radiation free and quick. This enables the detection of active small bowel disease in an early phase. According to current results, an MRE can help guide treatment. However, its use is limited to patients who are beyond school age because the method requires cooperation. An MRE clearly has a place in evaluating small bowel disease together with a WCE. Whereas an MRE shows the extramural complications better, a WCE shows mucosal disease better.

Proctocolectomy is the preferred curative treatment for UC patients, but many patients suffer from pouchitis postoperatively. Whereas most of the patients react to antibiotic therapy, some require medication, which is used to treat IBD. For the purposes of this dissertation, the actual type of inflammation in the pouch could not be confirmed as an activation of IBD by just studying MMPs and TIMPs, although some similarities were found. Pouchitis seems to be a heterogeneous disease, and the etiology of pouch-related problems may differ between patients.

Since the disease course is quite heterogeneous for pediatric IBD patients, it would be beneficial to separate patients with an aggressive form of the disease from those with a more benign form of the disease. The fourth study showed that the

presence of trypsinogen-1 in the diagnostic tissue samples differs between those patients who require surgery and those patients with a more benign disease course.

The treatment for pediatric IBD remains challenging, especially for CD patients. To better understand the etiology of IBD in children, and to find the optimal treatment modalities, further prospective studies are required. In particular, there is a need for studies that can ascertain the proper postoperative treatment to protect patients from disease relapses and studies that can discover the markers that predict the aggressive disease course.

ACKNOWLEDGEMENTS

This study was carried out at the Children`s Hospital, Helsinki University Central Hospital during the years 2009-2013. I would like to thank Docent Jari Petäjä, head of Children`s Hospital, Professor Mikael Knip, chair of Children`s Hospital, and Docent Eero Jokinen, head of the Department of Pediatrics, for providing me with the appropriate research facilities. As a member of the Pediatric Graduate School, I would like to thank Professor Markku Heikinheimo, head of the Institute of Clinical Medicine and former head of the Pediatric Graduate School, and Docent Jussi Merenmies, head of the Pediatric Graduate School, for their valuable work in gathering together and guiding young researchers.

My deepest gratitude goes out to my two supervisors, Docent Kaija-Leena Kolho and Professor Risto Rintala. Kaija-Leena, without your encouragement I never would have started researching this topic during my studies. I admire your enthusiasm and knowledge. You always had time to teach and guide me thought the whole process. Risto, I have been honored to be guided by one of the valued pediatric surgeons. Thank you for your interest in my project.

I warmly thank Docent Marja-Leena Lähdeaho and Docent Anna Lepistö for reviewing this dissertation and for providing valuable insight. I sincerely thank Docent Anders Paerregaard for accepting the role of opponent. I warmly thank Docent Merja Ashorn and Docent Taina Sipponen, the members of my follow-up group in the Pediatric Graduate School. You both supported me and gave me valuable guidance throughout the entire project.

Many thanks go out to my co-authors and collaborators: Docent Merja Ashorn, Professor Caj Haglund, Docent Teija Kalajoki-Helmiö, Docent Riitta Karikoski, Ph.D. Antti Koivusalo, Ph.D. Laura Martelius, M.D. Johanna Natunen, and Docent Maarit Tanskanen. In particular, I would like to thank Docent Mikko Pakarinen, Docent Jaana Hagström, and Ph.D. Laura Mäkitalo for their valuable guidance. Professor Keiichi Isaka and Professor Karl Tryggvason are gratefully acknowledged for providing essential materials. I would also like to thank Alli Tallqvist and Päivi Peltokangas for their expertise in the lab and Minna Kuurne-Koivisto, Leena Ripsaluoma, and Anne Nikkonen for their assistance in collecting patient data. Anne, your help was invaluable. You are the person that every young researcher wants to have help them.

Special thanks go out to my research colleagues: Ph.D. Hanne Rintamäki, Ph.D. Marianne Sidoroff, Ph.D. Hanne Laakkonen, M.D. Satu Pirilä, M.D. Hanna Lampela, B.Med. Mikaela Porkholm, and all those not specifically mentioned here from the Biomedicum 2 C building. You all gave me valuable guidance and support.

I warmly thank my dear friends. Emilia Ahola, Emilia Hintsala, Sini Sammalkorpi, Merit Järvelä, and Reetta Häivälä, you were there every day when I struggled with the research and with my studies. Emilia Lindström and Katja Heiskanen, our friendship is one of the best things that has ever happened to me. You have kept my feet on the ground and eased my stress. Through you, I have made many new friends. Karoliina Tuomola, you know me best: Next time in Greece. Johanna Koskilammi, thank you for providing me with the wonderful figures and for your support. Reetta Hälvä and Sanna-Kaisa Pykälä, I am so happy that we met each other in Lappeenranta. My friends from Kaskipartio: With you I have had unforgettable moments. I would also like to thank all my friends from Cursus Placebo and Cursus Omnia Probans who are not specifically mentioned here.

Finally, my family: Mum, dad, Olli, and Anni, without you this would never have happened. I love you all. I would also like to warmly thank my grandparents, nunni and pappa, mummi and ukki, Eija's and Mika's families, Atte (thanks for being my language support), and Taru for your support.

This study was supported by grants from the Helsinki University Central Hospital Research Funds (EVO), the Pediatric Research Foundation, the Päivikki and Sakari Sohlberg Foundation, the University of Helsinki Foundation, the Gust. Rud. Idmanin Foundation, the Pediatric Graduate School, Tuija and Matti Piekkala, and Saara and Kauko Piekkala.

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Table 3. Semi-quantitative immunopositivity (score 0 to 3) and number of positive samples in the diagnostic colonic tissue samples of pediatric ulcerative colitis (UC) patients and non inflammatory bowel disease (IBD) patients.

Number of positive samples (%)	Surgery (n=24)	Conservative treatment controls (n=27)	non-IBD controls (n=20)	P-value surgery vs. conservative treatment controls	P-value surgery vs. non-IBD controls
Tryp-1					
epithelium	13(57)	19(76)	15(79)	0.22	0.19
stroma; inflammatory cells	11(48)	8(32)	5(26)	0.38	0.21
Tryp-2					
epithelium	21(95)	21(81)	18(95)	0.20	0.22
TATI					
epithelium	18(78)	24(92)	15(75)	0.23	1.00
MMP-9					
epithelium	4(18)	2(8)	0(0)	0.40	0.17
stroma; inflammatory cells	11(50)	12(48)	1(5)	1.00	0.006*
Median immunopositivity					
Tryp-1					
epithelium	1.0	2.0	2.0	0.03**	0.04**
stroma; inflammatory cells	0.0	0.0	0.0	0.16	0.10
Tryp-2					
epithelium	1.0	1.0	1.0	0.56	0.38
TATI					
epithelium	1.0	1.0	1.0	0.81	0.97
MMP-9					
epithelium	0.0	0.0	0.0	0.33	0.17
stroma; inflammatory cells	0.5	0.0	0.0	0.89	0.003***

* Both UC patient groups had significantly more positively stained inflammatory cells in their colonic epithelium when compared to the non-IBD controls.
** The immunopositivity of the patients who had undergone surgery had significantly lower immunopositivity when compared to the conservatively treated and non-IBD controls.
*** Both UC patient groups had significantly higher immunopositivity when compared to the non-IBD controls.