CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) includes two distinctive forms of intestinal inflammation: Crohn's disease (CD) and ulcerative colitis (UC). In both CD and UC, the disease is characterized by bouts of inflammation (relapses or flares) and periods with remission. In CD, the intestinal histology shows inflammation, frequently transmural and often granulomatous, and the disease can involve the entire length of the gastro-intestinal tract in a discontinuous manner. UC on the other hand, only involves the colon where it causes a continuous mucosal inflammation, typically starting from the rectum and spreading proximally. In adult UC patients, the disease often present with inflammation limited to the rectum, while children with UC regularly present with more extensive and severe disease in the whole colon, or pancolitis.[1] During diagnostic endoscopy, approximately 5-15% of patients have a phenotype that bares similarities with both CD and UC.[2-4] These patients are then diagnosed as IBD-unclassified (IBD-U). After two years, 40% of these patients are reclassified as either CD or UC.[5]

A recent systematic review of population-based studies reported the highest prevalence values of IBD in Europe with UC found in approximately 5 of 1000 individuals and CD in approximately 3 of 1000 individuals.[6] In 3-25% of the patients with IBD the disease develops during childhood or adolescence.[7-12] This wide variation may be caused by differences in definitions of pediatric IBD, with upper age limits varying from 15 to 20 years.[9]

The pathogenesis of IBD is multifactorial, and generally believed to be the result of variations in four components; 1) the patient's genetic make-up, 2) the surrounding environment, 3) the composition of the gut microbiota, and 4) the reactivity of the intestinal immune response (see figure 1A).[13] As young children have been less exposed to environmental factors than adults, genetic factors and a dysregulated immune response might play a bigger role in the pathogenesis of early-onset IBD (see figure 1B).

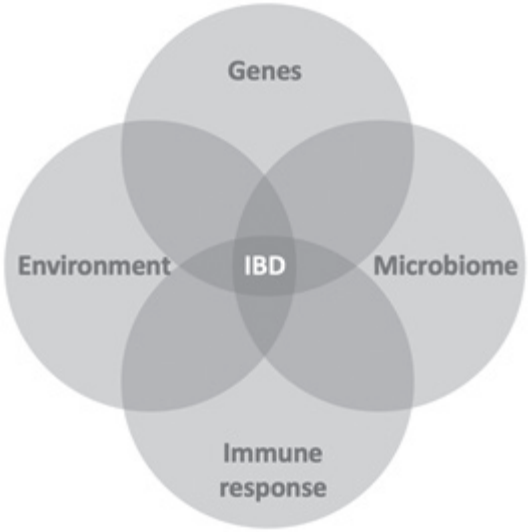
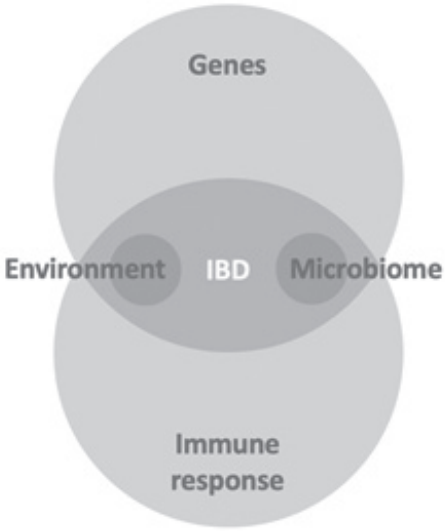


Figure 1. (A) The 4 components involved in IBD pathogenesis.



(B) In paediatric-onset disease genetic factors and a dysregulated immune response probably contribute more to the disease pathogenesis than environmental factors and the microbiome.

Clinical presentation and diagnosis of pediatric IBD

Figure 2 shows the diagnosic proces of IBD. CD and UC typically manifest with symptoms including abdominal pain, diarrhoea, fatigue and weight loss. Additional findings during physical examination include growth delay, abdominal tenderness or perianal disease (fistulae, anal skin tags, or fissures). Blood tests including thrombocytosis, anaemia or leukocytosis, and increased inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) may further increase the probability of IBD. Stool tests with raised calprotectin concentration in the absence of colon pathogens will further raise suspicion for IBD.[14] The gold standard to diagnose IBD is endoscopic evaluation of the mucosal layer in the upper and lower gastrointestinal tract with histopathological biopsies.[15] Endoscopy is an invasive and for the patient burdensome procedure, which is mostly performed under general anesthesia or deep sedation in children and teenagers. When endoscopy fails to provide a diagnosis or is incomplete, despite a high clinical suspicion of CD, evaluation of the small intestine is advised with intestinal

ultrasound, magnetic resonance enterography or small bowel capsule endoscopy.[16]

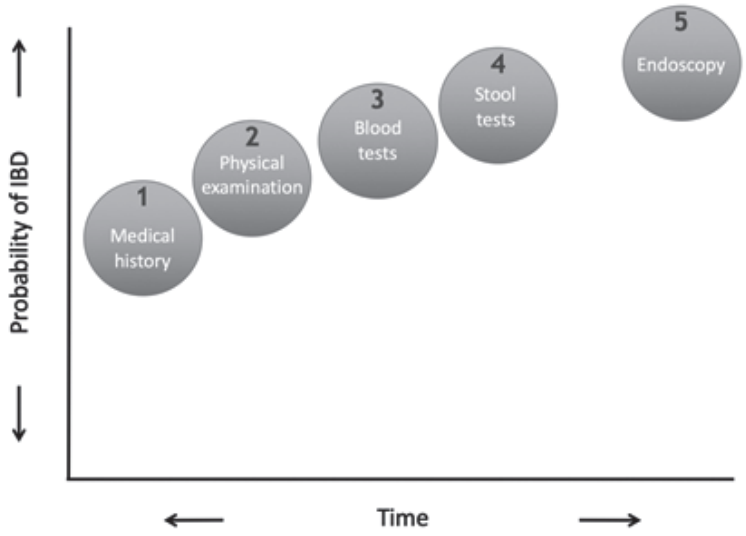


Figure 2. Diagnostic work-up IBD.

Current treatment strategies

When a diagnosis of IBD is confirmed, the patient is usually advised to start induction therapy as well as maintenance therapy with the aim to reach disease remission and reduce the number of flares, respectively. The ideal induction therapy has a rapid onset of action, and the maintenance therapy may have a slower onset of action and preferably has fewer side effects. Additional treatment goals in paediatric IBD compared to adult-onset IBD is to secure a normal growth and pubertal development. The conventional treatment strategy is based on the "step-up" principle.[17] Patients with active CD start remission-induction treatment with steroids and gradual dose tapering, or with exclusive enteral nutrition (EEN) for 6 weeks. Patients with active UC and IBD-U start remission-induction treatment with steroids and aminosalicylate dose escalation. Maintenance therapy in CD includes a thiopurine (azathioprine or 6-mercaptopurine) or methotrexate, and in UC or IBD-U aminosalicylate monotherapy or combination therapy with a thiopurine. Step up to anti-TNF therapy is indicated after failure (nonresponse, loss of response or intolerance) of conventional therapy.

The need for risk-profiling at diagnosis

When the first line of induction and maintenance therapy does not adequately control the underlying intestinal inflammation, it may take weeks or even months before a step-up to more potent medications is made. When active inflammation of the gut mucosa is not recognized and suppressed in a timely fashion, bowel damage will continue (see figure 3).[18] This may result in a complicated disease course defined by development of penetrating and/or stricturing Crohn's disease and an increased risk for bowel surgery. In UC patients, complicated disease is defined as the the need for colectomy.[19]

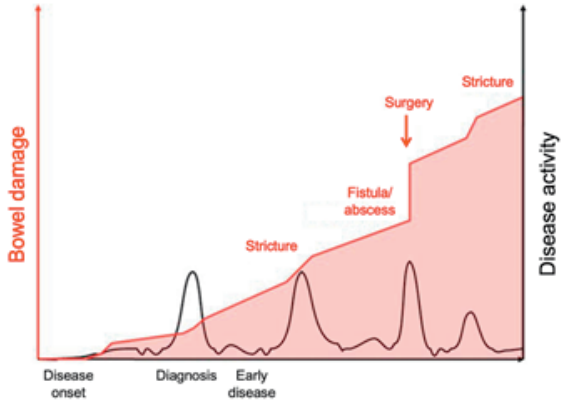


Figure 3. Complicated disease course of a patient with ongoing active CD [18]

Several trials have shown that early use of anti-TNF therapy is associated with improved outcomes and fewer complications in adult as well as in paediatric patients with CD.[20-26] Current paediatric guidelines do not advise the use of anti-TNFs for all patients with IBD, but specify a few exceptional conditions that warrant early and ongoing use of anti-TNF agents, including perianal fistulizing disease, growth failure, or extra-intestinal manifestations.[27] A considerable proportion of the patients with IBD have a relatively favorable disease course, with low risk of developing disease related complications. For those, treatment with anti-TNF therapy may be too agressive, with a higher risk of serious drug-related adverse events (e.g. infusion reactions, serious opportunistic infections and immune-mediated dermatological conditions such as vasculitis or drug-induced lupus) and higher

costs.[28-30] Therefore, in patients with a low risk for a complicated disease course, it may be better to follow a conventional step-up treatment schedule. Identification of predictors for a complicated disease course at diagnosis and in the early phases of treatment may allow physicians to tailor the treatment and select patients at risk of complicated disease for an “accelerated” step-up to anti-TNF therapy and prevent overtreatment in others. Previous studies[REFs] have identified a few prognostic factors that may predict the need for early anti-TNF use (see table 1).

Personalized medicine is defined by some as a medical model that separates people into different groups with medical decisions, practices, interventions and/or products being tailored to the individual patient based on their predicted response or risk of disease.[31] The identification of high and low risk patients may therefore be seen as an important part of personalized medicine.

Monitoring response to treatment

Once treatment has started, regular checks if the intervention is having the desired effect are warranted. Conventional monitoring in paediatric IBD used to be based on the control of symptoms, which means that a reduction in the severity of symptoms was already considered as a treatment success. The correlation between symptom scores and endoscopy-confirmed mucosal inflammation is poor.[37-39] Teenagers who claim to have no symptoms may still have macroscopic or microscopic intestinal inflammation. Such a smouldering disease activity may eventually lead to complications such as growth and pubertal delay.[20,22] Previous studies have shown that patients who achieve clinical as well as laboratory and endoscopic remission have better long-term outcomes than patients with only clinical remission.[40,41] In modern-day IBD care, the ultimate treatment goal is shifting from symptom control towards reaching and maintaining mucosal healing (MH).[42-46] MH is defined as the complete recovery of the intestinal mucosa, and physicians consider it as the most important therapeutic target when treating IBD patients.[44,45] MH is ideally evaluated with endoscopy and histological confirmation, but due to costs and its invasive nature, this method vis not practical when monitoring the response to treatment.

Table 1. Predictors of complicated disease in children, defined as the need for early anti-TNF treatment or surgery. [27,32-36]

Patient factors
■ Very early onset disease
■ Stunting (height-for-age below − 2.5 standard deviations at diagnosis)
■ Wasting (Weight-for-height below – 2.5 standard deviations at diagnosis)
Disease factors
■ Deep colonic ulcerations on endoscopy
■ Extensive (pan-enteric) disease (pancolitis)
■ Stricturing and penetrating disease (B2 and/or B3 disease behavior) at onset
■ Severe perianal disease
■ Acute Severe Colitis at diagnosis
■ Low serum albumin (<3.5 g/dL)
■ Extra-intestinal manifestations (joint, skin and ocular manifestations, and primary sclerosing cholangitis)
Initial treatment response
■ Lack of response on conventional induction therapy
■ PUCAI score of ≥10 at 3 months

The ideal monitoring test is non-invasive, simple to conduct, and easily interpretable. It should detect an imminent disease flare – often undetectable by symptom-based reporting alone – and makes provision for proactive treatment optimization.[47] Surrogate markers of intestinal inflammation, that might replace invasive procedures to determine the mucosal condition, have increasingly been studied in IBD. In Table 2, several frequently used markers of disease activity are compared and evaluated for their suitability as a monitoring test in IBD. Fecal calprotectin (FC) correlates well with colonic inflammation and is the most sensitive marker of active disease compared to other frequently used surrogate markers as CRP and symptom-based clinical scoring systems, including the Crohn's Disease Activity Index (CDAI) , Harvey Bradshaw Index, Pediatric CDAI, Simple Clinical Colitis Activity Index and the Pediatric Ulcerative Colitis Activity Index (PUCAI).[36,48-55] Imaging techniques like capsule endoscopy, MR enterography and intestinal ultrasound are mainly of value when the inflammation is located in the small bowel.[56-59]

Calprotectin is a protein released by activated or damaged granulocytes which can be measured in the stool. Measurement is most commonly performed by testing sent-in stool samples with an enzyme-linked immunosorbent assay (ELISA) at the hospital laboratory. For patients with IBD in remission, elevated FC has been identified as a predictor for relapse during follow-up.[60-68] The researchers of a recent systematic review concluded that repeated testing of FC is useful for early recognition of a disease flare and timely adjustment of therapy plans.[47] It seems reasonable that when rising FC levels can be used to detect intestinal inflammation, normalization of FC levels can be used to assess recovery of the mucosa and success of induction treatment. Multiple studies have used FC to evaluate the efficacy of new therapeutics in IBD, but the literature about the diagnostic accuracy of FC for monitoring response to treatment has not been systematically reviewed.
Whether FC is used to monitor response to treatment, or to detect an imminent relapse, in both cases it is important to realize that the FC test results can be influenced by multiple sources of variation, including biological variation, pre-analytical variation and analyticial variation (see Figure 4). Biological variation refers to fluctuations within the same subject inlcudering severity of mucosal inflammation and contact time between stool and mucosa for saturation with calprotectin. Pre-analytical variation

refers to differences in stool collection technique, stool transport and stool storage. Analytical variation refers to differences in precision and type of assay to measure calprotectin.[69] More information about these potential sources of variability is needed to create accurate handling procedures, to keep the variability as small as possible and to get reliable FC testing results.

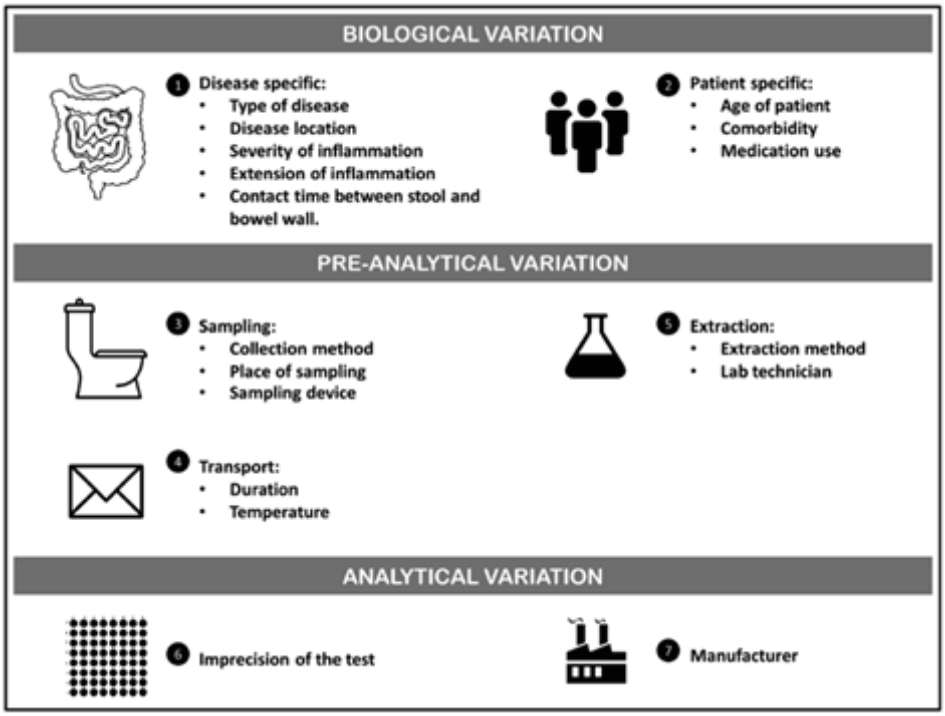


Figure 4. Presentation of biological, pre-analytical and analytical factors that can influence FC levels.[70]

Table 2. Markers of disease activity used in IBD patients.[16]

	Validity (correlation with gold standard)	Responsiveness to changes in condition
Endoscopy	Gold standard	Gold standard
Symptom-based clinical indices	Poor	Moderate Affected by subjectivity
C-reactive protein	Moderate	Moderate Late position in disease progression pathway
Faecal Calprotectin	Good	Good Rises quickly in case of relapse; falls rapidly with successful treatment
Capsule endoscopy	Good	Good
MR enterography	Moderate	Moderate Late position in disease progression pathway
Intestinal ultrasound	Unknown	Good

Signal-to-noise ratio (ability to differentiate changes in condition from background variability)	Practicality
Gold standard	Low Requires bowel preparation and in children general anaesthesia
Moderate Risk of false positive results (irritable bowel syndrome) and false negative results (dissimulation)	High Easy to perform; non-invasive
Moderate Risk of false positive results (acute infections and other inflammatory conditions) and false negative results (normal CRP despite active disease)	High Quick result; but requires venepuncture
Moderate Risk of false positive results	High Possible reluctance by patients for repeated stool collection.
Moderate Potential over-interpretation of insignificant mucosal lesions	Moderate Requires bowel preparation, but is generally well tolerated
Unknown	Moderate Requires oral preparation for bowel distension, and in children preparation through a naso- duodenal tube
Unknown	High Non-invasive, widely available, and well tolerated

Childhood-onset PSC-IBD

Approximately 10% of the IBD patients develop the cholestatic liver disease PSC. Reversly, 50-80% of the patients who present with PSC have or will develop concurrent IBD.[71] IBD in PSC has specific clinical and endoscopical features and should probably be distinguished from UC and CD without PSC.[72] Patients with PSC-IBD typically have a right-sided colonic involvement, rectal sparing, and relatively mild inflammatory disease behaviour.[72,73] It has recently be suggested that PSC-IBD not only has a different phenotype than isolated UC and CD, but may also have a distinct genetic pattern.[74]

In most patients, PSC presents between the age of 25 and 40 years, but it is also recognized as an important cause of chronic liver disease in children.[75-78] The disease causes inflammation and scarring of the bile ducts, which leads to complicated hepatobiliary disease, including cholangitis, hepatic cirrhosis, and liver failure and is potentially fatal.[77,79] Common symptoms include fatigue, pruritis, fever, chills, night sweats, and right upper quadrant pain. There is no curative therapy available for PSC and quality-of-life undermining complications may eventually justify a liver transplantation. In 20 to 25% of the transplanted patients the disease recurs within 10 years.[76]
In patients who already have the diagnosis of IBD, recognition of the early stages of PSC is not easy. The symptoms are initially nonspecific and intestinal disease is frequently more prominent In IBD patients a transient rise of liver enzymes can be caused by the acute inflammation in the intestine or secondary to medication.[71]

The most classical form of PSC is called large-duct PSC. Multifocal strictures and focal dilatations of the intra- and/or extrahepatic bile ducts or beading of the biliary tree is often detected by an abnormal cholangiogram. Small-duct PSC is diagnosed when the cholangiogram is normal, but liver histology shows characteristics strongly suggestive of PSC, like the presence of bile duct damage, onion-skinned peri-ductal fibrosis, inflammation, portal oedema or fibrosis, ductopenia, ductular proliferation, or cholestasis.[71,72,80,81] Overlap syndrome (also called autoimmune sclerosing cholangitis, ASC) is diagnosed when signs of large or small duct PSC are combined with increased levels of transaminases, hypergammaglobulinaemia and autoantibodies, and when

histologic findings of autoimmune hepatitis are detected. It is suggested by several studies that small-duct PSC and ASC are more common in childhood-onset PSC and that childhood-onset PSC tends to present with milder disease and have more favourable outcomes compared to adult-onset PSC.[78,82]

The aetiology of PSC is likely to be multifactorial, with environmental factors triggering the occurrence of PSC in genetically susceptible hosts, a so called complex disease. Genome-wide association studies (GWAS) have identified over thirthy risk loci (genetic locus associated with a certain trait) for PSC, but a large part of the estimated heritability remains unexplained. It has been speculated that rare variants with large effect size may play a role in the onset of complex disorders, but these variants are so rare in allele frequency (many private mutations) that their genetic signals are not detected in GWAS studies (see figure 5). Over 85% of known monogenic disease-causing mutations reside within gene coding regions.[83] Therefore, whole-exome sequencing (WES), in which the entire set of exons in the genome (the exome) is sequenced, has revolutionized our ability to identify rare variants in patients and assess their role in disease onset.

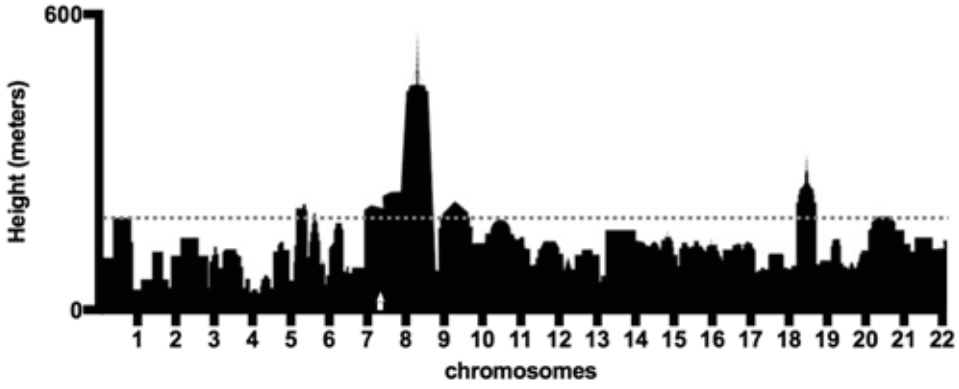


Figure 5. "Manhattan skyline" plot. From a distance, you can only appreciate the buildings (genetic variants) over 200 meters height (the grey dotted line). The tiny white house (rare genetic variant) does not get noticed as it is overshadowed by the concrete jungle of skyscrapers.

Since young children have been less exposed to environmental factors than adults, monogenic variants play a bigger role in the pathogenesis of childhood-onset disease (see figure 6). Monogenic forms of other complex diseases have already been discovered, for instance in very-early-onset IBD, early-onset chronic obstructive pulmonary disease (COPD) and early-onset diabetes.[84-87] In line with these findings, we suspect that this might also be the case for early-onset PSC but whether there are also monogenic forms of early-onset PSC, has not been investigated yet.

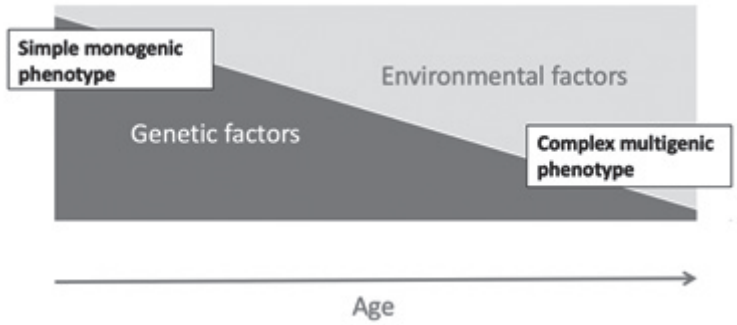


Figure 6. Highest concentration of monogenic forms of diseases at youngest age.

OUTLINE OF THIS THESIS

This thesis will address three themes;

1. Personalizing treatment-strategies
2. Optimizing accuracy of fecal calprotectin measurements in disease monitoring
3. Profiling patients with childhood-onset sclerosing cholangitis

Part I – Personalizing treatment-strategies

Reaching and maintaining mucosal healing, is considered to be the most important therapeutic target when managing adult IBD patients, and stool calprotectin concentrations in the target range are now frequently used as surrogate for mucosal healing. In **chapter 2** we perform a systematic review to determine if falling FC levels back to a predefined FC target range could predict success of induction treatment and healing of the intestinal lining.

Early use of anti-TNF therapy is associated with improved outcomes and fewer complications in adult as well as in paediatric patients.[20-24] In the Netherlands the paediatric IBD treatment guidelines are still based on a step-up principle and accelarated step-up to anti-TNF agents is only recommended in a few exceptional conditions. Risk stratification is needed to select those patients with a high risk of a complicated disease course in need of more aggressive therapies and prevention of overtreatment in low-risk patients. In **chapter 3** we prospectively follow newly diagnosed children with luminal CD or UC who are initially treated with conventional induction therapy and we evaluate whether time-to-reach target calprotectin levels can be another useful prognostic factor that justifies early treatment escalation to anti-TNF agents.

Despite therapeutic advances over the last decades, the arsenal of treatment options in IBD has remained relatively limited. Therefore, optimizing existing treatment schedules and disease monitoring possibilities are of utmost importance to improve patient outcomes. Methotrexate (MTX) is an immunomodulating drug that can be used to maintain remission in patients with CD,

but data on efficacy and tolerability in children and teenagers are scarce. Furthermore, unfamiliarity with MTX makes peadiatric gastroenterlogists frequently omit this drug and move on to anti-TNF agents. In **chapter 4** we evaluate the long-term efficacy and tolerability of MTX monotherapy in a retrospective multicentre cohort study and include patient data from 6 university and 4 general teaching hospitals in the Netherlands.

Part II – Optimizing accuracy of fecal calprotectin measurements in disease monitoring

Fecal calprotectin is widely believed to be a stable stool marker. Accordingly, many hospitals facilitate patients by allowing them to send their stool samples at room temperature to the laboratory. However, actual evidence for stability at room temperature is scarce. In **chapter 5** we test whether the calprotectin concentration remains stable in stool samples stored at room temperature and compare it to the calprotectin concentrations measured in stool samples stored at 4 and -20 degrees Celsius.

Calprotectin-guided disease monitoring is most commonly done by periodically measuring calprotectin in sent-in stool samples with ELISA in the hospital laboratory. Several manufacturers recently introduced a lateral flow–based test and a software application that turns an ordinary smartphone camera into a reader for quantitative measurements at home. In **chapter 6** we perform the first study that compares three home tests and their corresponding ELISA tests to assess which of the pairs has the best agreement.

Part III – Profiling patients with childhood-onset sclerosing cholangitis

It is suggested in several papers that childhood-onset PSC presents with milder disease and has a more favourable outcome compared to adult-onset PSC. In **chapter 7**, to compare outcomes between paediatric- and adult onset PSC, we evaluate time-to-complication curves in two independent pediatric-onset cohorts from the same geographical area in the Netherlands.

Following the recent discoveries of monogenic forms of other complex (auto-)immune diseases we hypothesize that the disease

in a fraction of patients with early-onset PSC is caused by rare genetic variants, resembling a monogenic inheritance pattern. In **chapter 8** we screen the exonic regions of all the genes in patients with childhood-onset PSC and their biological parents using whole-exome sequencing (WES) and perform patient-parents trio-analyses to identify possible monogenic forms of PSC.

In **chapter 9**, the implications for clinical practice and future studies will be discussed.

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Part I
Personalizing treatment strategies