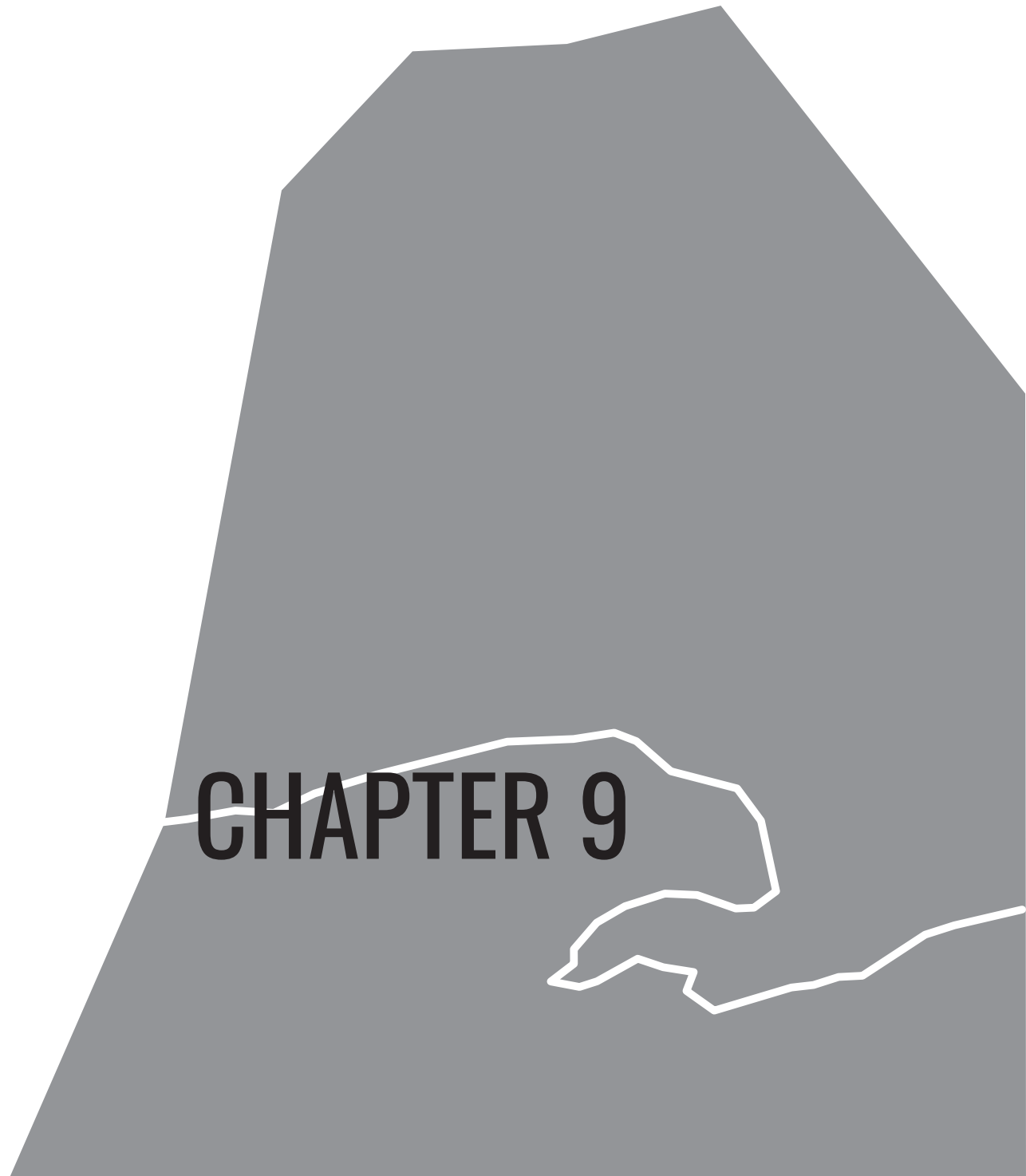
CHAPTER 9

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The various studies in this thesis focused around one of three major themes, including;

1. Personalizing treatment-strategies
   - What did we learn from recently published studies on calprotectin monitoring in patients on induction therapy?
   - Can we predict what type of patients should be treated with anti-TNF agents early in the course of their disease?
   - What is the “Dutch experience” with Methotrexate maintenance therapy in children with Crohn’s disease?

2. Optimizing accuracy of fecal calprotectin measurements in disease monitoring
   - Is the use of sent-in stool samples really the way to go?
   - Should we advise all patients with IBD to analyze their own stool at home?

3. Profiling patients with childhood-onset sclerosing cholangitis
   - What did we learn from comparing a cohort of patients with childhood-onset primary sclersoing cholangitis with a cohort with adult-onset primary sclerosing cholangitis?
   - Can the identification of disease-causing rare genetic variants in patients with early-onset primary sclerosing cholangitis lead to patient-specific therapies?

In the following chapter the main results are summarized, and clinical implications of the findings are discussed. Furthermore, a sketch of tomorrow’s IBD care is drawn.

PART I - PERSONALIZING TREATMENT-STRATEGIES

In chapter 2, we systematically searched the literature on the value of calprotectin monitoring in IBD patients on induction therapy. We identified four studies, including only adult patients, that qualified for inclusion in the meta-analysis. This limited number of suitable trials is indicative of the relatively new application of stool calprotectin to monitor disease activity. Based on the results of our meta-analysis we concluded that performing measurements at the start of induction therapy and at the end is useful to determine if the patient is responding adequately to the treatment or whether further treatment intensification is warranted. We suggest that, rather than merely a decline in stool calprotectin concentration, a pre-specified calprotectin target range needs to be reached which corresponds with disease in endoscopic remission, or mucosal healing, to be able to say that the treatment was truly successful.

In chapter 3, we evaluated whether calprotectin-based disease monitoring is helpful in determining the response to treatment in paediatric practice. We prospectively followed a cohort of children with newly-onset IBD during their first year after diagnosis and tested whether time-to-reach target calprotectin predicted sustained remission. We observed that a larger proportion of children with ulcerative colitis (UC) compared to Crohn's disease (CD) reached the calprotectin target range, and at an earlier time point after starting induction therapy. The state of remission in UC patients lasted shorter than in CD patients. Reaching the target within 12 weeks predicted sustained remission in CD, but not in UC. This finding suggests that a quick response to conventional induction therapy, based on calprotectin values in the target range, allows to identify those with a favourable disease course. Patients who do not have a quick response may need accelerated step-up to anti-TNF agents.

In chapter 4 we reported about the efficacy and tolerability of methotrexate (MTX) immunomodulation after thiopurine failure or intolerance. We showed that MTX is a good alternative for maintenance treatment in paediatric CD patients, especially for those who are thiopurine intolerant, before deciding to step up to anti-TNF therapy.

Clinical Implications

Over the last decade, we have seen advances in biological therapies - new drugs intervening with inflammatory responses, such as vedolizumab and ustekinumab - and small molecule therapies (tofacitinib) that target specific steps in the inflammatory pathways.[1-4] However, none of these drugs can be considered as precision drugs that take into account individual variability in genes, environment and lifestyle for each person. Although current therapeutics aim at countering the immune response, they are rather unrefined and therefore impact adversely on the rest of the body and increase the risk for infections and malignancy.[5,6] Both researchers and clinicians therefore aim to expose only those patients that are really in need of these new drugs, balancing the risk of ongoing inflammation leading to irreversible bowel damage and the need of surgery versus the risk of adverse events. The initiative described in chapter 3, to identify at an early stage those patients that require an accelerated step-up to anti-TNF is, in fact, nothing more than treating bad diseases at diagnosis in a more aggressive manner.

Many of the new drugs have shown to induce mucosal healing (MH), a state that describes the absence of both endoscopic and histologic inflammation, which has recently become the main treatment target in IBD. Reaching and maintaining MH is thought to stop disease progression in the long term. Increasingly researchers and clinicians put confidence in the motto “the first blow is half the battle” and promote the early institution of biologic therapy.[7-12] The short-term results of early intensive treatment are promising. Nevertheless, it is difficult to fully justify the start of such a new treatment strategy. Proper monitoring of the effects in a research setting is therefore crucial to keep moving forward. With the treat-to-target strategy, the response to treatment should be closely followed by using objective clinical and biological outcome measures. If there is a lack of response, initial treatment should be escalated. The CALM study published in 2017, investigated the effectiveness and safety of two treatment algorithms (clinical symptoms combined with biomarkers versus clinical symptoms alone) in achieving MH in CD patients.[13] This was the first treat-to-target trial in IBD, that also tested faecal calprotectin (FC) as a treatment target, with levels below 250 µg/g considered to indicate a satisfactory therapeutic response to treatment and treatment escalation with anti-TNF therapy when FC was ≥ 250 µg/g. The FC-based

strategy appeared to result in better clinical and endoscopic outcomes than symptom-driven decisions alone. The CALM study also indicated that choosing an appropriate FC target level was a critical element of FC-based monitoring. Choosing a target which is too low (e.g. 100 µg/g), may lead to a smaller percentage of false negative cases and will prevent undertreatment. However, this might also cause physicians to switch the treatment faster and therefore use the available arsenal of therapeutics at an increased rate. There is no consensus about the optimal FC target range yet and the question remains whether it is appropriate to pursue one action threshold for all patients or if a patient should have a personal action threshold.[14-18] This is because the calprotectin-concentration is not only dependent on the extent and severity of the inflammation, but also on the contact time between stool and inflamed tissue. The location of the inflamed area in the gastrointestinal tract and the intestinal transit rate are therefore important factors which (co-)influence the calprotectin concentration.

Since we are just at the start of the treat-to-target strategy, we do not yet know its long-term effects. Does this strategy really prevent disease progression and irreversible intestinal fibrosis? Do fewer patients need to undergo surgical interventions? During the 2019 European Crohn's and Colitis Organisation (ECCO) congress in Copenhagen it was reported that the early CD patients, of the previously mentioned CALM study, who achieved endoscopic or deep remission after 1 year of intensive treatment were less likely to have disease progression over a median of 3 years.[19] These first long(er)-term results of the treat-to-target strategy are expected to be published this year.

In 1971, thiopurine therapy was introduced as a useful treatment option for CD and the long-term effects were unknown as well.[20] For a long time the thiopurines have been used as the immunomodulators of choice for moderate to severe UC and CD. The increased use of anti-TNF agents in IBD led to a reappraisal of the role of immunomodulators in the treatment, since combination therapy of thiopurines and anti-TNF medication has been shown to be more effective and to reduce the risk of development of anti-drug antibodies.[20-22] However, some studies have reported an association between the use of thiopurines and an increased risk of developing lymphomas.[23] This is why, in paediatric practice, thiopurines are nowadays prescribed with

reservation. This has also resulted in a renewed interest in the use of methotrexate (MTX) immunomodulation.[24-31] In chapter 4 we showed that it is worthwhile to consider MTX as a second-line immunomodulator, especially in those who are thiopurine intolerant, before stepping up to anti-TNF therapy. More studies are needed to assess the long-term efficacy and safety of MTX in the treatment of CD patients to determine whether MTX might be used as primary immunomodulator instead of thiopurine. In IBD, there is only one study available discussing the use of MTX in combination with anti-TNF therapy, concluding that the combination of infliximab and methotrexate, although safe, was no more effective than infliximab alone in patients with CD receiving treatment with prednisone.[32] However, it is unclear whether the study had been confounded by the enrollment of patients with low disease activity and/or the concomitant use of corticosteroids in each treatment group. In patients with rheumatoid arthritis, there is more experience and published literature available about the efficacy and safety of MTX – anti-TNF combination therapy.[33-35] The authors of a recently published meta-analysis suggest that the combination of MTX and anti-TNF therapy better reduce disease activity and obtain remission than anti-TNF monotherapy.[36] With the increased use of anti-TNF treatment in IBD, it seems very justifiable to further explore the potential role of the MTX combination therapy, not only in CD patients, but also in UC patients.

PART II - OPTIMIZING THE ACCURACY AND PRACTICALITY OF CALPROTECTIN MEASUREMENTS IN DISEASE MONITORING

FC is increasingly used for monitoring response to treatment or early recognition of flares in patients with IBD. Many clinicians allow patients to send a stool sample to the laboratory by surface mail at ambient temperatures.[37,38] Since the stability of the calprotectin protein at room temperature has remained rather unclear, we performed a FC stability study (chapter 5). We observed that stool calprotectin at room temperature is rather unstable, with a steady decline in calprotectin concentration in the first 6 days after stool collection. In clinical practice this does not mean that all FC test results from sent-in stool samples underestimate the actual disease activity, but this discovery does

warn us that when the FC result is in the uncertain range (250 – 500 µg/g) we should be more careful with our interpretation. Enabling patients to perform the FC test at home directly after defecation could overcome the issue of FC instability and may provide a more reliable manner of monitoring disease activity. In Chapter 6 we compared three of these FC home tests and evaluated by how much the test results differed from the established ELISA method. The three lateral flow-based home tests have shown acceptable agreement with the ELISA reference test results within the low range values (≤ 500 µg/g). However, further test improvements should focus on better agreement in the high range values. Furthermore, the home test procedures need to be simplified for patients before they are implemented on a larger scale.

Clinical implications

FC is a valuable laboratory test in all phases of IBD care; from allowing differentiation between irritable bowel syndrome and inflammatory processes in patients with gastrointestinal symptoms, to monitoring response to treatment and predict short-term relapse.[39-58] It can therefore be a great help for clinicians in diagnosing and monitoring IBD, and can be used as a guidance for treatment adaptation. FC is commonly used, because of the practicality of the stool test. It is non-invasive and the samples are relatively easy to obtain. Although data indicate that FC is currently the best marker available (chapter 1), it is certainly not the ideal marker. One of the reasons why FC is not ideal is that there is no linear relationship between the extent and severity of the disease and the level of FC. This is why we advise to use a two-threshold strategy when interpreting FC for monitoring disease activity (see figure 1). Based on previous literature, FC values above 500 µg/g best correspond with active disease and is therefore chosen as the upper threshold.[59-61] In the action range (red zone), adjusting the treatment is advised to attempt to get the patient back into remission again. Despite the fact there is no consensus on the upper limit of the FC target range for remission (green zone), we use a value of 250 µg/g since multiple studies have shown that FC values below 250 µg/g correspond well with disease in remission.[13,46,56,61-64]

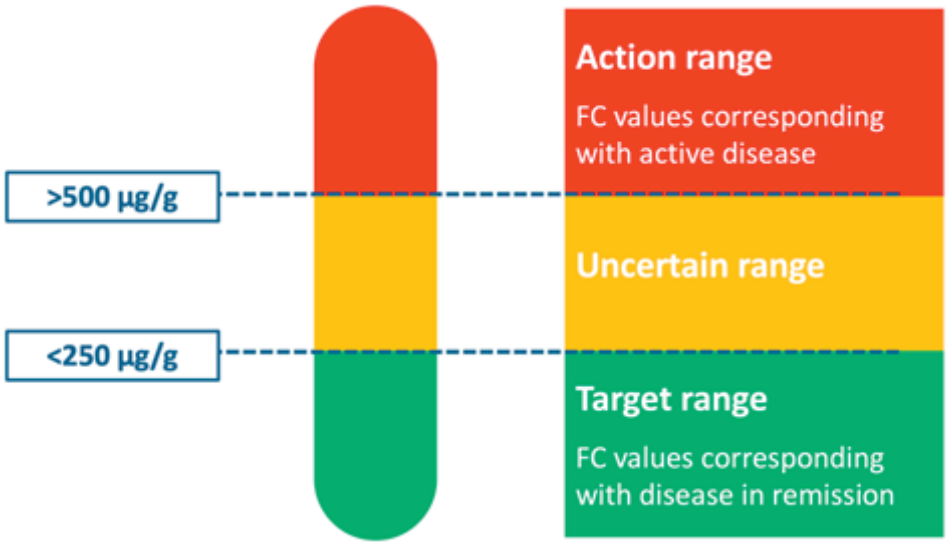

Figure 1. The two-threshold strategy with three calprotectin ranges. (i.e., <250 µg/g for target range, 250-500 µg/g for uncertain range, and >500 µg/g for active disease)

The ideal biomarker should be easily accessible, non-invasive, low in costs and reflect the degree of tissue damage and repair. Table 1. provides an overview of possible other biomarkers that can be found in different biological specimens including, blood, faeces, urine and saliva. Other blood inflammatory markers that have been suggested, include the non-protein amino acid citrullin, which is synthesized by the enterocytes of the small intestine and the intestinal fatty acid binding protein (I-FABP), which is an endogenous cytosolic enterocyte protein released by dying mature enterocytes and therefore a possible marker of enterocyte loss in the small intestine.[65-68] The downside of using blood markers is the required invasiveness to obtain the sample. Non-invasive biomarkers like FC are certainly less invasive, although sampling feces may not be considered convenient by patients. Different faecal markers have been evaluated, but only FC and lactoferrin are used in clinical practice. This is because most other faecal biomarkers that have been investigated, like calgranulin C and polymorphonuclear neutrophil elastase (PMN-e), deal with the same advantages and disadvantages as faecal calprotectin, since they also are neutrophil-derived proteins.[69,70] While a faecal biomarker may seem to be the obvious choice in an intestinal disease, we should not overlook the usefulness of other non-invasive biomarkers. Perhaps the measurement of

urinary claudin-3, which is a protein that indicates tight junction breakdown that is an early event in the development of intestinal damage, can be used in IBD patients.[71]or the measurement of cytokines like IL-1*β*, IL-6 and TNF-α in the saliva.[72,73]
In the meantime, FC remains the best known and most studied biomarker of inflammation currently available in IBD.(37,62,74-76) Our present data indicate that stool calprotectin is not stable at room temperature (chapter 5 and 6). Although we only tested a limited amount of samples, our message got picked up incredibly fast, expressing the urgent need for more literature on this topic. Within half a year not only the FC handling instruction in our own hospital has been adapted, but the issue is now also being addressed in the national paediatric IBD treatment guidelines. [77] This finding could be seen as a setback for the previously cherished practicallity of the FC test, however manufacturers have now realized that there is considerable profit in further improving the lateral flow-based technique to measure calprotectin with a smartphone at home.[78-81]

The home tests have now been investigated under ideal circumstances in the hospital laboratory as well as by the patients themselfes at home, but only in a research setting, not as part of regular care. The next step will involve incorporating the FC home test in routine clinical practice, to determine whether patients who use this telemonitoring possiblity fare better than those who do not. Furthermore, future studies will focus on connecting each FC home test result, in a series of repeated FC measurements during follow-up, immediately to an automatically generated treatment advise. This advise is based on the possible rise of calprotectin out of the target range in the early stage of an upcoming relapse what could allow the assess of early intervention. The future will tell us whether preemptive treatment optimization is a feasible strategy, resulting in a decrease of the number of relapses and prevention of progressive bowel damage in the long-term, both in adults as well as in children.

Table 1. Possible other biomarkers of intestinal inflammation

Potential biomarkers	What it measures
Blood	
■ Citrullin [67,82]	■ Indicator of enterocyte mass
■ Intestinal fatty acid binding protein (I-FABP) [65,66,83]	■ Enterocyte loss in the small intestine
Faeces	
■ Calgranulin C [84-86]	■ Protein released by activated or damaged granulocytes
■ Polymorphonuclear neutrophil elastase (PMN-e)[40,87]	■ Protein released by activated or damaged granulocytes
■ Human neutrophil peptides (HNPs) [88]	■ Protein released by activated or damaged granulocytes
■ Neutrophil gelatinase-associated lipocalin (NGAL)[89,90]	■ Protein released by activated or damaged granulocytes
■ Matrix metalloproteinase 9 (MMP9)[91,92]	■ Enzymes which recruit granulocytes and other inflammatory cells into inflamed tissues
■ Myeloperoxidase (MPO)[93,94]	■ Protein released by activated or damaged granulocytes
■ High-mobility group box 1 (HMGB1)[95]	■ Protein released by activated or damaged granulocytes, monocytes, macrophages, dendritic cells, and natural killer cells
■ Neopterin [96,97]	■ Metabolite released by activated T lymphocytes and macrophages
Urine	
■ Claudin-3 [71]	■ Protein that indicates intestinal tight junction loss
Saliva	
■ Cytokines □ In CD patients: IL-1*β*, IL-6 and TNF-α [72] □ In UC patients: TGF-*β*1 and NO [73]	■ Salivary concentrations of cytokines, including; interleukin 1*β* (IL-1*β*), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), transforming growth factor- *β*1 (TGF- *β*1), nitric oxide (NO).

PART III - PROFILING PATIENTS WITH CHILDHOOD-ONSET PRIMARY SCLEROSING CHOLANGITIS

PSC is a relatively rare (8-10%), but serious complication of IBD. It is suggested that childhood-onset PSC presents with milder disease and has a more favourable outcome compared to adult-onset PSC. To compare outcomes between paediatric- and adult onset PSC we evaluated time-to-complication curves in two independent Dutch PSC cohorts (chapter 7). We found that a quarter of the childhood-onset PSC patients developed biliary and portal hypertensive complications or progressed to liver transplantation in the first five years postdiagnosis. Furthermore, we showed that paediatric- and adult-onset PSC run a similar progressive disease course with no differences seen between both cohorts with regard to frequencies of liver transplantations or the mean disease duration until liver transplantation. Our findings suggest that both the paediatricians as well as the adult-oriented specialists need to be aware of serious complications that might accompany childhood-onset PSC possibly already early during the course of disease.

In analogy with recently published family-based studies on monogenic forms of other complex (auto-)immune diseases [98-100], we hypothesized that in a fraction of patients with early-onset PSC, the disease is caused by rare genetic variants as well. In chapter 8 we therefore performed a family-based study, in which we examined the DNA of patients with early-onset PSC and their biological parents. Whole exome sequencing (WES) of all protein-coding genes results in many variants of uncertain clinical significance. A trio-analysis design, i.e. the inclusion of patients and parents in the analysis of rare genetic variants, helped to immediately ascertain whether a variant is inherited or has arisen de novo, i.e. as a new mutation in the child during foetal development. This appeared to be a successful approach that revealed multiple candidate disease-causing variants with possibly large effects on protein function in several genes. Our findings suggest that PSC can also present in a rare, monogenic or oligogenic fashion, but this suggestion would be supported if functional experiments provide mechanistic insights in how this could possibly increase the risk of PSC.

Clinical Implications

For years there have hardly been any studies focusing on the paediatric PSC populations. Accordingly, paediatricians had almost completely to relie on information available from adult PSC registries.[101-105] Recently the topic has gained interest, and new studies of paediatric PSC cohorts are being published.[106-110] However, most of the currently available studies are retrospective, single-centre designs and the rarity of the disease hampers the studies on providing information about clinical course and prognosis. Different perspectives exist concerning childhood-onset PSC and the long-term consequences. It has been assumed that children with PSC generally have an earlier stage of the same disease as adult PSC patients, tend to present with milder disease, and finally, have more favourable outcomes.[107,108] However, there are also doubts about these assumptions since there are remarkable differences between adult-onset and childhood-onset disease, including higher incidence of the autoimmune-overlap syndrome and higher incidence of concomitant IBD in the latter.[111,112] These differences may indicate that childhood-onset PSC is not merely an early stage of the same disease, but might rather involve a (partially) different immune-mediating pathophysiology.[113]

Based on the findings shown in chapter 7, we argue that childhood-onset PSC follows at least the same progressive disease course as adult-onset disease. We can not even exclude that nearly all childhood-onset patients will need a liver transplantation at some point during their lives, although current registries do not have a sufficiently long time follow up to test this hypothesis. The prognostic factors we identified now in combination with those identified in previous studies, can be used to target and select those patients with a high risk of early hepatic deterioration. Eventhough the therapeutic options to modify the disease course are presently very limited, prognostic factors can be used for early recognition and prevention of complications, selecting patients for clinical trials, and promote timely listing for liver transplantation.

The aetiology of PSC will hopefully become more clear in the upcoming years. Important developments will occur in the field of genetics and immunology. Genome-wide association studies (GWAS) have identified a large number of disease susceptibility genes, showing a strong overlap with the genetic architecture of IBD and other autoimmune diseases in general.[114-121] The

expectations were high that these risk genes would quickly lead to new drug targets for PSC, but so far this is not the case. How to convert the increasing list of genetic risk loci into tailored management for the individual patient, remains unclear.[122] Furthermore the contribution of these already identified risk genes to overall PSC burden is only about 5%.[120,123]
Actual cure requires insight into causation of the disease. In chapter 8 we have shown a method to identify candidate disease causing genes in early-onset PSC patients and concluded that there may be monogenic or oligogenic forms of PSC as well. The family-based strategy permitted efficient discovery of de novo mutations and compound heterozygous genotypes in our cohort. Furthermore, we used multiple pathogenicity tools to predict if our variants are deleterious based on whether the site of the variant displays evolutionary conservation [124], or is predicted to be damaging according to known gene function. We do recognize that this is not sufficient to implicate a variant as playing a causal role in disease, since even healthy individuals carry many rare protein-disrupting variants.[125,126]

Assessment of evidence for variant implication in a certain disease is challenging and requires a step-wise approach (see Tabel 2), as suggested by a working group of experts in genomic research, analysis and clinical diagnostic sequencing convened by the US National Human Genome Research Institute.[126] For our WHELP-study, the next step will involve experimental validation of our findings to determine a poptential pathophysiologoical impact of one or more of our candidate variants. If we can demonstrate that a gene product is functionally disrupted by one (or more) of the present candidate mutations, this discovery may serve as an important clue that could lead to understanding of the individual underlying pathogenic mechanism.
Although monogenic or oligogenic variants may only explain disease-onset in a small proportion of the total PSC population, understanding how the loss of the involved protein functions results in the monogenic PSC-like phenotype can also be very informative for complex PSC. Uncovering genetic variants that provide causal evidence will provide valuable insights in terms of both biological understanding, but most important, it might help to take our main goal, to design new targeted therapies and personalize the medicine, to the next level.

Table 2. Assessment of evidence for candidate pathogenic variants (after MacArthur DG et al. Nature 2014 [126])

Genetic evidence
1. Determine and report the formal statistical evidence for segregation or association of each variant, and its frequency in large control populations matched as closely as possible to patients in terms of ancestry.
Informative evidence
2. Predict variant deleteriousness based on evolutionary conservation; ■ the site of the variant displays evolutionary conservation consistent with deleterious effects of sequence changes at that location, and/or; Predict that a variant is damaging in terms of biological function; ■ the variant is found at the location within the protein predicted to cause functional disruption (for example, enzyme active site, protein-binding region).
Experimental evidence
3. Validate experimentally the predicted damaging impact of candidate variants using assays of patient-derived tissue or well-established cell or animal models of gene function. 4. Avoid assuming that implicated variants are fully penetrant, or completely explanatory in any specific disease case.

CONCLUSION

In this thesis we aimed to contribute to the further development of personalized medicine in paediatric IBD. Most of the studies that have been published so far, including our present work, use prognostic factors to identify subgroups of patients and are not tailored to the individual patient. Personalized medicine in the way we conducted it now may therefore better be called 'stratified medicine'. Our results of the WHELP-study might give a glimpse of what we can expect in the near future. Knowledge of the underlying pathogenic mechanisms in each individual patient at diagnosis may allow early adaptation of therapeutic pathways with the aim to enhance treatment efficacy while minimizing or even avoiding drug-toxicity complications. Advances in molecular-medicine will allow more precise drug targeting. Based on the current developments, I expect that IBD care will gradually move from "stratified medicine" via "personalized medicine" towards "precision medicine".

REFERENCES

1. Panes J, Sandborn WJ, Schreiber S, Sands BE, Vermeire S, D'Haens G, et al. Tofacitinib for induction and maintenance therapy of Crohn's disease: results of two phase IIb randomised placebo-controlled trials. Gut 2017 June 01;66(6):1049-1059.
2. Sandborn WJ, Feagan BG, Fedorak RN, Scherl E, Fleisher MR, Katz S, et al. A randomized trial of Ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with moderate-to-severe Crohn's disease. Gastroenterology 2008 October 01;135(4):1130-1141.
3. Sands BE, Feagan BG, Rutgeerts P, Colombel JF, Sandborn WJ, Sy R, et al. Effects of vedolizumab induction therapy for patients with Crohn's disease in whom tumor necrosis factor antagonist treatment failed. Gastroenterology 2014 September 01;147(3):627.e3.
4. Danese S, Vuitton L, Peyrin-Biroulet L. Biologic agents for IBD: practical insights. Nat Rev Gastroenterol Hepatol 2015 September 01;12(9):537-545.
5. Andrade P, Lopes S, Gaspar R, Nunes A, Magina S, Macedo G. Anti-Tumor Necrosis Factor-alpha-Induced Dermatological Complications in a Large Cohort of Inflammatory Bowel Disease Patients. Dig Dis Sci 2018 March 01;63(3):746-754.
6. Pastore S, Naviglio S, Canuto A, Lepore L, Martelossi S, Ventura A, et al. Serious Adverse Events Associated with Anti-Tumor Necrosis Factor Alpha Agents in Pediatric-Onset Inflammatory Bowel Disease and Juvenile Idiopathic Arthritis in A Real-Life Setting. Paediatr Drugs 2018 April 01;20(2):165-171.
7. Kugathasan S, Denson LA, Walters TD, Kim MO, Marigorta UM, Schirmer M, et al. Prediction of complicated disease course for children newly diagnosed with Crohn's disease: a multicentre inception cohort study. Lancet 2017 April 29;389(10080):1710-1718.
8. Walters TD, Kim MO, Denson LA, Griffiths AM, Dubinsky M, Markowitz J, et al. Increased effectiveness of early therapy with anti-tumor necrosis factor-alpha vs an immunomodulator in children with Crohn's disease. Gastroenterology 2014 February 01;146(2):383-391.
9. Schreiber S, Reinisch W, Colombel JF, Sandborn WJ, Hommes DW, Robinson AM, et al. Subgroup analysis of the placebo-controlled CHARM trial: increased remission rates through 3 years for adalimumab-treated patients with early Crohn's disease. J Crohns Colitis 2013 April 01;7(3):213-221.

10. Colombel JF, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med 2010 April 15;362(15):1383-1395.
11. Khanna R, Bressler B, Levesque BG, Zou G, Stitt LW, Greenberg GR, et al. Early combined immunosuppression for the management of Crohn's disease (REACT): a cluster randomised controlled trial. Lancet 2015 November 07;386(10006):1825-1834.
12. D'Haens G, Baert F, van Assche G, Caenepeel P, Vergauwe P, Tuynman H, et al. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. Lancet 2008 February 23;371(9613):660-667.
13. Colombel JF, Panaccione R, Bossuyt P, Lukas M, Baert F, Vanasek T, et al. Effect of tight control management on Crohn's disease (CALM): a multicentre, randomised, controlled phase 3 trial. Lancet 2018 December 23;390(10114):2779-2789.
14. De Vos M, Dewit O, D'Haens G, Baert F, Fontaine F, Vermeire S, et al. Fast and sharp decrease in calprotectin predicts remission by infliximab in anti-TNF naive patients with ulcerative colitis. J Crohns Colitis 2012 June 01;6(5):557-562.
15. Zubin G, Peter L. Predicting Endoscopic Crohn's Disease Activity Before and After Induction Therapy in Children: A Comprehensive Assessment of PCDAI, CRP, and Fecal Calprotectin. Inflamm Bowel Dis 2015 June 01;21(6):1386-1391.
16. Sipponen T, Kolho KL. Fecal calprotectin in diagnosis and clinical assessment of inflammatory bowel disease. Scand J Gastroenterol 2015 January 01;50(1):74-80.
17. Zhulina Y, Cao Y, Amcoff K, Carlson M, Tysk C, Halfvarson J. The prognostic significance of faecal calprotectin in patients with inactive inflammatory bowel disease. Aliment Pharmacol Ther 2016 September 01;44(5):495-504.
18. Ferreiro-Iglesias R, Barreiro-de Acosta M, Lorenzo-Gonzalez A, Dominguez-Munoz JE. Accuracy of Consecutive Fecal Calprotectin Measurements to Predict Relapse in Inflammatory Bowel Disease Patients Under Maintenance With Anti-TNF Therapy: A Prospective Longitudinal Cohort Study. J Clin Gastroenterol 2018 March 01;52(3):229-234.
19. Yzet C, Ungaro R, Bossuyt P, Baert F, Vanasek T, D'Haens G, et al. Endoscopic and deep remission at 1 year prevents disease progression in early Crohn's disease: long-term data from CALM. 2019 March.
20. Willoughby JM, Beckett J, Kumar PJ, Dawson AM. Controlled

trial of azathioprine in Crohn's disease. Lancet 1971 October 30;2(7731):944-947.
21. Panaccione R, Ghosh S, Middleton S, Marquez JR, Scott BB, Flint L, et al. Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. Gastroenterology 2014 February 01;146(2):400.e3.
22. Jones JL, Kaplan GG, Peyrin-Biroulet L, Baidoo L, Devlin S, Melmed GY, et al. Effects of Concomitant Immunomodulator Therapy on Efficacy and Safety of Anti-Tumor Necrosis Factor Therapy for Crohn's Disease: A Meta-analysis of Placebo-controlled Trials. Clin Gastroenterol Hepatol 2015 December 01;13(13):8.
23. Kotlyar DS, Lewis JD, Beaugerie L, Tierney A, Brensinger CM, Gisbert JP, et al. Risk of lymphoma in patients with inflammatory bowel disease treated with azathioprine and 6-mercaptopurine: a meta-analysis. Clin Gastroenterol Hepatol 2015 May 01;13(5):50.
24. Turner D, Grossman AB, Rosh J, Kugathasan S, Gilman AR, Baldassano R, et al. Methotrexate following unsuccessful thiopurine therapy in pediatric Crohn's disease. Am J Gastroenterol 2007 December 01;102(12):12; quiz 2803, 2813.
25. Mack DR, Young R, Kaufman SS, Ramey L, Vanderhoof JA. Methotrexate in patients with Crohn's disease after 6-mercaptopurine. J Pediatr 1998 May 01;132(5):830-835.
26. Uhlen S, Belbouab R, Narebski K, Goulet O, Schmitz J, Cezard JP, et al. Efficacy of methotrexate in pediatric Crohn's disease: a French multicenter study. Inflamm Bowel Dis 2006 November 01;12(11):1053-1057.
27. Ravikumara M, Hinsberger A, Spray CH. Role of methotrexate in the management of Crohn disease. J Pediatr Gastroenterol Nutr 2007 April 01;44(4):427-430.
28. Boyle B, Mackner L, Ross C, Moses J, Kumar S, Crandall W. A single-center experience with methotrexate after thiopurine therapy in pediatric Crohn disease. J Pediatr Gastroenterol Nutr 2010 December 01;51(6):714-717.
29. Willot S, Noble A, Deslandres C. Methotrexate in the treatment of inflammatory bowel disease: an 8-year retrospective study in a Canadian pediatric IBD center. Inflamm Bowel Dis 2011 December 01;17(12):2521-2526.
30. Weiss B, Lerner A, Shapiro R, Broide E, Levine A, Fradkin A, et al. Methotrexate treatment in pediatric Crohn disease patients intolerant or resistant to purine analogues. J Pediatr

Gastroenterol Nutr 2009 May 01;48(5):526-530.
31. Sunseri W, Hyams JS, Lerer T, Mack DR, Griffiths AM, Otley AR, et al. Retrospective cohort study of methotrexate use in the treatment of pediatric Crohn's disease. Inflamm Bowel Dis 2014 August 01;20(8):1341-1345.
32. Feagan BG, McDonald JW, Panaccione R, Enns RA, Bernstein CN, Ponich TP, et al. Methotrexate in combination with infliximab is no more effective than infliximab alone in patients with Crohn's disease. Gastroenterology 2014 March 01;146(3):688.e1.
33. Stamm TA, Machold KP, Aletaha D, Alasti F, Lipsky P, Pisetsky D, et al. Induction of sustained remission in early inflammatory arthritis with the combination of infliximab plus methotrexate: the DINORA trial. Arthritis Res Ther 2018 August 09;20(1):z.
34. Breedveld FC, Weisman MH, Kavanaugh AF, Cohen SB, Pavelka K, van Vollenhoven R, et al. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheum 2006 January 01;54(1):26-37.
35. St Clair EW, van der Heijde, D M, Smolen JS, Maini RN, Bathon JM, Emery P, et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial. Arthritis Rheum 2004 November 01;50(11):3432-3443.
36. Donahue KE, Gartlehner G, Schulman ER, Jonas B, Coker-Schwimmer E, Patel SV, et al. No title. 2018 July 01.
37. Roseth AG, Fagerhol MK, Aadland E, Schjonsby H. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand J Gastroenterol 1992 September 01;27(9):793-798.
38. Lasson A, Stotzer PO, Ohman L, Isaksson S, Sapnara M, Strid H. The intra-individual variability of faecal calprotectin: a prospective study in patients with active ulcerative colitis. J Crohns Colitis 2015 January 01;9(1):26-32.
39. Lasson A, Ohman L, Stotzer PO, Isaksson S, Uberbacher O, Ung KA, et al. Pharmacological intervention based on fecal calprotectin levels in patients with ulcerative colitis at high risk of a relapse: A prospective, randomized, controlled study. United European Gastroenterol J 2015 February 01;3(1):72-79.
40. Langhorst J, Boone J, Lauche R, Rueffer A, Dobos G. Faecal Lactoferrin, Calprotectin, PMN-elastase, CRP, and White Blood Cell Count as Indicators for Mucosal Healing and Clinical

Course of Disease in Patients with Mild to Moderate Ulcerative Colitis: Post Hoc Analysis of a Prospective Clinical Trial. J Crohns Colitis 2016 July 01;10(7):786-794.
41. Yamamoto T, Shimoyama T, Bamba T, Matsumoto K. Consecutive Monitoring of Fecal Calprotectin and Lactoferrin for the Early Diagnosis and Prediction of Pouchitis after Restorative Proctocolectomy for Ulcerative Colitis. Am J Gastroenterol 2015 June 01;110(6):881-887.
42. Molander P, Farkkila M, Ristimaki A, Salminen K, Kemppainen H, Blomster T, et al. Does fecal calprotectin predict short-term relapse after stopping TNFalpha-blocking agents in inflammatory bowel disease patients in deep remission? J Crohns Colitis 2015 January 01;9(1):33-40.
43. De Vos M, Louis EJ, Jahnsen J, Vandervoort JG, Noman M, Dewit O, et al. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. Inflamm Bowel Dis 2013 September 01;19(10):2111-2117.
44. De Vos M, Louis EJ, Jahnsen J, Vandervoort JG, Noman M, Dewit O, et al. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. Inflamm Bowel Dis 2013 September 01;19(10):2111-2117.
45. Dabritz J, Langhorst J, Lugering A, Heidemann J, Mohr M, Wittkowski H, et al. Improving relapse prediction in inflammatory bowel disease by neutrophil-derived S100A12. Inflamm Bowel Dis 2013 May 01;19(6):1130-1138.
46. Jauregui-Amezaga A, Lopez-Ceron M, Aceituno M, Jimeno M, Rodriguez de Miguel C, Pino-Donnay S, et al. Accuracy of advanced endoscopy and fecal calprotectin for prediction of relapse in ulcerative colitis: a prospective study. Inflamm Bowel Dis 2014 July 01;20(7):1187-1193.
47. Heida A, Van de Vijver E, van Ravenzwaaij D, Van Biervliet S, Hummel TZ, Yuksel Z, et al. Predicting inflammatory bowel disease in children with abdominal pain and diarrhoea: calgranulin-C versus calprotectin stool tests. Arch Dis Child 2018 June 01;103(6):565-571.
48. Holtman GA, Lisman-van Leeuwen Y, Kollen BJ, Norbruis OF, Escher JC, Kindermann A, et al. Diagnostic Accuracy of Fecal Calprotectin for Pediatric Inflammatory Bowel Disease in Primary Care: A Prospective Cohort Study. Ann Fam Med 2016 September 01;14(5):437-445.
49. Van de Vijver E, Schreuder AB, Cnossen WR, Muller Kobold AC,

van Rheenen PF, North Netherlands Pediatric IBD Consortium. Safely ruling out inflammatory bowel disease in children and teenagers without referral for endoscopy. Arch Dis Child 2012 December 01;97(12):1014-1018.
50. Van de Vijver E, Schreuder AB, Cnossen WR, Muller Kobold AC, van Rheenen PF, North Netherlands Pediatric IBD Consortium. Safely ruling out inflammatory bowel disease in children and teenagers without referral for endoscopy. Arch Dis Child 2012 December 01;97(12):1014-1018.
51. Ashorn S, Honkanen T, Kolho KL, Ashorn M, Valineva T, Wei B, et al. Fecal calprotectin levels and serological responses to microbial antigens among children and adolescents with inflammatory bowel disease. Inflamm Bowel Dis 2009 February 01;15(2):199-205.
52. Sidler MA, Leach ST, Day AS. Fecal S100A12 and fecal calprotectin as noninvasive markers for inflammatory bowel disease in children. Inflamm Bowel Dis 2008 March 01;14(3):359-366.
53. Canani RB, de Horatio LT, Terrin G, Romano MT, Miele E, Staiano A, et al. Combined use of noninvasive tests is useful in the initial diagnostic approach to a child with suspected inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2006 January 01;42(1):9-15.
54. Fagerberg UL, Loof L, Myrdal U, Hansson LO, Finkel Y. Colorectal inflammation is well predicted by fecal calprotectin in children with gastrointestinal symptoms. J Pediatr Gastroenterol Nutr 2005 April 01;40(4):450-455.
55. Hassan EA, Ramadan HK, Ismael AA, Mohamed KF, El-Attar MM, Alhelali I. Noninvasive biomarkers as surrogate predictors of clinical and endoscopic remission after infliximab induction in patients with refractory ulcerative colitis. Saudi J Gastroenterol 2017 August 01;23(4):238-245.
56. Kristensen V, Roseth A, Ahmad T, Skar V, Moum B. Fecal Calprotectin: A Reliable Predictor of Mucosal Healing after Treatment for Active Ulcerative Colitis. Gastroenterol Res Pract 2017;2017:2098293.
57. Wagner M, Peterson CG, Ridefelt P, Sangfelt P, Carlson M. Fecal markers of inflammation used as surrogate markers for treatment outcome in relapsing inflammatory bowel disease. World J Gastroenterol 2008 September 28;14(36):9; discussion 5588.
58. Sipponen T, Savilahti E, Karkkainen P, Kolho KL, Nuutinen H, Turunen U, et al. Fecal calprotectin, lactoferrin, and

endoscopic disease activity in monitoring anti-TNF-alpha therapy for Crohn's disease. Inflamm Bowel Dis 2008 October 01;14(10):1392-1398.
59. Heida A, Dijkstra A, Muller Kobold A, Rossen JW, Kindermann A, Kokke F, et al. Efficacy of Home Telemonitoring versus Conventional Follow-up: A Randomized Controlled Trial among Teenagers with Inflammatory Bowel Disease. J Crohns Colitis 2018 March 28;12(4):432-441.
60. Paul S, Del Tedesco E, Marotte H, Rinaudo-Gaujous M, Moreau A, Phelip JM, et al. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. Inflamm Bowel Dis 2013 November 01;19(12):2568-2576.
61. Zhulina Y, Cao Y, Amcoff K, Carlson M, Tysk C, Halfvarson J. The prognostic significance of faecal calprotectin in patients with inactive inflammatory bowel disease. Aliment Pharmacol Ther 2016 September 01;44(5):495-504.
62. Zittan E, Kelly OB, Kirsch R, Milgrom R, Burns J, Nguyen GC, et al. Low Fecal Calprotectin Correlates with Histological Remission and Mucosal Healing in Ulcerative Colitis and Colonic Crohn's Disease. Inflamm Bowel Dis 2016 March 01;22(3):623-630.
63. D'Haens G, Vermeire S, Lambrecht G, Baert F, Bossuyt P, Pariente B, et al. Increasing Infliximab Dose Based on Symptoms, Biomarkers, and Serum Drug Concentrations Does Not Increase Clinical, Endoscopic, and Corticosteroid-Free Remission in Patients With Active Luminal Crohn's Disease. Gastroenterology 2018 April 01;154(5):1351.e1.
64. Heida A, Dijkstra A, Muller Kobold A, Rossen JW, Kindermann A, Kokke F, et al. Efficacy of Home Telemonitoring versus Conventional Follow-up: A Randomized Controlled Trial among Teenagers with Inflammatory Bowel Disease. J Crohns Colitis 2018 March 28;12(4):432-441.
65. Sarikaya M, Ergul B, Dogan Z, Filik L, Can M, Arslan L. Intestinal fatty acid binding protein (I-FABP) as a promising test for Crohn's disease: a preliminary study. Clin Lab 2015;61(1-2):87-91.
66. Pelsers MM, Hermens WT, Glatz JF. Fatty acid-binding proteins as plasma markers of tissue injury. Clin Chim Acta 2005 February 01;352(1-2):15-35.
67. Crenn P, Vahedi K, Lavergne-Slove A, Cynober L, Matuchansky C, Messing B. Plasma citrulline: A marker of enterocyte mass in villous atrophy-associated small bowel disease. Gastroenterology 2003 May 01;124(5):1210-1219.

68. Windmueller HG, Spaeth AE. Source and fate of circulating citrulline. Am J Physiol 1981 December 01;241(6):473.
69. Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic, or unnecessary toys? Gut 2006 March 01;55(3):426-431.
70. Langhorst J, Elsenbruch S, Mueller T, Rueffer A, Spahn G, Michalsen A, et al. Comparison of 4 neutrophil-derived proteins in feces as indicators of disease activity in ulcerative colitis. Inflamm Bowel Dis 2005 December 01;11(12):1085-1091.
71. Thuijls G, Derikx JP, de Haan JJ, Grootjans J, de Bruine A, Masclee AA, et al. Urine-based detection of intestinal tight junction loss. J Clin Gastroenterol 2010 January 01;44(1):14.
72. Szczeklik K, Owczarek D, Pytko-Polonczyk J, Kesek B, Mach TH. Proinflammatory cytokines in the saliva of patients with active and non-active Crohn's disease. Pol Arch Med Wewn 2012;122(5):200-208.
73. Rezaie A, Khalaj S, Shabihkhani M, Nikfar S, Zamani MJ, Mohammadirad A, Daryani NE, Abdollahi M. Study on the correlations among disease activity index and salivary transforming growth factor- beta 1 and nitric oxide in ulcerative colitis patients. Ann N Y Acad Sci 2007;1095:305–14.
74. Tibble J, Teahon K, Thjodleifsson B, Roseth A, Sigthorsson G, Bridger S, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000 October 01;47(4):506-513.
75. Lee SH, Kim MJ, Chang K, Song EM, Hwang SW, Park SH, et al. Fecal calprotectin predicts complete mucosal healing and better correlates with the ulcerative colitis endoscopic index of severity than with the Mayo endoscopic subscore in patients with ulcerative colitis. BMC Gastroenterol 2017 October 23;17(1):7.
76. Patel A, Panchal H, Dubinsky MC. Fecal Calprotectin Levels Predict Histological Healing in Ulcerative Colitis. Inflamm Bowel Dis 2017 September 01;23(9):1600-1604.
77. Escher JC, Hagemeijer JW, de Ridder L, Rings EHHM. Guideline on diagnosis and treatment of pediatric IBD. 2018; Available at: http://www.nvk.nl/Portals/0/richtlijnen/ inflammatoire darmziekten/ inflammatoiredarmziekten.pdf.
78. Vinding KK, Elsberg H, Thorkilgaard T, Belard E, Pedersen N, Elkjaer M, et al. Fecal Calprotectin Measured By Patients at Home Using Smartphones--A New Clinical Tool in Monitoring Patients with Inflammatory Bowel Disease. Inflamm Bowel Dis 2016 February 01;22(2):336-344.

79. Bello C, Roseth A, Guardiola J, Reenaers C, Ruiz-Cerulla A, Van Kemseke C, et al. Usability of a home-based test for the measurement of fecal calprotectin in asymptomatic IBD patients. Dig Liver Dis 2017 September 01;49(9):991-996.
80. Piekkala M, Alfthan H, Merras-Salmio L, Puustinen Wikstrom A, Heiskanen K, Jaakkola T, et al. Fecal Calprotectin Test Performed at Home: A Prospective Study of Pediatric Patients With Inflammatory Bowel Disease. J Pediatr Gastroenterol Nutr 2018 June 01;66(6):926-931.
81. Heida A, Knol M, Kobold AM, Bootsman J, Dijkstra G, van Rheenen PF. Agreement Between Home-Based Measurement of Stool Calprotectin and ELISA Results for Monitoring Inflammatory Bowel Disease Activity. Clin Gastroenterol Hepatol 2017 November 01;15(11):1749.e2.
82. Windmueller HG, Spaeth AE. Source and fate of circulating citrulline. Am J Physiol 1981 December 01;241(6):473.
83. Sarikaya M, Ergul B, Dogan Z, Filik L, Can M, Arslan L. Intestinal fatty acid binding protein (I-FABP) as a promising test for Crohn's disease: a preliminary study. Clin Lab 2015;61(1-2):87-91.
84. de Jong NS, Leach ST, Day AS. Fecal S100A12: a novel noninvasive marker in children with Crohn's disease. Inflamm Bowel Dis 2006 July 01;12(7):566-572.
85. Sidler MA, Leach ST, Day AS. Fecal S100A12 and fecal calprotectin as noninvasive markers for inflammatory bowel disease in children. Inflamm Bowel Dis 2008 March 01;14(3):359-366.
86. Dabritz J, Langhorst J, Lugering A, Heidemann J, Mohr M, Wittkowski H, et al. Improving relapse prediction in inflammatory bowel disease by neutrophil-derived S100A12. Inflamm Bowel Dis 2013 May 01;19(6):1130-1138.
87. Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. Am J Gastroenterol 2008 January 01;103(1):162-169.
88. Kanmura S, Hamamoto H, Morinaga Y, Oda K, Fujita T, Arima S, et al. Fecal Human Neutrophil Peptide Levels Correlate with Intestinal Inflammation in Ulcerative Colitis. Digestion 2016;93(4):300-308.
89. Thorsvik S, Damas JK, Granlund AV, Flo TH, Bergh K, Ostvik AE, et al. Fecal neutrophil gelatinase-associated lipocalin as a biomarker for inflammatory bowel disease. J Gastroenterol

Hepatol 2017 January 01;32(1):128-135.
90. Nielsen OH, Gionchetti P, Ainsworth M, Vainer B, Campieri M, Borregaard N, et al. Rectal dialysate and fecal concentrations of neutrophil gelatinase-associated lipocalin, interleukin-8, and tumor necrosis factor-alpha in ulcerative colitis. Am J Gastroenterol 1999 October 01;94(10):2923-2928.
91. Annahazi A, Molnar T, Farkas K, Rosztoczy A, Izbeki F, Gecse K, et al. Fecal MMP-9: a new noninvasive differential diagnostic and activity marker in ulcerative colitis. Inflamm Bowel Dis 2013 February 01;19(2):316-320.
92. Farkas K, Balint A, Bor R, Foldesi I, Szucs M, Nagy F, et al. Faecal matrix metalloprotease-9 is a more sensitive marker for diagnosing pouchitis than faecal calprotectin: results from a pilot study. Expert Rev Gastroenterol Hepatol 2015 March 01;9(3):387-392.
93. Peterson CG, Eklund E, Taha Y, Raab Y, Carlson M. A new method for the quantification of neutrophil and eosinophil cationic proteins in feces: establishment of normal levels and clinical application in patients with inflammatory bowel disease. Am J Gastroenterol 2002 July 01;97(7):1755-1762.
94. Sugi K, Saitoh O, Hirata I, Katsu K. Fecal lactoferrin as a marker for disease activity in inflammatory bowel disease: comparison with other neutrophil-derived proteins. Am J Gastroenterol 1996 May 01;91(5):927-934.
95. Palone F, Vitali R, Cucchiara S, Pierdomenico M, Negroni A, Aloi M, et al. Role of HMGB1 as a suitable biomarker of subclinical intestinal inflammation and mucosal healing in patients with inflammatory bowel disease. Inflamm Bowel Dis 2014 August 01;20(8):1448-1457.
96. Nancey S, Boschetti G, Moussata D, Cotte E, Peyras J, Cuerq C, et al. Neopterin is a novel reliable fecal marker as accurate as calprotectin for predicting endoscopic disease activity in patients with inflammatory bowel diseases. Inflamm Bowel Dis 2013 April 01;19(5):1043-1052.
97. Husain N, Tokoro K, Popov JM, Naides SJ, Kwasny MJ, Buchman AL. Neopterin concentration as an index of disease activity in Crohn's disease and ulcerative colitis. J Clin Gastroenterol 2013 March 01;47(3):246-251.
98. Uhlig HH, Schwerd T, Koletzko S, Shah N, Kammermeier J, Elkadri A, et al. The diagnostic approach to monogenic very early onset inflammatory bowel disease. Gastroenterology 2014 November 01;147(5):1007.e3.
99. Pazmandi J, Kalinichenko A, Ardy RC, Boztug K. Early-onset

inflammatory bowel disease as a model disease to identify key regulators of immune homeostasis mechanisms. Immunol Rev 2019 January 01;287(1):162-185.
100. Qiao D, Lange C, Beaty TH, Crapo JD, Barnes KC, Bamshad M, et al. Exome Sequencing Analysis in Severe, Early-Onset Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 2016 June 15;193(12):1353-1363.
101. Wilschanski M, Chait P, Wade JA, Davis L, Corey M, St Louis P, et al. Primary sclerosing cholangitis in 32 children: clinical, laboratory, and radiographic features, with survival analysis. Hepatology 1995 November 01;22(5):1415-1422.
102. Debray D, Pariente D, Urvoas E, Hadchouel M, Bernard O. Sclerosing cholangitis in children. J Pediatr 1994 January 01;124(1):49-56.
103. Gregorio GV, Portmann B, Karani J, Harrison P, Donaldson PT, Vergani D, et al. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. Hepatology 2001 March 01;33(3):544-553.
104. Feldstein AE, Perrault J, El-Youssif M, Lindor KD, Freese DK, Angulo P. Primary sclerosing cholangitis in children: a long-term follow-up study. Hepatology 2003 July 01;38(1):210-217.
105. Miloh T, Arnon R, Shneider B, Suchy F, Kerkar N. A retrospective single-center review of primary sclerosing cholangitis in children. Clin Gastroenterol Hepatol 2009 February 01;7(2):239-245.
106. Deneau M, Jensen MK, Holmen J, Williams MS, Book LS, Guthery SL. Primary sclerosing cholangitis, autoimmune hepatitis, and overlap in Utah children: epidemiology and natural history. Hepatology 2013 October 01;58(4):1392-1400.
107. Deneau MR, El-Matary W, Valentino PL, Abdou R, Alqoaer K, Amin M, et al. The natural history of primary sclerosing cholangitis in 781 children: A multicenter, international collaboration. Hepatology 2017 August 01;66(2):518-527.
108. Valentino PL, Wiggins S, Harney S, Raza R, Lee CK, Jonas MM. The Natural History of Primary Sclerosing Cholangitis in Children: A Large Single-Center Longitudinal Cohort Study. J Pediatr Gastroenterol Nutr 2016 December 01;63(6):603-609.
109. Yoon J, Oh SH, Kim HJ, Park SH, Ye BD, Yang SK, et al. Primary Sclerosing Cholangitis with Inflammatory Bowel Disease in Korean Children. Pediatr Gastroenterol Hepatol Nutr 2015 December 01;18(4):268-275.
110. Rojas CP, Bodicharla R, Campuzano-Zuluaga G, Hernandez L, Rodriguez MM. Autoimmune hepatitis and primary sclerosing

cholangitis in children and adolescents. Fetal Pediatr Pathol 2014 August 01;33(4):202-209.
111. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Autoimmune hepatitis. J Hepatol 2015 October 01;63(4):971-1004.
112. Boberg KM, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrumpf E, et al. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. J Hepatol 2011 February 01;54(2):374-385.
113. Chapman R, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, et al. Diagnosis and management of primary sclerosing cholangitis. Hepatology 2010 February 01;51(2):660-678.
114. Srivastava B, Mells GF, Cordell HJ, Muriithi A, Brown M, Ellinghaus E, et al. Fine mapping and replication of genetic risk loci in primary sclerosing cholangitis. Scand J Gastroenterol 2012 July 01;47(7):820-826.
115. Folseraas T, Melum E, Rausch P, Juran BD, Ellinghaus E, Shiryaev A, et al. Extended analysis of a genome-wide association study in primary sclerosing cholangitis detects multiple novel risk loci. J Hepatol 2012 August 01;57(2):366-375.
116. Karlsen TH, Franke A, Melum E, Kaser A, Hov JR, Balschun T, et al. Genome-wide association analysis in primary sclerosing cholangitis. Gastroenterology 2010 March 01;138(3):1102-1111.
117. Melum E, Franke A, Schramm C, Weismuller TJ, Gotthardt DN, Offner FA, et al. Genome-wide association analysis in primary sclerosing cholangitis identifies two non-HLA susceptibility loci. Nat Genet 2011 January 01;43(1):17-19.
118. Ellinghaus D, Folseraas T, Holm K, Ellinghaus E, Melum E, Balschun T, et al. Genome-wide association analysis in primary sclerosing cholangitis and ulcerative colitis identifies risk loci at GPR35 and TCF4. Hepatology 2013 September 01;58(3):1074-1083.
119. Liu JZ, Hov JR, Folseraas T, Ellinghaus E, Rushbrook SM, Doncheva NT, et al. Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis. Nat Genet 2013 June 01;45(6):670-675. Ellinghaus D, Jostins L, Spain SL, Cortes A, Bethune J, Han B, et al. Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. Nat Genet 2016 May 01;48(5):510-518.
120. Ji SG, Juran BD, Mucha S, Folseraas T, Jostins L, Melum E, et al. Genome-wide association study of primary sclerosing

cholangitis identifies new risk loci and quantifies the genetic relationship with inflammatory bowel disease. Nat Genet 2017 February 01;49(2):269-273.
121. Jiang X, Karlsen TH. Genetics of primary sclerosing cholangitis and pathophysiological implications. Nat Rev Gastroenterol Hepatol 2017 May 01;14(5):279-295.
122. Karlsen TH, Melum E, Franke A. The utility of genome-wide association studies in hepatology. Hepatology 2010 May 01;51(5):1833-1842.
123. Cooper GM, Shendure J. Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data. Nat Rev Genet 2011 August 18;12(9):628-640.
124. Tennessen JA, Bigham AW, O'Connor TD, Fu W, Kenny EE, Gravel S, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science 2012 July 06;337(6090):64-69.
125. Veltman JA, Brunner HG. De novo mutations in human genetic disease. Nat Rev Genet 2012 July 18;13(8):565-575.
126. MacArthur DG, Manolio TA, Dimmock DP, Rehm HL, Shendure J, Abecasis GR, et al. Guidelines for investigating causality of sequence variants in human disease. Nature 2014 April 24;508(7497):469-476.