# Implementation of intestinal ultrasound and optimal use of biologics in inflammatory bowel disease

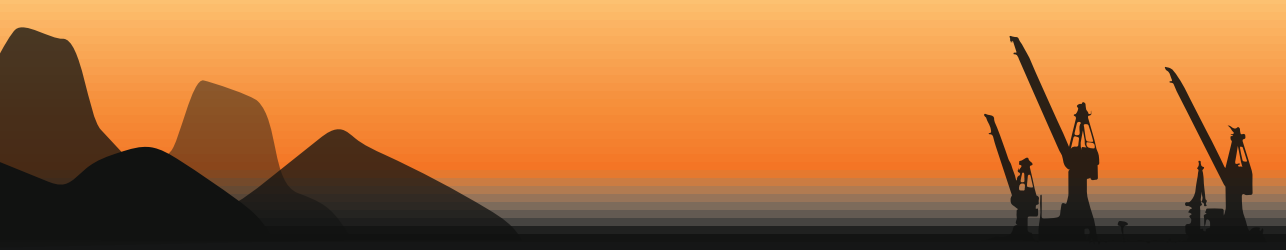
Bots, S.J.A.

**Publication date**
2022

**Document Version**
Final published version

# IMPLEMENTATION OF INTESTINAL ULTRASOUND AND OPTIMAL USE OF BIOLOGICS IN INFLAMMATORY BOWEL DISEASE

Steven J.A. Bots



# IMPLEMENTATION OF INTESTINAL ULTRASOUND AND OPTIMAL USE OF BIOLOGICS IN INFLAMMATORY BOWEL DISEASE

Steven Joannes Alexander Bots

**Implementation of intestinal ultrasound and optimal use of biologics in inflammatory bowel disease**

ISBN: 978-94-6423-729-0

Cover design by: J.E. Dantuma. This figure illustrates the journey of my PhD thesis. I have lived in different harbor cities (Rotterdam, Amsterdam, Bergen). The sunrise at the horizon with land in sight symbolizes the finalization of the book. The color gradients reflect ultrasound waves and frequencies. Biologic antibodies are schematically drawn.

Printed by: Proefschriftmaken

The printing of this thesis was financially supported by Amsterdam UMC and NVGE.

# IMPLEMENTATION OF INTESTINAL ULTRASOUND AND OPTIMAL USE OF BIOLOGICS IN INFLAMMATORY BOWEL DISEASE

**ACADEMISCH PROEFSCHRIFT**

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. ir. K.I.J. Maex
ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op woensdag 20 april 2022, te 16:00 uur

door Steven Joannes Alexander Bots
geboren te Leidschendam

## Promotiecommissie

| | | |
|---|---|---|
| *Promotor:* | Prof. Dr. G.R.A.M. D'Haens | Universiteit van Amsterdam |
| *Copromotores:* | Dr. M. Löwenberg | Universiteit van Amsterdam |
| | Dr. K.B. Gecse | Universiteit van Amsterdam |
| *Overige leden:* | Prof. dr. J. Stoker | Universiteit van Amsterdam |
| | Prof. dr. W.A. Bemelman | Universiteit van Amsterdam |
| | Prof. dr. C.Y. Ponsioen | Universiteit van Amsterdam |
| | Prof. dr B. Oldenburg | Universiteit Utrecht |
| | Dr. P.C.F. Stokkers | OLVG |
| | Prof. Dr. C. Maaser | Klinikum Lüneburg |

Faculteit der Geneeskunde

# Contents

## Contents

# Chapter 1

## General introduction and outline of the thesis

# General introduction and outline of the thesis

## Inflammatory bowel disease

Inflammatory bowel disease (IBD) is comprised of the disorders Crohn's disease (CD) and ulcerative colitis (UC). Symptoms of IBD include abdominal pain, diarrhea, increased stool frequency, rectal blood loss, bloating, fever, weight loss, loss of appetite and fatigue.[1, 2] The pathogenesis of IBD is complex and incompletely understood with genetics, environmental factors, the microbiome and immune dysfunction playing a role. It is thought that inflammation of the bowel is caused by an aberrant immune response to commensal gut microbiota in genetically susceptible individuals.[3]

UC and CD are characterized by relapsing and remitting episodes of bowel inflammation. They have distinct and overlapping pathologic and clinical characteristics. UC is characterized by inflammation limited to the mucosal layer of the colon. It usually involves the rectum and typically extends in a continuous way along the colon.[1] CD is characterized by transmural inflammation and patchy inflammation. Transmural inflammation can lead to complications, such as fibrosis and strictures, which are not typically seen in UC. Transmural inflammation might also result in sinus tracts, perforations and fistula formation. CD may involve the gastrointestinal tract from mouth to perianal area, but the most commonly affected areas are the ileum and the colon.[2] A small proportion of IBD patients in which the disease phenotype cannot be defined are classified as IBD-uncertain (IBD-U).[4]

IBD patients can develop extra-intestinal manifestations, such as ankylosing spondylitis, iritis/uveitis, pyoderma gangrenosum, erythema nodosum and primary sclerosing cholangitis, which are more often seen in CD patients. IBD is also associated with diseases, such as asthma, bronchitis, pericarditis, psoriasis, rheumatoid arthritis, and multiple sclerosis.[1, 2, 5-7]

The onset of IBD is usually at a young age, with most patients being affected by the disease before the age of 40.[5] The incidence and prevalence of the disease are increasing and are highest in Western countries compared to regions such as Asia, Africa and South America.[8] In the Netherlands estimated prevalence are 50.000 and 40.000 for UC and CD patients, respectively and incidence and prevalence are increasing.[9, 10]

*Diagnosis and monitoring of IBD patients*

Tools for diagnosis and monitoring of IBD patients include assessment of clinical disease activity (i.e. symptoms and physical examination), endoscopy, histopathology, biomarkers and cross-sectional imaging.[11] Clinical disease activity indices that are widely used are the Harvey Bradshaw Index (HBI) in CD patients and the partial Mayo score and simple clinical colitis activity index (SCCAI) in UC patients.[12, 13] It has been shown that clinical disease activity indices more accurately reflect presence of inflammation in UC than in CD patients, since CD patients with active disease are often asymptomatic.[12-15]

The most frequently used biomarkers to detect presence of inflammation are fecal calprotectin (FCP) and C-reactive Protein (CRP). Although these are useful in clinical practice, they are not always accurate and cannot be used to assess the location and extent of inflammation.[16-19]

Endoscopy is used for assessment of luminal inflammation and to take biopsies for histopathologic assessment. Furthermore,it is used to assess treatment response and to treat strictures with endoscopic balloon dilation. Endoscopy may show erythema, decrease/absence of the vascular pattern, friability, erosions, ulcerations and spontaneous bleeding In UC patients. In CD patients, endoscopy may show ulcerations in a skip lesion pattern and strictures. Endoscopic scoring indices for grading of disease activity include the Crohn's Disease Endoscopic Index of Severity (CDEIS), Simple Endoscopic Score for Crohn's Disease (SES-CD) for CD patients and endoscopic Mayo score and ulcerative colitis endoscopic index of severity (UCEIS) for UC patients.[12, 13, 20, 21]

Cross-sectional imaging modalities include Magnetic Resonance Imaging (MRI), Computed Tomography (CT) and ultrasound (US). These modalities are all useful for detection of inflammation and complications.[22, 23] Given the complexity of IBD and the different diagnostic modalities, there is no gold standard for follow-up of IBD patients. Therefore, treatment decisions are usually based on a combination of information provided by these modalities.[11]

*Treatment of IBD*

Treatment of IBD patients is complex, consisting of anti-inflammatory medications and surgical interventions. A wide variety of anti-inflammatory agents are currently available including 5-aminosaliclyates, corticosteroids (intravenous, oral and topical), immunosuppressives (i.e. thiopurines and methotrexate), various biologics and JAK inhibitors (tofacitinib). In this thesis we focus on treatment with biologics.

Biologics are available for the treatment of IBD since the nineties and are increasingly being used.[24] Over the years several types have been approved. They can be divided into different classes, namely the tumor necrosis factor-alpha (TNF- $\alpha$) antagonists (infliximab, adalimumab, golimumab, certolizumab pegol), anti-interleukin-12 and 23 agents (ustekinumab) and integrin inhibitors (vedolizumab, natalizumab, etrolizumab).

Biologics are the most advanced medication for IBD and are effective for inducing and maintaining remission.[25, 26] However, a considerable number of patients experience primary non-response, secondary loss of response or adverse effects. An important clinical problem in this respect is immunogenicity (i.e. development of anti-drug antibodies [ADA]) which is associated with lower serum drug levels (mainly for the anti-TNF agents), loss of response, and adverse effects such as infusion and injection site reactions.[27-30] Strategies for treatment optimization include dose optimization by therapeutic drug monitoring (TDM) and combination treatment with immunosuppresives, which will be discussed in more detail later in this thesis.

IBD patients are often treated with biologics for many years when the treatment is effective, which is associated with significant costs.[31] Although, in recent years these costs have reduced for anti-TNF agents with the introduction of biosimilars.[32] Nevertheless, management of treatment costs is important. An important question in this respect is how and when to stop biologic treatment.. There are many potential reasons to discontinue biologic treatment, such as loss of efficacy, ADA formation, adverse effects, pregnancy, disease remission, patient preference and costs. In case of severe adverse effects or inefficacy the decision to discontinue treatment is usually simple. The decision to discontinue treatment in patients who are in remission is more difficult and evidence based guidelines regarding this topic are still lacking.[33]

*Treatment goals in IBD*

Treatment targets in IBD are becoming more ambitious with 'restoration of intestinal integrity' as the main goal.[34] Previously, doctors and patients were satisfied if patient's symptoms such as diarrhea and abdominal pain improved. However, in recent years endoscopy increasingly guides the therapeutic decision process, since evidence suggests that 'mucosal healing' is associated with improved long-term outcomes.[35-37] Mucosal healing in UC patients is defined as an endoscopic Mayo score of 0 or 1 in most clinical trials (i.e. absence of friability, erosions, blood and ulcers in all colon segments and rectum).[12, 37] Mucosal healing in CD patients is defined as absence of ulcers in all segments in most clinical trials.[13, 37] However, there is mounting evidence that complete endoscopic healing (i.e. completely normal mucosa) and histological healing are preferable treatment goals.[37, 38]Reaching the desired treatment target is referred to as a 'treat-to-target' strategy. Furthermore, early evaluation of the effectiveness of treatment is of increasing importance to give clinicians the possibility to optimize treatment at an early stage. In IBD patients, this strategy would require multiple endoscopies to assess the mucosa, which creates logistic and economic problems as well as a considerable burden for the patient.[39, 40] [40] Hence, alternative methods for repeated assessment of disease activity are needed. Biomarkers can be used for this purpose, but are not location and disease specific and are not suitable for assessment of disease severity. CT-scans are usually performed in the acute setting due to radiation exposure and MRI is limited by waiting lists.[22, 23, 41] Since IUS is a rapid, efficient, non-invasive and a relatively cheap imaging technique it is the most attractive tool for repeated assessment of disease activity.[42]

## Ultrasound

*Technical aspects of ultrasound*

Ultrasound (US) is high frequency sound that the human ear cannot detect. Diagnostic US uses wave frequencies between 1 to 40 Mega-hertz (MHz). Imaging of the alimentary tract is usually performed with frequencies between 3 and 15 Mhz. US probes emit and receive soundwaves with piezoelectric (PE) crystals. The US waves are attenuated in human tissue by reflection, absorption and diffraction. The reflected sound waves are received by the US probe and transformed into images by the computer. Images from higher frequencies have better spatial resolution. However, higher frequencies penetrate less trough tissue due to more attenuation. On the contrary, images from lower frequencies have less spatial resolution but are more useful to examine deeper lying structures.

Colour Doppler Signal (CDS) is an important US tool which is based on the Doppler effect. The Doppler effect is a frequency shift in sound that is reflected from a moving object. Movement away from the observer results in lower frequencies and movement towards the observer results in higher frequencies, whilst the emitted sound frequency of the moving object stays the same. This phenomenon is used to measure presence, speed and direction of blood flow in blood vessels and tissues. Blue colors usually represent movement away from the probe and red colors movement towards the probe.

*Intestinal ultrasound*

Intestinal ultrasound (IUS) is increasingly being used for the assessment of disease activity and complications in IBD patients. It is accurate in the diagnosis of IBD and to determine the extent and location of inflammation.[23, 43] Furthermore, it can be used to detect complications of the disease, such as stenosis, fistulas and abscesses in CD patients.[23, 43-45] Longitudinal studies have shown that ultrasound can be used for monitoring treatment effects.[46-49] Therefore, it is an attractive tool in the outpatient clinic for point-of-care (POC) decision making.[14, 42, 50-52] POC testing is rapid testing near or at the site of the patient, allowing for quick results and on the spot decision making. [42, 51, 52]

Several IUS parameters are useful when assessing disease activity in IBD patients. Most important is the assessment of the intestinal wall. Prior IUS studies showed that there is an association between intestinal layers visualized by IUS and histology. [53, 54] Soundwaves are reflected or absorbed differently by the different layers of the bowel wall, which is called wall layer stratification (WLS). Depending on the quality of the images, 5 to 9 layers can be depicted (figure 1).

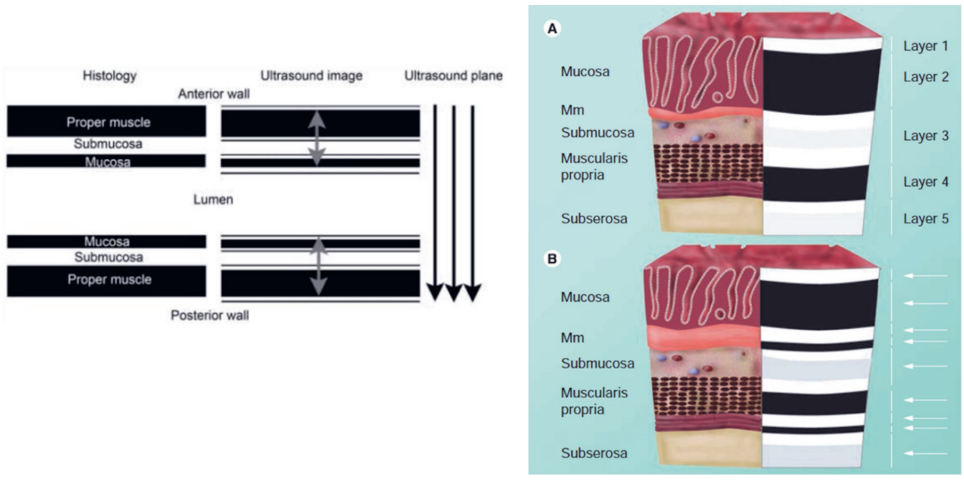



**Figure 1.** *Depiction of layers of the intestinal wall as visualized by IUS (reused with copyright agreement).*[55, 56]

Bowel wall thickness (BWT) is generally considered the most important IUS parameter. The bowel wall measures 1-2 mm in thickness in healthy individuals.[56] Inflammation results in thickening of the bowel wall. However, also fibrosis caused by chronic inflammation can lead to bowel wall thickening. Increased BWT and loss of WLS have been associated with increased disease activity in IBD patients, while fibrosis is more common in a thickened GI wall with visibile stratification.[43, 44] Some studies have shown that inflammation is associated with a thickening of the submucosa layer while fibrosis is associated with thickening of the proper muscle layer.[57-59] However, these associations are not useful for a reliable assessment of inflammation versus fibrosis. Hyperaemia within and around the bowel wall measured with CDS is another important parameter that is associated with inflammation. Bowel wall thickening without CDS has been associated with fibrosis, but absence of CDS in a thickened bowel wall does not exclude active inflammation and is not specific, nor sensitive for detection of fibrosis. [60-62] Other IUS parameters that are associated with active inflammation include presence of reactive lymph nodes, disruption of haustrations, absence of motility, fatty wrapping around the bowel and presence of complications such as fistulas and abscesses.[23, 43, 63] Figure 2 shows an example of terminal ileitis in a CD patient.

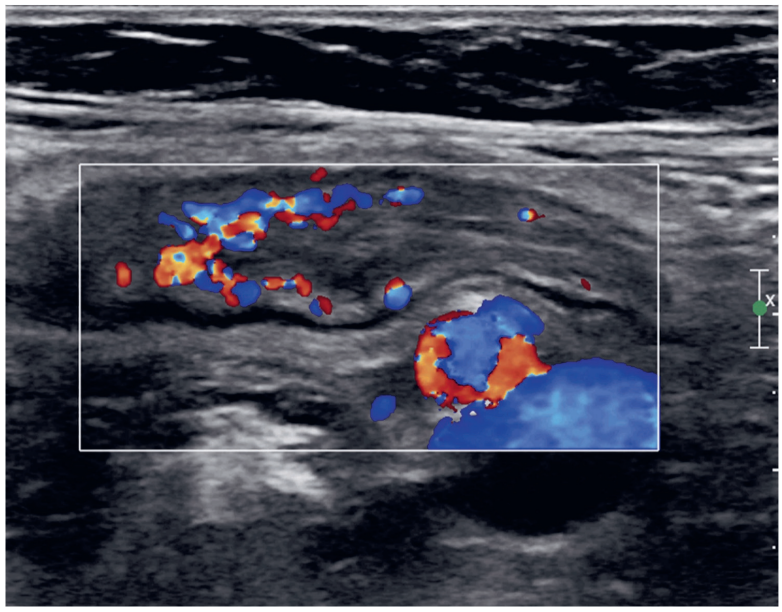


**Figure 2.** *Terminal ileitis in a CD patient.*

*Contrast enhanced ultrasound*

Contrast enhanced ultrasound (CEUS) is an imaging modality which is being studied increasingly for the assessment of inflammation and fibrosis in IBD patients (mainly CD). CEUS is performed by the injection of microbubbles. Microbubbles are phospholipid cells filled with gas, which can reach and pass the smallest capillaries in the body. The gas inside is sulphur hexafluoride which is breathed out when the microbubbles burst. Microbubbles always remain intravascular, and thus do not leak trough the capillaries into the surrounding tissue. Furthermore, they are not phagocytized and as such behave similarly to erythrocytes. Because of these properties, microbubbles can be used to study blood flow and tissue perfusion. Inflammation in CD leads to increased micro-vessel density and a local dysregulation of blood flow in the gastro-intestinal wall.[64-66] This causes changes in the bowel wall perfusion which can be quantified with CEUS.[57] Studies have shown an association between contrast enhancement and disease activity.[67-71] Few studies have investigated CEUS for treatment follow-up.[58] The details of the complex physics underlying CEUS won't be discussed in this thesis. However, basic knowledge regarding several CEUS parameter is useful and will be discussed further.

*CEUS parameters*

CEUS parameters are derivatives of the contrast enhancement time-intensity curve (figure 3).[58] The amount of contrast enhancement is usually described in arbitrary units based on the

contrast signal intensity over time, which can be measured and quantified by a specialized software (Vuebox; Bracco Suisse SA, Geneva, Switzerland). Peak enhancement (PE) describes the highest signal intensity in the curve. The wash-in area under the curve (WiAUC) is the area under the curve (AUC) during the time to PE. The rise time (RT) is the time to PE from the beginning of enhancement. The mean transit time corresponds with the average time the microbubbles spend within a determinate volume of capillary circulation. The washin-rate (WiR) is the velocity of the upslope of the curve. The wash-out AUC (WoAUC) is the AUC from PE to the end of the curve and the wash-in/wash out AUC is the WiAUC and WoAUC combined. The fall time (FT) is the time from PE to the end of the curve and finally, the wash-out rate (WoR) is the velocity of the down slope of the curve. Increase in these parameters represents increased perfusion and vascularity of the intestinal wall and is associated with inflammation.[57, 58, 72-74]

| | |
|---|---|
| PE | Peak Enhancement |
| WiAUC | Wash-in area under the curve |
| RT | Rise time |
| mTT | Mean transit time |
| WiR | Wash-in rate |
| WoAUC | Wash-out area under the curve |
| WiWoAUC | Wash in/wash out area under the curve |
| FT | Fall time |
| Wor | Wash out rate |

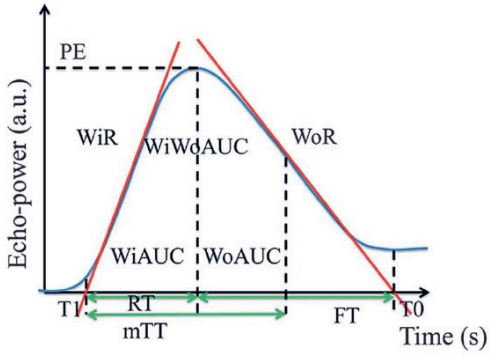



**Figure 3.** *Contrast enhancement time-intensity curve and associated parameters (reused with copyright agreement).*[58]

## Outline of the thesis

In part I of this thesis, we study the implementation of IUS in IBD care. We aimed to contribute to the further optimization of IUS for detection of disease activity, follow-up of treatment and potential for POC assessment in IBD patients. In part II we study biologic treatment, focusing on immunogenicity of biologics, combination treatment with immunomodulators, anti-TNF use in the Netherlands and relapse after anti-TNF discontinuation

### Part I: Implementation of intestinal ultrasound in IBD

In **chapter 2** we performed a systematic review on available ultrasound indices to study their characteristics as well as their strengths and weaknesses in development methodology. Based on the results of **chapter 2,** we developed a novel IUS index for grading of disease activity in UC patients in **chapter 3**. We studied all potential IUS parameters based on the literature and our own experience and compared them with endoscopic disease activity. The most predictive parameters and cut-offs were used to construct a point-based index. In addition, inter- and intra-observer agreement were studied for different parameters. In **chapter 4,** we studied treatment decisions after POC IUS in a real-life cohort of IBD patients at the Amsterdam University Medical Centre. Additionally we compared IUS outcomes with clinical and biochemical parameters and other diagnostic modalities. Furthermore, we assessed the evolution of IUS implementation in our clinic over time. In **chapter 5,** we studied conventional IUS parameters and CEUS for follow-up of anti-TNF treatment in CD patients. We hypothesized that improvement in CEUS and regular IUS parameters are suitable for the assessment of early treatment response and can be used to predict endoscopic treatment response and remission.

### Part II: Biological and combination therapy in IBD

In **chapter 6,** we reviewed optimal use of anti-TNF agents and in **chapter 7** we reviewed combination immunosuppression in IBD. We discussed important topics such as therapeutic drug monitoring (TDM), anti-drug antibody (ADA) formation, treatments costs, screening before treatment, dosing strategies, management of loss of response, elective discontinuation, re-initiation, mechanisms of action of combination therapy. In **chapter 8,** we systematically reviewed incidence of ADA formation against biologic agents for the treatment of IBD, since ADA formation is an important factor for treatment failure of biologics. Furthermore, we studied the effect of combination therapy with immunosuppressives on ADA formation. In **chapter 9,** we studied patterns of anti-TNF use in the Netherlands using data from a healthcare provider, allowing us to study large patient numbers. Finally, in **chapter 10** we studied patients who discontinued anti-TNF treatment who were in clinical remission. We aimed to study relapse rates and to identify predictors for relapse in a real-life cohort.

## References


1. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017;389(10080):1756-70.

2. Torres J, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. Lancet. 2017;389(10080):1741-55.

3. de Souza HSP, Fiocchi C, Iliopoulos D. The IBD interactome: an integrated view of aetiology, pathogenesis and therapy. Nat Rev Gastroenterol Hepatol. 2017;14(12):739-49.

4. Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Canadian journal of gastroenterology = Journal canadien de gastroenterologie. 2005;19 Suppl A:5a-36a.

5. Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 2011;140(6):1785-94.

6. Peyrin-Biroulet L, Loftus EV, Jr., Colombel JF, Sandborn WJ. Long-term complications, extraintestinal manifestations, and mortality in adult Crohn's disease in population-based cohorts. Inflammatory bowel diseases. 2011;17(1):471-8.

7. Monsén U, Sorstad J, Hellers G, Johansson C. Extracolonic diagnoses in ulcerative colitis: an epidemiological study. The American journal of gastroenterology. 1990;85(6):711-6.

8. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012;142(1):46-54.e42; quiz e30.

9. van den Heuvel TR, Jonkers DM, Jeuring SF, Romberg-Camps MJ, Oostenbrug LE, Zeegers MP, et al. Cohort Profile: The Inflammatory Bowel Disease South Limburg Cohort (IBDSL). Int J Epidemiol. 2017;46(2):e7.

10. de Groof EJ, Rossen NG, van Rhijn BD, Karregat EP, Boonstra K, Hageman I, et al. Burden of disease and increasing prevalence of inflammatory bowel disease in a population-based cohort in the Netherlands. European journal of gastroenterology & hepatology. 2016;28(9):1065-72.

11. Maaser C, Sturm A, Vavricka SR, Kucharzik T, Fiorino G, Annese V, et al. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. Journal of Crohn's & colitis. 2019;13(2):144-64.

12. D'Haens G, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. Gastroenterology. 2007;132(2):763-86.

13. Sandborn WJ, Feagan BG, Hanauer SB, Lochs H, Löfberg R, Modigliani R, et al. A review of activity indices and efficacy endpoints for clinical trials of medical therapy in adults with Crohn's disease. Gastroenterology. 2002;122(2):512-30.

14. Novak K, Tanyingoh D, Petersen F, Kucharzik T, Panaccione R, Ghosh S, et al. Clinic-based Point of Care Transabdominal Ultrasound for Monitoring Crohn's Disease: Impact on Clinical Decision Making. Journal of Crohn's & colitis. 2015;9(9):795-801.

15. Zittan E, Kabakchiev B, Kelly OB, Milgrom R, Nguyen GC, Croitoru K, et al. Development of the Harvey-Bradshaw Index-pro (HBI-PRO) Score to Assess Endoscopic Disease Activity in Crohn's Disease. Journal of Crohn's & colitis. 2017;11(5):543-8.

16. 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. Inflammatory bowel diseases. 2013;19(10):2111-7.

17. Travis S, Satsangi J, Lemann M. Predicting the need for colectomy in severe ulcerative colitis: a critical appraisal of clinical parameters and currently available biomarkers. Gut. 2011;60(1):3-9.

18. Stevens TW, Gecse K, Turner JR, de Hertogh G, Rubin DT, D'Haens GR. Diagnostic Accuracy of Fecal Calprotectin Concentration in Evaluating Therapeutic Outcomes of Patients With Ulcerative Colitis. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2020.

19. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflammatory bowel diseases. 2012;18(12):2218-24.

20. Travis SP, Schnell D, Krzeski P, Abreu MT, Altman DG, Colombel JF, et al. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Gut. 2012;61(4):535-42.

21. Daperno M, D'Haens G, Van Assche G, Baert F, Bulois P, Maunoury V, et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointestinal endoscopy. 2004;60(4):505-12.

22. Panes J, Bouhnik Y, Reinisch W, Stoker J, Taylor SA, Baumgart DC, et al. Imaging techniques for assessment of inflammatory bowel disease: joint ECCO and ESGAR evidence-based consensus guidelines. Journal of Crohn's & colitis. 2013;7(7):556-85.

23. Panes J, Bouzas R, Chaparro M, Garcia-Sanchez V, Gisbert JP, Martinez de Guerenu B, et al. Systematic review: the use of ultrasonography, computed tomography and magnetic resonance imaging for the diagnosis, assessment of activity and abdominal complications of Crohn's disease. Alimentary pharmacology & therapeutics. 2011;34(2):125-45.

24. van der Valk ME, Mangen MJ, Leenders M, Dijkstra G, van Bodegraven AA, Fidder HH, et al. Healthcare costs of inflammatory bowel disease have shifted from hospitalisation and surgery towards anti-TNFalpha therapy: results from the COIN study. Gut. 2014;63(1):72-9.

25. Torres J, Bonovas S, Doherty G, Kucharzik T, Gisbert JP, Raine T, et al. ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment. Journal of Crohn's & colitis. 2020;14(1):4-22.

26. Harbord M, Eliakim R, Bettenworth D, Karmiris K, Katsanos K, Kopylov U, et al. Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 2: Current Management. Journal of Crohn's and Colitis. 2017;11(7):769-84.

27. Baert F, Noman M, Vermeire S, Van Assche G, G DH, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. The New England journal of medicine. 2003;348(7):601-8.

28. Karmiris K, Paintaud G, Noman M, Magdelaine-Beuzelin C, Ferrante M, Degenne D, et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. Gastroenterology. 2009;137(5):1628-40.

29. West RL, Zelinkova Z, Wolbink GJ, Kuipers EJ, Stokkers PC, van der Woude CJ. Immunogenicity negatively influences the outcome of adalimumab treatment in Crohn's disease. Alimentary pharmacology & therapeutics. 2008;28(9):1122-6.

30. O'Meara S, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in patients with inflammatory bowel disease: a systematic review and meta-analysis. Inflammatory bowel diseases. 2014;20(1):1-6.

31. van Linschoten RCA, Visser E, Niehot CD, van der Woude CJ, Hazelzet JA, van Noord D, et al. Systematic review: societal cost of illness of inflammatory bowel disease is increasing due to biologics and varies between continents. Alimentary pharmacology & therapeutics. 2021.

32. Gulacsi L, Pentek M, Rencz F, Brodszky V, Baji P, Vegh Z, et al. Biosimilars for the Management of Inflammatory Bowel Diseases: Economic Considerations. Curr Med Chem. 2019;26(2):259-69.

33. Pauwels RWM, van der Woude CJ, Nieboer D, Steyerberg EW, Casanova MJ, Gisbert JP, et al. Prediction of Relapse After Anti-Tumor Necrosis Factor Cessation in Crohn's Disease: Individual Participant Data Meta-analysis of 1317 Patients From 14 Studies. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2021.

34. Peyrin-Biroulet L, Sandborn W, Sands BE, Reinisch W, Bemelman W, Bryant RV, et al. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. The American journal of gastroenterology. 2015;110(9):1324-38.

35. Schnitzler F, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, et al. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. Inflammatory bowel diseases. 2009;15(9):1295-301.

36. Colombel JF, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. Gastroenterology. 2011;141(4):1194-201.

37. Turner D, Ricciuto A, Lewis A, D'Amico F, Dhaliwal J, Griffiths AM, et al. STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): Determining Therapeutic Goals for Treat-to-Target strategies in IBD. Gastroenterology. 2021;160(5):1570-83.

38. Park S, Abdi T, Gentry M, Laine L. Histological Disease Activity as a Predictor of Clinical Relapse Among Patients With Ulcerative Colitis: Systematic Review and Meta-Analysis. The American journal of gastroenterology. 2016;111(12):1692-701.

39. Miles A, Bhatnagar G, Halligan S, Gupta A, Tolan D, Zealley I, et al. Magnetic resonance enterography, small bowel ultrasound and colonoscopy to diagnose and stage Crohn's disease: patient acceptability and perceived burden. European radiology. 2019;29(3):1083-93.

40. Sharara AI, El Reda ZD, Harb AH, Abou Fadel CG, Sarkis FS, Chalhoub JM, et al. The burden of bowel preparations in patients undergoing elective colonoscopy. United European gastroenterology journal. 2016;4(2):314-8.

41. Ordas I, Rimola J, Rodriguez S, Paredes JM, Martinez-Perez MJ, Blanc E, et al. Accuracy of magnetic resonance enterography in assessing response to therapy and mucosal healing in patients with Crohn's disease. Gastroenterology. 2014;146(2):374-82.e1.

42. de Voogd FAE, Verstockt B, Maaser C, Gecse KB. Point-of-care intestinal ultrasonography in inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2021.

43. Goodsall TM, Nguyen TM, Parker CE, Ma C, Andrews JM, Jairath V, et al. Systematic Review: Gastrointestinal Ultrasound Scoring Indices for Inflammatory Bowel Disease. Journal of Crohn's & colitis. 2021;15(1):125-42.

44. Maconi G, Carsana L, Fociani P, Sampietro GM, Ardizzone S, Cristaldi M, et al. Small bowel stenosis in Crohn's disease: clinical, biochemical and ultrasonographic evaluation of histological features. Alimentary pharmacology & therapeutics. 2003;18(7):749-56.

45. Maconi G, Ardizzone S, Parente F, Bianchi Porro G. Ultrasonography in the evaluation of extension, activity, and follow-up of ulcerative colitis. Scandinavian journal of gastroenterology. 1999;34(11):1103-7.

46. Parente F, Molteni M, Marino B, Colli A, Ardizzone S, Greco S, et al. Are colonoscopy and bowel ultrasound useful for assessing response to short-term therapy and predicting disease outcome of moderate-to-severe forms of ulcerative colitis?: a prospective study. The American journal of gastroenterology. 2010;105(5):1150-7.

47. Castiglione F, Testa A, Rea M, De Palma GD, Diaferia M, Musto D, et al. Transmural healing evaluated by bowel sonography in patients with Crohn's disease on maintenance treatment with biologics. Inflammatory bowel diseases. 2013;19(9):1928-34.

48. Castiglione F, de Sio I, Cozzolino A, Rispo A, Manguso F, Del Vecchio Blanco G, et al. Bowel wall thickness at abdominal ultrasound and the one-year-risk of surgery in patients with Crohn's disease. The American journal of gastroenterology. 2004;99(10):1977-83.

49. Kunihiro K, Hata J, Manabe N, Mitsuoka Y, Tanaka S, Haruma K, et al. Predicting the need for surgery in Crohn's disease with contrast harmonic ultrasound. Scandinavian journal of gastroenterology. 2007;42(5):577-85.

50. Bryant RV, Friedman A, Wright EK, Taylor K, Begun J, Maconi G, et al. Gastrointestinal ultrasound in inflammatory bowel disease: an underused resource with potential paradigm-changing application. Gut. 2018.

51. Allocca M, Furfaro F, Fiorino G, Peyrin-Biroulet L, Danese S. Point-of-Care Ultrasound in Inflammatory Bowel Disease. Journal of Crohn's & colitis. 2021;15(1):143-51.

52. Sathananthan D, Rajagopalan A, Van De Ven L, Martin S, Fon J, Costello S, et al. Point-of-care gastrointestinal ultrasound in inflammatory bowel disease: An accurate alternative for disease monitoring. JGH Open. 2020;4(2):273-9.

53. Kimmey MB, Martin RW, Haggitt RC, Wang KY, Franklin DW, Silverstein FE. Histologic correlates of gastrointestinal ultrasound images. Gastroenterology. 1989;96(2 Pt 1):433-41.

54. Wiersema MJ, Wiersema LM. High-resolution 25-megahertz ultrasonography of the gastrointestinal wall: histologic correlates. Gastrointestinal endoscopy. 1993;39(4):499-504.

55. Ødegaard S, Nesje LB, Lærum OD, Kimmey MB. High-frequency ultrasonographic imaging of the gastrointestinal wall. Expert Rev. Med. Devices. 2012;9(3):263-73.

56. Nylund K, Hausken T, Ødegaard S, Eide GE, Gilja OH. Gastrointestinal wall thickness measured with transabdominal ultrasonography and its relationship to demographic factors in healthy subjects. Ultraschall in der Medizin (Stuttgart, Germany : 1980). 2012;33(7):E225-32.

57. Nylund K, Jirik R, Mezl M, Leh S, Hausken T, Pfeffer F, et al. Quantitative contrast-enhanced ultrasound comparison between inflammatory and fibrotic lesions in patients with Crohn's disease. Ultrasound in medicine & biology. 2013;39(7):1197-206.

58. Saevik F, Nylund K, Hausken T, Odegaard S, Gilja OH. Bowel perfusion measured with dynamic contrast-enhanced ultrasound predicts treatment outcome in patients with Crohn's disease. Inflammatory bowel diseases. 2014;20(11):2029-37.

59. Ellrichmann M, Wietzke-Braun P, Dhar S, Nikolaus S, Arlt A, Bethge J, et al. Endoscopic ultrasound of the colon for the differentiation of Crohn's disease and ulcerative colitis in comparison with healthy controls. Alimentary pharmacology & therapeutics. 2014;39(8):823-33.

60. Limberg B. [Diagnosis of chronic inflammatory bowel disease by ultrasonography]. Zeitschrift fur Gastroenterologie. 1999;37(6):495-508.

61. Sasaki T, Kunisaki R, Kinoshita H, Yamamoto H, Kimura H, Hanzawa A, et al. Use of color Doppler ultrasonography for evaluating vascularity of small intestinal lesions in Crohn's disease: correlation with endoscopic and surgical macroscopic findings. Scandinavian journal of gastroenterology. 2014;49(3):295-301.

62. Sasaki T, Kunisaki R, Kinoshita H, Kimura H, Kodera T, Nozawa A, et al. Doppler ultrasound findings correlate with tissue vascularity and inflammation in surgical pathology specimens from patients with small intestinal Crohn's disease. BMC research notes. 2014;7:363.

63. Novak KL, Nylund K, Maaser C, Petersen F, Kucharzik T, Lu C, et al. Expert consensus on optimal acquisition and development of the International Bowel Ultrasound Segmental Activity Score (IBUS-SAS): a reliability and inter-rater variability study on intestinal ultrasonography in Crohn's Disease. Journal of Crohn's & colitis. 2020.

64. Danese S, Sans M, de la Motte C, Graziani C, West G, Phillips MH, et al. Angiogenesis as a novel component of inflammatory bowel disease pathogenesis. Gastroenterology. 2006;130(7):2060-73.

65. Hatoum OA, Binion DG, Otterson MF, Gutterman DD. Acquired microvascular dysfunction in inflammatory bowel disease: Loss of nitric oxide-mediated vasodilation. Gastroenterology. 2003;125(1):58-69.

66. Konerding MA, Turhan A, Ravnic DJ, Lin M, Fuchs C, Secomb TW, et al. Inflammation-induced intussusceptive angiogenesis in murine colitis. Anat Rec (Hoboken). 2010;293(5):849-57.

67. Girlich C, Schacherer D, Jung EM, Schreyer A, Buttner R. Comparison between a clinical activity index (Harvey-Bradshaw-Index), laboratory inflammation markers and quantitative assessment of bowel wall vascularization by contrast-enhanced ultrasound in Crohn's disease. Eur J Radiol. 2012;81(6):1105-9.

68. Migaleddu V, Scanu AM, Quaia E, Rocca PC, Dore MP, Scanu D, et al. Contrast-enhanced ultrasonographic evaluation of inflammatory activity in Crohn's disease. Gastroenterology. 2009;137(1):43-52.

69. Serra C, Menozzi G, Labate AM, Giangregorio F, Gionchetti P, Beltrami M, et al. Ultrasound assessment of vascularization of the thickened terminal ileum wall in Crohn's disease patients using a low-mechanical index real-time scanning technique with a second generation ultrasound contrast agent. Eur J Radiol. 2007;62(1):114-21.

70. Medellin-Kowalewski A, Wilkens R, Wilson A, Ruan J, Wilson SR. Quantitative contrast-enhanced ultrasound parameters in Crohn disease: Their role in disease activity determination with ultrasound. American Journal of Roentgenology. 2016;206(1):64-73.

71. Romanini L, Passamonti M, Navarria M, Lanzarotto F, Villanacci V, Grazioli L, et al. Quantitative analysis of contrast-enhanced ultrasonography of the bowel wall can predict disease activity in inflammatory bowel disease. Eur J Radiol. 2014;83(8):1317-23.

72. Quaia E, Gennari AG, van Beek EJR. Differentiation of Inflammatory from Fibrotic Ileal Strictures among Patients with Crohn's Disease through Analysis of Time-Intensity Curves Obtained after Microbubble Contrast Agent Injection. Ultrasound in medicine & biology. 2017;43(6):1171-8.

73. Quaia E, Sozzi M, Angileri R, Gennari AG, Cova MA. Time-Intensity Curves Obtained after Microbubble Injection Can Be Used to Differentiate Responders from Nonresponders among Patients with Clinically Active Crohn Disease after 6 Weeks of Pharmacologic Treatment. Radiology. 2016;281(2):606-16.

74. Quaia E, Cabibbo B, De Paoli L, Toscano W, Poillucci G, Cova MA. The value of time-intensity curves obtained after microbubble contrast agent injection to discriminate responders from non-responders to anti-inflammatory medication among patients with Crohn's disease. European radiology. 2013;23(6):1650-9.

# Part I

## Implementation of Intestinal Ultrasound

# Chapter 2

## Ultrasound For Assessing Disease Activity in IBD Patients: A Systematic Review of Activity Scores

S. Bots, K. Nylund, M. Löwenberg, K. Gecse, O.H. Gilja, G. D'Haens

*Journal of Crohn's and Colitis 2018;12(8):920-92*

## Abstract

### Background and aims

Ultrasound (US) indices for assessing disease activity in IBD patients have never been critically reviewed. We aimed to systematically review the quality and reliability of available ultrasound (US) indices compared with reference standards for grading disease activity in IBD patients.

### Methods

Pubmed, Embase and Medline were searched for relevant literature published within the period 1990 to June 2017. Relevant publications were identified through full text review after initial screening by two investigators. Data on methodology and index characteristics were collected. Study quality was assessed using a modified version of the Quadas-2 tool for risk of bias assessment.

### Results

Of 20 studies with an US index, 11 studies met the inclusion criteria. Out of these 11 studies, 7 and 4 studied Crohn's disease (CD) and ulcerative colitis (UC) activity indices, respectively. Parameters that were used in these indices included bowel wall thickness (BWT), Doppler signal (DS), wall layer stratification (WLS), compressibility, peristalsis, haustrations, fatty wrapping, contrast enhancement (CE), and strain pattern. Study quality was graded high in 5 studies, moderate in 3 studies and low in 3 studies. Ileocolonoscopy was used as the reference standard in 9 studies. In 1 study a combined index of ileocolonoscopy and barium contrast radiography and in 1 study histology was used as the reference standard. Only 5 studies used an established endoscopic index for comparison with US.

### Conclusions

Several US indices for assessing disease activity in IBD are available; however, the methodology for development was suboptimal in most studies. For the development of future indices, stringent methodological design is required.

## Introduction

Assessing disease activity in inflammatory bowel disease (IBD) patients is becoming increasingly important. Treatment targets in IBD patients are shifting from symptom control to intestinal repair, an end point that has been associated with improved long-term outcomes.[1, 2] Ileocolonoscopy is the gold standard for the assessment of disease activity in IBD patients. Therefore, it is increasingly being implemented to guide treatment decisions and to evaluate treatment outcomes in clinical trials. Several endoscopic activity scores have been developed and validated and can be used to assess endoscopic disease activity.[3-8]

For optimal monitoring of disease activity in IBD patients, ileocolonoscopy should be performed on a regular basis. However, repeated colonoscopies represent a logistic and economic challenge, as well as significant burden for the patient. Moreover, there is a small risk of bowel perforation and transmural or extra-luminal disease activity, and complications such as abscesses cannot be assessed. Finally, the ileum cannot be intubated in a significant proportion of patients due technical or anatomical difficulties.

Biomarkers such as serum C-reactive protein (CRP) and fecal calprotectin have limited reliability for assessing and grading IBD disease activity.[9] Therefore, cross-sectional imaging modalities, such as trans-abdominal ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) are increasingly being used in the management of IBD.[10-12] These imaging techniques can be used to determine the extent and location of inflammation and to detect disease complication, such as stenosis, fistulas and abscesses in patients with Crohn's disease (CD).[2, 10, 11, 13-20] Magnetic resonance imaging and CT show good results for grading disease activity, but they are not ideal for repeated use due to logistical reasons (MRI) or radiation exposure (CT).[10, 11] Since US is rapid, non-invasive, relatively cheap, and can even be performed in a point-of-care setting, it appears to be the most suitable modality for systematic monitoring in IBD patients.[21]

An accurate US index for grading disease activity would therefore be of great clinical value. Although various US activity indices for IBD patients exist, and have also been evaluated in previous reviews, the applicability of US in grading disease activity remains uncertain.[11, 19, 22, 23] Also, a comprehensive evaluation of the characteristics and methods of all available studies focusing on US activity indices for assessing disease activity in IBD has never been conducted.

Here, we aim to critically review the quality and reliability of available US activity indices compared with reference standards for grading disease activity in IBD patients. This could serve as a basis for improving US activity indices and for the development of novel scoring systems.

## Methods

This systematic review has been conducted in accordance with the Preferred Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[24] The protocol has not been published in advance.

*Literature search*

PUBMED, MEDLINE, CENTRAL, and EMBASE were electronically searched for literature published within the period January 1990 until March 2017 on studies examining the use of US for grading disease activity in CD and UC. Details of the search criteria are provided in the supplementary material (Appendix E1). All reference lists of the included studies were searched for potentially relevant records.

*Inclusion and exclusion criteria*

Study inclusion was based on the following criteria: (1) Study of an US index consisting of at least three categories for disease activity grading (i.e. quiescent, moderate, or severe); (2) comparison with a reference test/standard such as ileocolonoscopy, MRI, barium contrast radiography, or histology; (3) a sample size of at least 20 patients; (4) articles written in English; (5) full text available (i.e. no abstracts). Studies that used a clinical activity index as the reference standard were not included, since these instruments poorly correlate with inflammatory disease activity, especially in CD.[25]

*Study selection*

All retrieved studies were assessed by one observer (SB). Irrelevant studies were excluded based on title, abstract, and study type (i.e. review, case report, comment, letter). The remaining titles and abstracts were independently assessed by two observers (SB, KN) for eligibility for full text review. Subsequently, the selected full texts were assessed by both observers in order to identify studies with US indices. Finally, the remaining studies were assessed for inclusion by both observers. Disagreements were resolved through discussion after every phase in the selection process.

*Data collection and analysis*

The following data were collected on study characteristics: study design, diagnosis, number of included patients, number of US exams, segments analysed, patient selection and inclusion methods, reference test and index used, blinding methods, and time between reference and US exams. Additionally, the following data were collected on the US indices: index parameters, severity grades, cut-offs, index calculation methods, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and correlation

coefficients with reference test. A meta-analysis was not performed due the heterogeneity in study methodology and index characteristics.

*Study quality grading*

All included studies were graded for methodological quality by two investigators (SB and KN) with a modified version of the QUADAS-2 tool.[26] The QUADAS-2 tool is designed to assess the quality of diagnostic accuracy studies with signaling questions in 4 domains (patient selection, index test, reference test, and patient flow). The signaling questions of the modified tool are shown in table 1. Established reference indices were considered as good quality reference standards. If existing reference indices were modified for the purpose of the study, they were considered as lower quality reference standards. The questions in each domain could be answered with 'yes', 'no', or 'unclear'. Unclear answers were considered as 'no' for the final quality grading. Each subdomain was graded as high risk of bias if ≥50% of the signaling questions were answered with 'no'. A study was graded as high quality in the case of a low risk of bias in at least 6 out of the 7 subdomains. A study was graded as low quality in the case of a high risk of bias in 4 or more subdomains. All other studies were graded as moderate quality. Any disagreements were resolved through discussion.

**Table 1.** *Modified QUADAS-2 risk of bias assessment tool.*

| | |
|---|---|
| Domain 1 | Patient selection |
| 1A | Was a consecutive or random sample used? |
| | Was a case-control or retrospective design avoided? |
| | Were inappropriate exclusions avoided? |
| | Was the samplesize appropriate (10 patients per index parameter)?[a] |
| 1B | Did the patients match the review question? (confirmed IBD) |
| Domain 2 | Index test |
| 2A | Blinding for the results of the reference test? |
| | Were the thresholds not pre-specified?[b] |
| 2B | Concerns regarding applicability of the index (reproducibility)? |
| Domain 3 | Reference standard |
| 3A | Was the reference standard used to classify the condition? |
| | Blinding for results of index test? |
| | Use of an established reference index?[a] |
| 3B | Concerns regarding applicability of the reference test (reproducibility)? |
| Domain 4 | Flow and timing |
| 4A | Appropriate interval between index and reference test ( ≥ 1 month) ? |
| | Did all patients receive reference test? |
| | Did all patients receive the same reference test? |
| | Were all patients included in the analysis? |

[a]This item was not part of the original Quadas-2 tool

[b]This question was adapted from the original tool

## Results

*Study selection*

A total of 2103 records were identified through electronic search, and 1656 remained after removal of duplicates. One additional record was identified through other sources. This particular study was published after the search date, but we decided to include it due to its relevance.[27] After screening titles and abstracts, 140 potentially eligible studies were selected for full text review. After full text review, 21 records were identified that studied an US activity index (supplementary table 1). Out of these 21 studies, 11 met the inclusion criteria. A chart flow of the selection process is shown in figure 1.

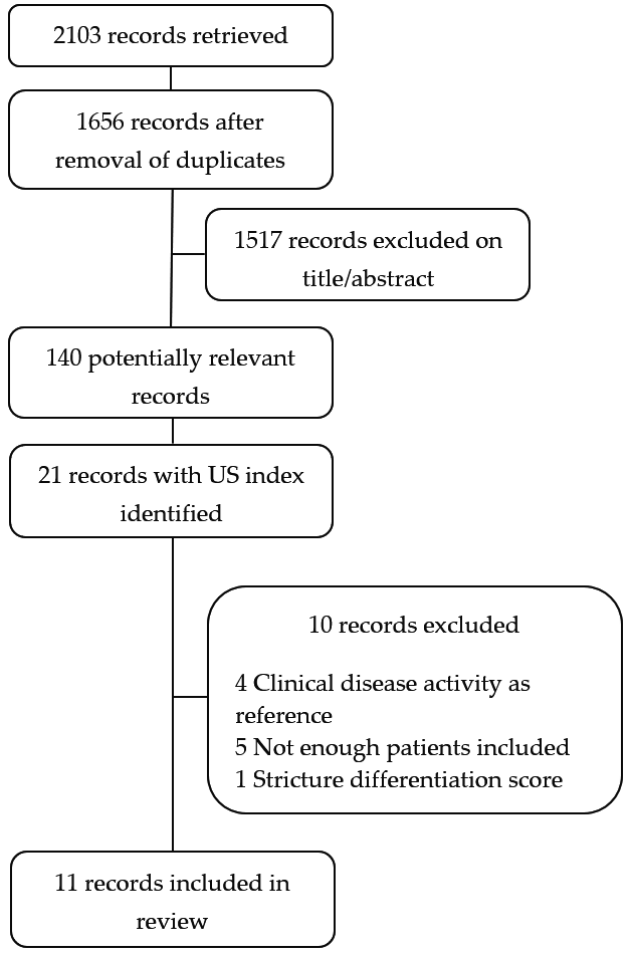



**Figure 1.** *Flow chart of study selection process.*

*Study characteristics*

The study characteristics are shown in table 2. Eight studies used a prospective and two studies a retrospective design. One study consisted of a retrospective development phase and a prospective validation phase. The total number of studied subjects was 771 (mean 70.1; SD 56.2), and a total of 1088 (mean 98.9; SD 93.9) US exams were performed. In 4 studies, only the ileum was investigated. Ileocolonoscopy was used as the reference standard in 9 studies, in 1 study a combined index of ileocolonoscopy or barium contrast radiography was used as the reference standard, and in 1 study histology was used as the reference standard.

*Crohn's disease ultrasonographic activity indices*

Seven CD indices were identified from eight records. The parameters used in the CD indices included bowel wall thickness (BWT), Doppler signal (DS), wall layer stratification (WLS), compressibility, peristalsis, haustrations, fatty wrapping and contrast enhancement (CE). Crohn's disease index details are provided in table 3.

Futagami et al. developed an US index with BWT and WLS as parameters.[28] The thresholds of the index were defined before the study. They compared the index with either endoscopy or barium contrast radiography in 55 patients. An endoscopic/radiological index was developed for comparison; thus, not all patients received the same reference standard. The overall correlation with the reference index was average ($r^2 = 0.62$; $p < 0.01$).

Neye et al. developed an US index with BWT and DS as parameters.[29] The thresholds of the index were defined before the study. The index was compared with a newly developed endoscopic activity index in 22 patients (i.e. for each bowel segment: 1 (no lesions), 2 (aphtes), 3 (aphtes and ulcers < 50%) to 4 (aphtes and ulcers >50%). The highest concordance was found in the descending colon ($\kappa = 0.91$; 95% CI 0.56–0.99) and the lowest in the ascending colon ($\kappa = 0.75$; 95% CI 0.56 – 0.94). Concordance for all bowel segments separately is shown in supplementary table 2.

Drews et al. conducted a retrospective study comparing the Limberg score with histologic inflammation in ileum biopsies obtained by ileocolonoscopy in 32 CD patients.[30] This index was first proposed by Limberg and semiquantitatively measures DS in thickened bowel segments (>4 mm).[31] A histologic index for severity of inflammation was developed for the study. The association between the Limberg score and histologic grades of disease activity was poor ($\kappa = 0.4375$).

Sasaki et al. conducted a retrospective study comparing the Limberg score with the SES-CD score in 108 CD patients.[32] Only the ileum was investigated. The correlation between US and endoscopy was good ($Þ = 0.709$; $p < 0.001$).

Paredes et al. developed an US index with BWT and DS for grading of post-surgical recurrence in 33 patients.[33] The index was compared with the endoscopic Rutgeerts score for post-operative recurrence in 33 patients.[34] The Rutgeerts score is a prognostic score to predict post-operative disease course. The thresholds of the US index were determined before the study. The correlation of the US index with the Rutgeerts score was poor ($\kappa = 0.29$; p = unknown). For the diagnosis of moderate–severe recurrence, the correlation with endoscopy was average ($\kappa = 0.57$; $p = 0.009$). A follow-up study with similar methods was conducted, combining the index with contrast enhanced ultrasound (CEUS).[35] Postoperative recurrence was assessed in 60 CD patients. A cut-off of 34.5% of maximum contrast enhancement predicted endoscopic recurrence most accurately. In combination with the other US parameters, the accuracy was 94.4% and the correlation was good ($\kappa = 0.82$; $p < 0.001$). A cut-off >46% contrast enhancement was best for the prediction of moderate–severe endoscopic recurrence.

Pascu et al. developed an index with BWT, DS, compressibility, WLS and fatty wrapping as parameters.[36] The index was compared with ileocolonoscopy using a modified Baron score in 37 CD patients.[6] The thresholds of the index were defined before the study. The overall activity index was calculated by the sum of segmental indices. The overall correlation between US and ileocolonoscopy was good [$r = 0.830$; $p < 0.001$].

Novak et al. developed an index with BWT and DS as parameters. The study consisted of a retrospective phase for developing the index and a prospective phase for validating the index. The SES-CD or Rutgeerts score was used as the reference standard. The index was developed using univariate and multivariate logistic regression models. Cut-offs for discriminating between inactive/mild endoscopic disease and moderate/severe endoscopic disease were determined from the area under the receiver operating characteristic curve (AUROC). The SES-CD cut-off for active versus inactive disease was >5. Also, there were 7 UC patients in the development cohort. Additionally, there were 63 patients and 87 examinations in the validation cohort; thus, for 24 patients 2 US examinations were used for the statistical calculations. In both phases, ultrasonographers and endoscopists were not blinded for the results of the other examinations. The final US score could be calculated using a formula (table 3). The AUROC was 0.836 for discerning disease activity in the validation cohort.

**Table 2.** *Characteristics of included studies.*

| Study (index) | Diagnosis | Design | Subjects | US nr. | Index PM | Segments | Ref. | Ref. index | Days index/ref |
|---|---|---|---|---|---|---|---|---|---|
| Futagami 1999 | CD | Prospective | 55 | 126 | BWT, WLS<br>Haustrations<br>Compressibility<br>Peristalsis | Jejunum<br>Ileum<br>Ascending<br>Transverse<br>Descending<br>Sigmoid<br>Rectum | BCR/ ICC | Developed | 3 |
| Neye 2004 | CD | Prospective | 22 | 22 | BWT, DS | Ileum<br>Cecum<br>Ascending<br>Transverse<br>Descending<br>Sigmoid | ICC | Developed | 3 |
| Drews 2009 (Limberg) | CD | Retrospective | 32 | 32 | BWT, DS | Ileum | Biopsies | Developed | 5 |
| Sasaki 2014 (Limberg) | CD | Retrospective | 108 | 108 | BWT, DS | Ileum | ICC | SES-CD | 30 |
| Paredes 2010 | CD | Prospective | 40 (33) | 40 | BWT, DS<br>Complications | Ileum | ICC | Rutgeerts | 3 |
| Paredes 2013 | CD | Prospective | 60 | 60 | BWT, DS, CE, complications | Ileum | ICC | Rutgeerts | 3 |

**Table 2. (***Continued).*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Novak 2017 | CD | Phase 1: retrospective<br>Phase 2: Prospective | 223 | 247 | BWT, DS | Ileum<br>Cecum<br>Ascending<br>Transverse<br>Descending<br>Sigmoid<br>Rectum | ICC | SES-CD & Rutgeerts | Phase 1: 60<br>Phase 2: 14 |
| Pascu 2004 | UC and CD | Prospective | 37 CD<br>24 UC | 61 | BWT, DS, WLS, FW<br>Compressibility | Ileum<br>Cecum<br>Ascending<br>Transverse<br>Descending<br>Sigmoid | ICC | Modified Baron | 5 |
| Civitelli 2014 | UC | Prospective | 60 (50) | 50 | BWT, DS, WLS, haustrations | Right colon<br>Transverse<br>Left colon | ICC | Mayo | 1 |
| Parente 2009/2010 | UC | Prospective | 83 | 305 | BWT, DS | Ascending<br>Transverse<br>Descending<br>Sigmoid | ICC | Modified Baron | 3 |
| Ishikawa 2011 | UC | Prospective | 37 | 37 | Strain patterns | Ascending<br>Transverse<br>Descending<br>Sigmoid | ICC | Developed | 1 |

*BWT = bowel wall thickness; DS = Doppler signal; WLS = wall layer stratification; FW = fatty wrapping; CE = contrast enhancement; ICC = ileocolonoscopy; BCR = barium contrast radiography; pm = parameters; Ref.= reference; Developed = Reference index was newly developed for study*

**Table 3.** *Characteristics of Crohn's disease indices.*

| Index | | | | | |
|---|---|---|---|---|---|
| | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Limberg<br>Drews<br>Sasaki | - BWT < 4mm<br>-no vessels | - BWT > 4mm<br>- no vessels | - BWT >4mm<br>- Spots of vascularity | - BWT > 4 mm<br>- longer stretches of vascularity | - BWT > 4mm<br>- long stretches of vascularity into mesentery |
| | Normal | Type A | Type B | Type C | - |
| Futagami | - BWT < 4mm<br>- normal compressibility and peristalsis<br>- Haustrations present | - BWT <4mm<br>- reduced compressibility and peristalsis<br>- Loss of haustrations | - BWT > 4mm<br>- stratification intact | - BWT > 4mm<br>- loss of stratification | - |
| | The formula: 1 point for type A lesions [BWT -2] * 2 for type B lesions [BWT-2] * 4 for type C lesions | | | | |
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 | - |
| Neye | BWT < 5mm, no vessels/cm$^2$ | - BWT < 5mm, 1-2 vessels/cm$^2$<br>- BWT > 5 mm, no vessels/ cm$^2$ | - BWT < 5mm, >2 vessels/cm$^2$<br>- BWT > 5mm, 1-2 vessels/cm$^2$ | - BWT > 5mm, 2 vessels/cm$^2$ | |
| | Normal | Recurrence | Mod/Sev recurrence | | |
| Paredes 2010 | - BWT < 3mm<br>- No DS | BWT >3mm and/or positive DS | BWT >5mm and DS grade 2 or 3. | | |
| | Normal | Recurrence | Mod recurrence | Sev recurrence | |
| Paredes 2013 | - BWT < 3mm<br>- CE <34.5% | -BWT 3-5 mm<br>-CE <46%; | -BWT >5mm or CE >46% | -BWT >5 mm, or CE>70%, or presence of fistula. | |

**Table 3. (***Continued).*

| | Grade 0 | Grade 1 | Grade 2 | Grade 3 |
|---|---|---|---|---|
| Pascu | -BWT < 3mm<br>-No DS | -BWT 3-5mm<br>-Increased DS<br>-Loss of compressibility<br>-Accentuated WLS | -BWT 5-8mm<br>-Increased DS<br>-Loss of compressibility<br>-Loss of WLS | -BWT > 8mm<br>-Increased DS<br>-Loss of compressibility<br>-Loss of WLS<br>-Fatty wrapping |
| | Grade 0 | Grade 1 | Grade 2 | Grade 3 |
| Novak | -BWT < 3mm<br>-No DS | -BWT 3.1-6mm<br>-DS mild | -BWT 6.1-7.0mm<br>-DS mod/sev | -BWT >7.0mm<br>-DS mod/sev |
| | Score = (0.0563 * bwt1) + (2.0047 * bwt2) + (3.0881 * bwt3) + (1.0204 * doppler1) + (1.5460 * doppler2) | | | |

*BWT = bowel wall thickness; DS = Doppler signal; WLS = wall layer stratification; CE = contrast enhancement; Mod = moderate; Sev = severe.*

*Ulcerative colitis ultrasonographic activity indices*

Four US indices were identified. The parameters used in the indices included BWT, DS, WLS, compressibility, fatty wrapping, and strain pattern. Ulcerative colitis index details are provided in table 4.

Parente et al. developed an US index with BWT and DS for the assessment of mucosal healing.[2, 20] The index was compared with the endoscopic Baron score in 83 UC patients.[6] The thresholds of the US index were defined before the study. Patients were assessed at 0, 3 , 9, and 15 months. At baseline, all patients had US scores and baron scores of 2–3. Concordance of the severity classes was average, with a weighted κ coefficient of 0.59 (95 % CI: 0.40–0.78).

Ishikawa et al. 2011 proposed an US index with real-time elastography (RTE) based on normal, homogenous, random, and hard patterns[37] and compared it with ileocolonoscopy in 37 UC patients. Ileocolonoscopic findings were classified as (A) normal mucosa, (B) mucosal edema and erosion without ulcer, (C) punched-out ulcer, and (D) extensive ulcer. A significant correlation was reported between type A, B , C, and D and normal, homogenous, random, and hard, respectively (chi-square $p < 0.001$).

Civitelli et al. 2014 developed an US index for the assessment of disease activity in paediatric UC.[38] Ultrasound parameters were compared with the endoscopic Mayo score as dependent variables in 50 patients. Multiple regression analysis showed that BWT (P = 0.0008), increased vascularity (P = 0.002), loss of stratification (P = 0.021), and absence of colon haustrations (P = 0.031) were significantly associated with endoscopic disease severity. A US score >2 had a sensitivity of 100% and a specificity of 93% (AUC 0.98) for detecting severe endoscopic disease.

The US index correlated strongly with endoscopic disease activity (r = 0.94; P < 0.0001). Concordance between US and ileocolonoscopy for inactive, mild, moderate, and severe disease was very good (κ = 0.94; 95% CI 0.88–1).

Pascu et al. developed an US index with BWT, DS, compressibility, WLS, and fatty wrapping as parameters.[36] The index was compared with a modified Baron score in 24 UC patients. The US activity index showed a strong correlation with ileocolonoscopy (r = 0.974, P < 0.001).

**Table 4.** *Characteristics of ulcerative colitis indices.*

| Index | | | | | |
|---|---|---|---|---|---|
| | Grade 0 | Grade 1 | Grade 2 | - | |
| Parente | - BWT < 4 mm<br>- no or scarce intramural blood flow | -BWT 4 – 6 mm and blood flow | -BWT6 – 8 mm and blood flow | | |
| | Grade 0 | Grade 1 | Grade 2 | Grade 3 | |
| Ishikawa | Normal color pattern | Homogenous color pattern | Random color pattern | Hard color pattern | |
| | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Civitelli | no findings | 1 finding | 2 findings | 3 findings | 4 findings |
| | Findings: BWT > 3mm, increased DS, loss of WLS, absence of haustrations | | | | |
| | Grade 0 | Grade 1 | Grade 2 | Grade 3 | |
| Pascu | -BWT < 3mm<br>-No DS | -BWT 3-4.5mm<br>-Increased DS<br>-Loss of compressibility<br>-Accentuated WLS | -BWT 4.5-6mm<br>-Increased DS<br>-Loss of compressibility<br>-Loss of WLS | -BWT >6mm<br>-Increased DS<br>-Loss of compressibility<br>-Loss of WLS<br>-FW | |

*BWT = bowel wall thickness; DS = Doppler signal; WLS = wall layer stratification; FW = fatty wrapping*

*Grading of study quality*

Study quality was graded high in five studies, moderate in three studies, and low in three studies. Most concerns were raised in the subdomains regarding the index test and the reference standard. Blinding was performed properly in most studies, but in nine studies the thresholds of the index were defined before the study was performed. Civitelli et al. developed the US index using the reference standard as a dependent variable. Novak et al. developed the index in a retrospective study and validated it in a prospective study. Both studies were therefore used for quality grading. Five studies used an established endoscopic reference

index (i.e. SES-CD, Mayo, Rutgeerts score). In the other studies, either a newly developed index or a modified Baron index was used. Methods for patient selection were suboptimal in three studies. Flow and timing were good in all studies. The results of the Quadas-2 assessment are shown in table 5. There were no studies that used central reading or inter- and intra-observer variability assessment, and only the study performed by Novak et al. used a development and validation phase.

**Table 5.** *Quadas-2 assessment results: risk of bias in all subdomains.*

| Study | Domain1: patient selection | Domain 2: Index test | Domain 3: reference standard | Domain 4: Flow and timing | Overall quality |
|---|---|---|---|---|---|
| Futagami 1999 | A: Low<br>B: Low | A: High<br>B: Low | A: High<br>B: High | A: Low | Moderate |
| Neye 2004 | A: Low<br>B: Low | A: High<br>B: Low | A: High<br>B: High | A: Low | Moderate |
| Drews 2009 | A: High<br>B: Low | A: High<br>B: High | A: High<br>B: High | A: Low | Low |
| Sasaki 2014 | A: High<br>B: Low | A: High<br>B: Low | A: Low<br>B: Low | A: Low | Moderate |
| Paredes 2010 | A: Low<br>B: Low | A: High<br>B: Low | A: Low<br>B: Low | A: Low | High |
| Paredes 2013 | A: Low<br>B: Low | A: High<br>B: Low | A: Low<br>B: Low | A: Low | High |
| Novak 2017 | A: Low<br>B: Low | A: High<br>B: Low | A: Low<br>B: Low | A: Low | High |
| Pascu 2004 | A: High<br>B: Low | A: High<br>B: Low | A: High<br>B: High | A: Low | Low |
| Civitelli 2014 | A: Low<br>B: Low | A: Low<br>B: Low | A: Low<br>B: Low | A: Low | High |
| Parente 2009/2010 | A: Low<br>B: Low | A: High<br>B: Low | A: Low<br>B: Low | A: Low | High |
| Ishikawa 2011 | A: High<br>B: Low | A: High<br>B: High | A: High<br>B: High | A: Low | Low |

## Discussion

To our knowledge, this is the first comprehensive systematic review on US scoring indices that can be used to assess disease activity in IBD patients. The methods that were used for the development of these indices were suboptimal in most studies. Although 20 studies were identified that studied an US activity index, 9 were excluded due to small patient numbers or because clinical activity indices were used as the reference standard, indicating poor methodology. Out of 11 included studies, only 5 of them were graded as high quality using the modified Quadas-2 tool. Based on these findings, we conclude that the methodology for the development of US indices for grading disease activity in IBD patients should be improved in future studies.

Important criteria for the development of a diagnostic index are appropriate patient selection, a proper sample size, implementation of blinding, use of an established reference index, inclusion of patients with different disease activity, and proper study flow and timing (i.e. time between index and reference test and comparison of all patients with the same reference standard).[26] In addition, a diagnostic index should ideally be developed using the reference index as the dependent variable. Parameters of the imaging modality that can predict outcomes of the reference index should be determined and used for further development of the index. Subsequently, the most predictive cut-off values should be determined with appropriate statistical methods.[39] The methods that were used for the development of the so-called simple endoscopic indices for CD (CDEIS and SES-CD) are good examples of such an approach.[3, 8]

The most commonly used parameters in both the CD and UC indices were BWT, DS, and WLS (10, 9, and 3 indices in CD and 3, 3, and 2 indices in UC, respectively). Bowel wall thickness is the only quantifiable measurement, and in theory is probably the easiest to reproduce. However, it is important to standardize measurement methods in order to get reproducible results (i.e. measurement location and probe handling). DS is usually measured semi-quantitatively and thus is more prone to interpretation. Additionally, the amount of DS is influenced by equipment and patient characteristics such as the amount of body fat and location of inflammation. To optimize reproducibility, clear definitions should be used and settings on the US scanner should be optimized and remain constant when assessing different patients (i.e. slow-flow settings). The assessment of WLS is also more subjective and thus clear definitions should be used. Fatty wrapping (FW), haustrations, compressibility, and peristalsis were rarely used as index parameters. However, FW is considered as an important finding and should be considered for score development in the future, especially in CD patients.

Ileocolonoscopy was used as the reference standard in most of the included studies (n = 9), but only five studies compared US with an established endoscopic index (i.e. SES-CD, Mayo,

Rutgeerts' score). In the other four studies, a newly developed or a modified index was used as the reference standard. Pascu et al. used, for example, the modified Baron score for assessing disease activity in both CD and UC. Since CD and UC are different entities, activity cannot be scored with the same scoring system. Futugami et al. used an activity score that was based on both endoscopic and barium contrast radiography findings in CD patients. It is likely that the comparison with these non-established reference indices has biased the results in these studies. This is also reflected by the wide range in statistical association between US and endoscopic indices in these studies.

Additionally, in all these studies, the thresholds for ultrasonographic parameters were determined before the study. Establishment of index thresholds prior to a study is likely to result in overestimation of the diagnostic value.[39] Civitelli et al. used an endoscopic index (Mayo endoscopic score) as a dependent variable in order to determine thresholds of US parameters for the development of an US index for paediatric UC patients.[38] Additionally, Novak et al. conducted a retrospective study in which they determined parameters, cut-off values, and the formula for calculating the activity score.[27] As a next step, they validated the index formula prospectively. However, a major limitation of this study was that ultrasonographers and endoscopists were not blinded for the results of the other examinations. Moreover, the SES-CD cut-off that was used for active disease was quite liberal (SES-CD >5), and there were 7 UC patients in the development cohort.

Drews et al. compared the Limberg score (see table 3 for index characteristics) with histologic inflammation in biopsies in CD patients. Correlation between this score and the histology index was poor to average, depending on the cut-off values that were used. This could be explained by the fact that the location of, or small amount of tissue obtained through, biopsies may not accurately reflect disease activity. Additionally, a non-validated histology index was used. The Limberg score does seem to correlate better with endoscopic disease activity, as was shown by Sasaki et al.[32] However, the data for this study were collected retrospectively, which may have introduced bias. Additionally, only ileal disease was compared in these studies, since the Limberg score was initially developed to assess the ileum.

Interestingly, we found no studies that used an alternate cross-sectional imaging modality (e.g. MRI or CT) as the reference standard. This could be explained by the fact that disease activity indices for these modalities are also relatively rare, and that no standard and widely used activity index exists (i.e. such as the SES-CD or Mayo score). A comprehensive systematic review by Puylaert et al. described 11 studies on MRI and 3 studies on CT for grading of disease activity, which all used endoscopy, biopsies, or surgical specimens as the reference standard.[11] This confirms our finding that thus far, US has not been compared with activity indices from other cross-sectional modalities. Such comparisons could be of value and should be conducted in future studies.

Small intestine contrast ultrasonography has also been studied for the grading of disease activity in IBD. We identified two studies describing a SICUS activity index.[40, 41] However, both studies used clinical disease activity as the reference standard and therefore did not meet the inclusion criteria. Some studies have shown higher sensitivity and specificity of SICUS for the detection of inflammation than regular US.[42-44] The development of SICUS indices with use of a good reference standard could therefore be of important value. SICUS is, however, more time consuming than regular US and thus is probably less useful in a point-of-care setting.

The value of contrast enhancement for the assessment of disease activity in IBD is increasingly being studied. It seems to have promising potential for the assessment of disease activity.[45-47] For instance, the pattern of bowel wall enhancement and perfusion quantification may have value for disease activity assessment.[35, 46, 48-51] The only index using CEUS that met our inclusion criteria was developed by Paredes et al.[35] They showed a high accuracy of CEUS for the assessment of postoperative recurrence in 33 patients. We identified one other index using CEUS.[52] However, this study was excluded because a clinical activity index was used as the reference standard. It is to be expected that CEUS will be increasingly used for the development of new indices in the future. However, it is important to note that CEUS parameters are more equipment dependent than classical US parameters. Additionally, results from perfusion quantification can currently not be compared between different ultrasound scanners.[53] It has also been postulated that CEUS could be useful for differentiating between fibrosis and inflammation. However, results from different studies regarding this topic are conflicting.[52, 54-56] Therefore, it remains to be seen if CEUS truly will have additional value for differentiation between disease activity and fibrosis. Finally, CEUS is more expensive and time -consuming than regular US.

We identified one index using real -time elastography for the assessment of disease activity in UC patients.[37] Although the concept seems interesting, many factors in this study may have introduced bias. For instance, endoscopic findings from specific locations were compared with US, but in reality it is difficult to compare precise locations between two modalities. The elastographic patterns also seemed difficult to interpret. This complicates the applicability and reproducibility of the index. Finally, no established endoscopic index was used as a reference standard. Elastography probably has more value for the detection of fibrotic intestinal tissue, as was shown in several studies.[57, 58]

US for grading disease activity in IBD has been reviewed by other groups. Rimola et al. evaluated four US studies in a systematic review on different imaging modalities in CD patients.[23] They reported good accuracy of the different indices, but they did not assess the quality of these studies. Puylaert et al. reviewed several imaging modalities for the grading of disease activity in CD, but they included only two US studies.[11] They concluded that US has low accuracy for disease activity grading in CD, but the number of patients (n = 86) used in

their analysis was relatively low. Panes et al. discussed 12 US studies for grading the disease severity of 1231 patients and concluded that US findings correlate well with endoscopy and histology, but not with clinical activity indices and biomarkers.[19] However, study and index quality were not assessed. Moreover, most studies that were reviewed used clinical and/or biochemical activity as a reference standard. Calabrese et al. recently reviewed a variety of aspects of US in CD, but only briefly elaborated on the use of US for grading CD activity.[22] They stated that the role of US in the evaluation of inflammatory activity remains controversial. Hence, the contradictory conclusions of these reviews exemplify the uncertainty regarding the use of US for disease activity grading in IBD and are probably caused by the heterogeneity of the different US activity indices that have been developed so far.

Our study has some limitations. First, we decided not to perform a meta-analysis. In our opinion, a meta-analysis could not be performed due to the considerable differences between the studies and would probably have resulted in highly biased results. Second, some factors that are important for the development of diagnostic indices (such as implementation of central reading, inter-observer variability, and the conduction of a development and validation study) are not part of the Quadas-2 tool. However, there were no studies that used central reading or inter-observer variability assessment, and only the study performed by Novak et al. used a development and validation phase.

In conclusion, gastrointestinal US seems a promising tool for the assessment of disease activity in IBD patients, but most available activity indices have been developed with suboptimal methodology. New indices should be developed with better methods in future studies. A reliable and standardized US activity index would be useful for facilitating the clinical decision -making process and for assessing and monitoring treatment outcomes in daily practice and in clinical trials.

## References


1. Schnitzler F, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, et al. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. Inflammatory bowel diseases. 2009;15(9):1295-301.

2. Parente F, Molteni M, Marino B, Colli A, Ardizzone S, Greco S, et al. Bowel ultrasound and mucosal healing in ulcerative colitis. Digestive diseases (Basel, Switzerland). 2009;27(3):285-90.

3. Daperno M, D'Haens G, Van Assche G, Baert F, Bulois P, Maunoury V, et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointestinal endoscopy. 2004;60(4):505-12.

4. Travis SP, Schnell D, Krzeski P, Abreu MT, Altman DG, Colombel JF, et al. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Gut. 2012;61(4):535-42.

5. Khanna R, Nelson SA, Feagan BG, D'Haens G, Sandborn WJ, Zou GY, et al. Endoscopic scoring indices for evaluation of disease activity in Crohn's disease. The Cochrane database of systematic reviews. 2016(8):Cd010642.

6. Baron JH, Connell AM, Lennard-Jones JE. Variation between observers in describing mucosal appearances in proctocolitis. Br Med J. 1964;1(5375):89-92.

7. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. The New England journal of medicine. 1987;317(26):1625-9.

8. Mary JY, Modigliani R. Development and validation of an endoscopic index of the severity for Crohn's disease: a prospective multicentre study. Groupe d'Etudes Therapeutiques des Affections Inflammatoires du Tube Digestif (GETAID). Gut. 1989;30(7):983-9.

9. 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. Inflammatory bowel diseases. 2013;19(10):2111-7.

10. Horsthuis K, Bipat S, Bennink RJ, Stoker J. Inflammatory bowel disease diagnosed with US, MR, scintigraphy, and CT: meta-analysis of prospective studies. Radiology. 2008;247(1):64-79.

11. Puylaert CA, Tielbeek JA, Bipat S, Stoker J. Grading of Crohn's disease activity using CT, MRI, US and scintigraphy: a meta-analysis. European radiology. 2015;25(11):3295-313.

12. Nylund K, Odegaard S, Hausken T, Folvik G, Lied GA, Viola I, et al. Sonography of the small intestine. World journal of gastroenterology : WJG. 2009;15(11):1319-30.

13. Maconi G, Parente F, Bollani S, Cesana B, Bianchi Porro G. Abdominal ultrasound in the assessment of extent and activity of Crohn's disease: clinical significance and implication of bowel wall thickening. The American journal of gastroenterology. 1996;91(8):1604-9.

14. Maconi G, Bollani S, Bianchi Porro G. Ultrasonographic detection of intestinal complications in Crohn's disease. Digestive diseases and sciences. 1996;41(8):1643-8.

15. Maconi G, Carsana L, Fociani P, Sampietro GM, Ardizzone S, Cristaldi M, et al. Small bowel stenosis in Crohn's disease: clinical, biochemical and ultrasonographic evaluation of histological features. Alimentary pharmacology & therapeutics. 2003;18(7):749-56.

16. Maconi G, Ardizzone S, Parente F, Bianchi Porro G. Ultrasonography in the evaluation of extension, activity, and follow-up of ulcerative colitis. Scandinavian journal of gastroenterology. 1999;34(11):1103-7.

17. Kucharzik T, Wittig BM, Helwig U, Borner N, Rossler A, Rath S, et al. Use of Intestinal Ultrasound to Monitor Crohn's Disease Activity. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2016.

18. Martinez MJ, Ripolles T, Paredes JM, Blanc E, Marti-Bonmati L. Assessment of the extension and the inflammatory activity in Crohn's disease: comparison of ultrasound and MRI. Abdominal imaging. 2009;34(2):141-8.

19. Panes J, Bouzas R, Chaparro M, Garcia-Sanchez V, Gisbert JP, Martinez de Guerenu B, et al. Systematic review: the use of ultrasonography, computed tomography and magnetic resonance imaging for the diagnosis, assessment of activity and abdominal complications of Crohn's disease. Alimentary pharmacology & therapeutics. 2011;34(2):125-45.

20. Parente F, Molteni M, Marino B, Colli A, Ardizzone S, Greco S, et al. Are colonoscopy and bowel ultrasound useful for assessing response to short-term therapy and predicting disease outcome of moderate-to-severe forms of ulcerative colitis?: a prospective study. The American journal of gastroenterology. 2010;105(5):1150-7.

21. Novak K, Tanyingoh D, Petersen F, Kucharzik T, Panaccione R, Ghosh S, et al. Clinic-based Point of Care Transabdominal Ultrasound for Monitoring Crohn's Disease: Impact on Clinical Decision Making. Journal of Crohn's & colitis. 2015;9(9):795-801.

22. Calabrese E, Maaser C, Zorzi F, Kannengiesser K, Hanauer SB, Bruining DH, et al. Bowel Ultrasonography in the Management of Crohn's Disease. A Review with Recommendations of an International Panel of Experts. Inflammatory bowel diseases. 2016;22(5):1168-83.

23. Rimola J, Ordas I, Rodriguez S, Ricart E, Panes J. Imaging indexes of activity and severity for Crohn's disease: current status and future trends. Abdominal imaging. 2012;37(6):958-66.

24. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS medicine. 2009;6(7):e1000097.

25. Falvey JD, Hoskin T, Meijer B, Ashcroft A, Walmsley R, Day AS, et al. Disease activity assessment in IBD: clinical indices and biomarkers fail to predict endoscopic remission. Inflammatory bowel diseases. 2015;21(4):824-31.

26. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Annals of internal medicine. 2011;155(8):529-36.

27. Novak KL, Kaplan GG, Panaccione R, Afshar EE, Tanyingoh D, Swain M, et al. A Simple Ultrasound Score for the Accurate Detection of Inflammatory Activity in Crohn's Disease. Inflammatory bowel diseases. 2017.

28. Futagami Y, Haruma K, Hata J, Fujimura J, Tani H, Okamoto E, et al. Development and validation of an ultrasonographic activity index of Crohn's disease. European journal of gastroenterology & hepatology. 1999;11(9):1007-12.

29. Neye H, Voderholzer W, Rickes S, Weber J, Wermke W, Lochs H. Evaluation of criteria for the activity of Crohn's disease by power Doppler sonography. Digestive diseases (Basel, Switzerland). 2004;22(1):67-72.

30. Drews BH, Barth TF, Hanle MM, Akinli AS, Mason RA, Muche R, et al. Comparison of sonographically measured bowel wall vascularity, histology, and disease activity in Crohn's disease. European radiology. 2009;19(6):1379-86.

31. Limberg B. [Diagnosis of chronic inflammatory bowel disease by ultrasonography]. Zeitschrift fur Gastroenterologie. 1999;37(6):495-508.

32. Sasaki T, Kunisaki R, Kinoshita H, Yamamoto H, Kimura H, Hanzawa A, et al. Use of color Doppler ultrasonography for evaluating vascularity of small intestinal lesions in Crohn's disease: correlation with endoscopic and surgical macroscopic findings. Scandinavian journal of gastroenterology. 2014;49(3):295-301.

33. Paredes JM, Ripolles T, Cortes X, Reyes MD, Lopez A, Martinez MJ, et al. Non-invasive diagnosis and grading of postsurgical endoscopic recurrence in Crohn's disease: usefulness of abdominal ultrasonography and (99m)Tc-hexamethylpropylene amineoxime-labelled leucocyte scintigraphy. Journal of Crohn's & colitis. 2010;4(5):537-45.

34. Rutgeerts P, Geboes K, Vantrappen G, Beyls J, Kerremans R, Hiele M. Predictability of the postoperative course of Crohn's disease. Gastroenterology. 1990;99(4):956-63.

35. Paredes JM, Ripolles T, Cortes X, Moreno N, Martinez MJ, Bustamante-Balen M, et al. Contrast-enhanced ultrasonography: usefulness in the assessment of postoperative recurrence of Crohn's disease. Journal of Crohn's & colitis. 2013;7(3):192-201.

36. Pascu M, Roznowski AB, Muller HP, Adler A, Wiedenmann B, Dignass AU. Clinical relevance of transabdominal ultrasonography and magnetic resonance imaging in patients with inflammatory bowel disease of the terminal ileum and large bowel. Inflammatory bowel diseases. 2004;10(4):373-82.

37. Ishikawa D, Ando T, Watanabe O, Ishiguro K, Maeda O, Miyake N, et al. Images of colonic real-time tissue sonoelastography correlate with those of colonoscopy and may predict response to therapy in patients with ulcerative colitis. BMC gastroenterology. 2011;11:29.

38. Civitelli F, Di Nardo G, Oliva S, Nuti F, Ferrari F, Dilillo A, et al. Ultrasonography of the colon in pediatric ulcerative colitis: a prospective, blind, comparative study with colonoscopy. The Journal of pediatrics. 2014;165(1):78-84.e2.

39. Leeflang MM, Moons KG, Reitsma JB, Zwinderman AH. Bias in sensitivity and specificity caused by data-driven selection of optimal cutoff values: mechanisms, magnitude, and solutions. Clinical chemistry. 2008;54(4):729-37.

40. Zorzi F, Stasi E, Bevivino G, Scarozza P, Biancone L, Zuzzi S, et al. A sonographic lesion index for Crohn's disease helps monitor changes in transmural bowel damage during therapy. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2014;12(12):2071-7.

41. Calabrese E, Zorzi F, Zuzzi S, Ooka S, Onali S, Petruzziello C, et al. Development of a numerical index quantitating small bowel damage as detected by ultrasonography in Crohn's disease. Journal of Crohn's & colitis. 2012;6(8):852-60.

42. Calabrese E, La Seta F, Buccellato A, Virdone R, Pallotta N, Corazziari E, et al. Crohn's disease: a comparative prospective study of transabdominal ultrasonography, small intestine contrast ultrasonography, and small bowel enema. Inflammatory bowel diseases. 2005;11(2):139-45.

43. Pallotta N, Vincoli G, Montesani C, Chirletti P, Pronio A, Caronna R, et al. Small intestine contrast ultrasonography (SICUS) for the detection of small bowel complications in crohn's disease: a prospective comparative study versus intraoperative findings. Inflammatory bowel diseases. 2012;18(1):74-84.

44. Pallotta N, Tomei E, Viscido A, Calabrese E, Marcheggiano A, Caprilli R, et al. Small intestine contrast ultrasonography: an alternative to radiology in the assessment of small bowel disease. Inflammatory bowel diseases. 2005;11(2):146-53.

45. Saevik F, Nylund K, Hausken T, Odegaard S, Gilja OH. Bowel perfusion measured with dynamic contrast-enhanced ultrasound predicts treatment outcome in patients with Crohn's disease. Inflammatory bowel diseases. 2014;20(11):2029-37.

46. Migaleddu V, Scanu AM, Quaia E, Rocca PC, Dore MP, Scanu D, et al. Contrast-enhanced ultrasonographic evaluation of inflammatory activity in Crohn's disease. Gastroenterology. 2009;137(1):43-52.

47. Quaia E, Cabibbo B, De Paoli L, Toscano W, Poillucci G, Cova MA. The value of time-intensity curves obtained after microbubble contrast agent injection to discriminate responders from non-responders to anti-inflammatory medication among patients with Crohn's disease. European radiology. 2013;23(6):1650-9.

48. Serra C, Menozzi G, Labate AM, Giangregorio F, Gionchetti P, Beltrami M, et al. Ultrasound assessment of vascularization of the thickened terminal ileum wall in Crohn's disease patients using a low-mechanical index real-time scanning technique with a second generation ultrasound contrast agent. Eur J Radiol. 2007;62(1):114-21.

49. Ripolles T, Rausell N, Paredes JM, Grau E, Martinez MJ, Vizuete J. Effectiveness of contrast-enhanced ultrasound for characterisation of intestinal inflammation in Crohn's disease: a comparison with surgical histopathology analysis. Journal of Crohn's & colitis. 2013;7(2):120-8.

50. Ripolles T, Martinez MJ, Paredes JM, Blanc E, Flors L, Delgado F. Crohn disease: correlation of findings at contrast-enhanced US with severity at endoscopy. Radiology. 2009;253(1):241-8.

51. De Franco A, Di Veronica A, Armuzzi A, Roberto I, Marzo M, De Pascalis B, et al. Ileal Crohn disease: mural microvascularity quantified with contrast-enhanced US correlates with disease activity. Radiology. 2012;262(2):680-8.

52. Schirin-Sokhan R, Winograd R, Tischendorf S, Wasmuth HE, Streetz K, Tacke F, et al. Assessment of inflammatory and fibrotic stenoses in patients with Crohn's disease using contrast-enhanced ultrasound and computerized algorithm: a pilot study. Digestion. 2011;83(4):263-8.

53. Zink F, Kratzer W, Schmidt S, Oeztuerk S, Mason RA, Porzner M, et al. Comparison of Two High-End Ultrasound Systems for Contrast-Enhanced Ultrasound Quantification of Mural Microvascularity in Crohn's Disease. Ultraschall in der Medizin (Stuttgart, Germany : 1980). 2016;37(1):74-81.

54. Quaia E, Gennari AG, van Beek EJR. Differentiation of Inflammatory from Fibrotic Ileal Strictures among Patients with Crohn's Disease through Analysis of Time-Intensity Curves Obtained after Microbubble Contrast Agent Injection. Ultrasound in medicine & biology. 2017;43(6):1171-8.

55. Nylund K, Jirik R, Mezl M, Leh S, Hausken T, Pfeffer F, et al. Quantitative contrast-enhanced ultrasound comparison between inflammatory and fibrotic lesions in patients with Crohn's disease. Ultrasound in medicine & biology. 2013;39(7):1197-206.

56. Wilkens R, Hagemann-Madsen RH, Peters DA, Nielsen AH, Norager CB, Glerup H, et al. Validity of Contrast-enhanced Ultrasonography and Dynamic Contrast-enhanced MR Enterography in the Assessment of Transmural Activity and Fibrosis in Crohn's Disease. Journal of Crohn's & colitis. 2018;12(1):48-56.

57. Baumgart DC, Muller HP, Grittner U, Metzke D, Fischer A, Guckelberger O, et al. US-based Real-time Elastography for the Detection of Fibrotic Gut Tissue in Patients with Stricturing Crohn Disease. Radiology. 2015;275(3):889-99.

58. Giannetti A, Biscontri M, Matergi M, Stumpo M, Minacci C. Feasibility of CEUS and strain elastography in one case of ileum Crohn stricture and literature review. Journal of ultrasound. 2016;19(3):231-7.

## Supplementary material

**Supplementary table 1.** *Excluded studies describing an US index.*

| Study/Index | Modality | Reference standard | Participants | Exclusion reason |
|---|---|---|---|---|
| Hata 1994 | Grayscale | Surgical specimens | 17 | Not enough patients |
| Spalinger 2000 | Grayscale/CDI | CDAI | 92 | Clinical disease activity as reference |
| Schirin-Sokhan 2011 | Grayscale/CDI/CEUS | ICC | 18 | Not enough patients |
| Calabrese 2012 | SICUS/Grayscale/CDI | CDAI | 110 | Clinical disease activity as reference |
| Sasaki 2014 | Grayscale/CDI | Surgical specimens | 10 | Not enough patients |
| Zorzi 2014 | SICUS/Grayscale/CDI | CDAI | 29 | Clinical disease activity as reference |
| Baumgart 2015 | Grayscale/CDI/RTE | Histology | 10 | Not enough patients |
| Fufezan 2015 | RTE | ICC/MRI | 14 | Not enough patients |
| Medellin-Kowalewski 2016 | Grayscale/CDI/CEUS | HBI | 127 | Clinical disease activity as reference |

*ICC = Ileocolonoscopy; CDAI = Crohn's disease activity index; HBI = Harvey Bradshaw index.*

**Supplementary table 2.** *Index statistics. Confidence intervals of sensitivity, specificity, PPV, NPV and accuracy are not shown.*

| Study (CD) | Correlation with reference | Sensitivity | Specificity | PPV | NPV | accuracy |
|---|---|---|---|---|---|---|
| Futagami 1999 | | | | | | |
| | $r^2 = 0.62$; P <0.01 | - | - | - | - | - |
| | | | | | | |
| Neye 2004 | | - | - | - | - | - |
| Sigmoid | K = 0.81 (95% CI 0.63–0.99) | - | - | - | - | - |
| Descendens | K = 0.78 (95% CI 0.56–0.99) | - | - | - | - | - |
| Ascending | K = 0.84 (95% CI 0.70–0.97) | - | - | - | - | - |
| Transverse | K = 0.75 (95% CI 0.56–0.94) | - | - | - | - | - |
| Cecum/TI | K = 0.82 (95% CI 0.71–0.93) | - | - | - | - | - |
| | | | | | | |
| Pascu 2004 | | | | | | |
| All segments | r = 0.830; P < 0.001 | 74% | 97% | 96% | 79% | 85% |
| TI | r = 0.970; P < 0.001 | 96% | 100% | 100% | 92% | 97% |
| Cecum | r = 0.844; P < 0.001 | 74% | 95% | 93% | 78% | 84% |
| Ascending | r = 0.869; P < 0.001 | 69% | 100% | 100% | 81% | 87% |
| Right transverse | r = 0.784; P < 0.001 | 60% | 100% | 100% | 79% | 84% |
| Left transverse | r = 0.741; P < 0.001 | 59% | 100% | 100% | 75% | 82% |
| Descending | r = 0.588; P < 0.001 | 61% | 90% | 85% | 72% | 76% |
| Sigmoid | r = 0.791; P < 0.001 | 82% | 94% | 95% | 80% | 87% |
| | | | | | | |
| Drews 2009 | | | | | | |
| Grade 0-1 vs 2-4 | K = 0.4375; p = unknown | 68% | 77% | 63% | 81% | - |
| Grade 0 vs 1-4 | K = 0.66; p = unknown | 95% | 69% | 90% | 82% | - |

**Supplementary table 2.** *(Continued).*

| | | | | | | |
|---|---|---|---|---|---|---|
| Paredes 2010 | | | | | | |
| recurrence | K = 0.29; p = unknown | 76.9% | 57.1% | 87.0% | 40% | 72.7% |
| moderate-severe recurrence | K = 0.40; p=0.009 | 86.7% | 66.7% | 68.4% | 85.7% | 75.8% |
| Paredes 2013 | | | | | | |
| recurrence | K=0.95; p: 0.0001 | 98.0% | 81.8% | 96.0% | 90.0% | 98.3% |
| moderate-severe recurrence | K=0.69; p: 0.0001 | 95.1% | 73.1% | 82.1% | 90.5% | 85.0% |
| | | | | | | |
| Sasaki 2014 | | | | | | |
| | Þ = 0.709 (p < 0.001) | 39.1% | 100% | - | - | 61.1% |
| Novak 2017 development | | | | | | |
| All segments | AUROC = 0.866 | 93.3% | 76.8% | 88.2% | 86.3% | 87.5% |
| Colon | - | 94.6% | 81.3% | 94.6% | 81.3% | 91.7% |
| Ileum | - | 91.7% | 75% | 81.5% | 88.2% | 84.1% |
| Novak 2017 validation | | | | | | |
| All segments | AUROC = 0.836 | 92.1% | 81.6% | 79.6% | 93.2% | 86.2% |
| Colon | - | 80% | 90% | 88.9% | 81.8% | 85% |
| Ileum | - | 96.4% | 78.9% | 77.1% | 96.8% | 86.4% |

**Supplementary table 2.** *(Continued).*

| Study (UC) | Correlation with reference | Sensitivity | Specificity | PPV | NPV | accuracy |
|---|---|---|---|---|---|---|
| Pascu 2004 (UC) | | | | | | |
| All segments | r = 0.974; P < 0.001 | 95% | 96% | 96% | 95% | 95% |
| TI | r = 0.790; P < 0.001 | 80% | 94% | 80% | 94% | 61% |
| Cecum | r = 0.993; P < 0.001 | 100% | 100% | 100% | 100% | 100% |
| Ascending | r = 0.99; P < 0.0013 | 100% | 100% | 100% | 100% | 100% |
| Right transverse | r = 0.935; P < 0.001 | 100% | 85% | 83% | 100% | 91% |
| Left transverse | r = 0.812; P < 0.001 | 93% | 100% | 100% | 90% | 96% |
| Descending | r = 0.939; P < 0.001 | 89% | 100% | 100% | 67% | 91% |
| Sigmoid | r = 0.891; P < 0.001 | 100% | 100% | 100% | 100% | 100% |
| | | | | | | |
| Parente 2009/2010 | | | | | | |
| | κ 0.59, 95 % CI: 0.40 – 0.78 | - | - | - | - | - |
| Civitelli 2014 (UC) | | | | | | |
| All segments | r = 0.90 (P < .0001)<br>K = 0.94 (95% CI 0.88-1) for classifying activity | 100% | 93% | - | - | - |
| Right colon | | 75% | 100% | 100% | 83% | - |
| Transverse | | 86% | 100% | 100% | 85% | - |
| Left colon | | 96% | 100% | 100% | 80% | - |
| | | | | | | |
| Ishikawa 2011 | $Chi^2$ p < 0.001 | | | | | |

*TI = terminal ileum; PPV = positive predictive value; NPV = negative predictive value; AUROC = area under receiver operating characteristic curve.*

**Search strategy**

PubMed

("Inflammatory Bowel Diseases"[Mesh] OR inflammatory bowel disease*[tiab] OR crohn disease*[tiab] OR crohn* disease*[tiab] OR ulcerative colitis[tiab] OR gastrointestinal wall*[tiab]) AND ("Ultrasonography" [Mesh] OR ultrasound[tiab] OR sonograph*[tiab] OR ultrasonic[tiab] OR ultrasonograph*[tiab] OR echo*[tiab]) AND ("Gastrointestinal Tract"[Mesh] OR abdom*[tiab] OR transabdom*[tiab]) AND ("Sensitivity and Specificity"[MeSH Terms] OR "Reproducibility of Results"[MeSH Terms] OR "Longitudinal Studies"[MeSH Terms] OR "Follow-Up Studies"[MeSH Terms] OR "Clinical Trial"[pt] OR "Comparative Study"[pt] OR "Prospective Studies"[MeSH Terms] OR "Evaluation Studies"[pt] OR "Retrospective Studies"[MeSH Terms] OR "Reference Values"[MeSH Terms] OR "diagnosis" [Subheading] OR diagnos*[tiab] OR sensitiv*[tiab] OR specific*[tiab] OR accura*[tiab])

EMBASE (OVID)

(*inflammatory bowel disease/ or *ulcerative colitis/ or *Crohn disease/ or (inflammatory bowel disease* or crohn disease* or crohn* disease* or ulcerative colitis or gastrointestinal wall*).ti,ab.) AND (exp echography/ or (ultrasound or sonograph* or ultrasonic or ultrasonograph* or echo*).ti,ab.) AND (gastrointestinal tract/ or intestine wall/ or intestine/ or intestine fistula/ or blood vessel wall/ or intestine blood flow/ or gastrointestinal symptom/ or (abdom* or transabdom*).ti,ab.) AND ("sensitivity and specificity"/ or comparative study/ or controlled study/ or prospective study/ or reproducibility/ or longitudinal study/ or follow up/ or clinical trial/ or evaluation study/ or retrospective study/ or major clinical study/ or reference value/ or di.fs. or (diagnos* OR sensitiv* OR specific* OR accura*).ti,ab.)

Cochrane Central Register of Controlled Trials (CENTRAL)

#1 MeSH descriptor: [Inflammatory Bowel Diseases] explode all trees

#2 inflammatory bowel disease* or crohn disease* or crohn* disease* or ulcerative colitis or gastrointestinal wall*:ti,ab,kw (Word variations have been searched)

#3 #1 or #2

#4 MeSH descriptor: [Ultrasonography] explode all trees

#5 ultrasound or sonograph* or ultrasonic or ultrasonograph* or echo*:ti,ab,kw (Word variations have been searched)

#6 #4 or #5

#7 MeSH descriptor: [Gastrointestinal Tract] explode all trees

#8 abdom* or transabdom*:ti,ab,kw (Word variations have been searched)

#9 #7 or #8

#10 #3 and #6 and #9 in Trials

# Chapter 3

## Intestinal Ultrasound to Assess Disease Activity in Ulcerative Colitis: Development of a novel UC-Ultrasound Index

S. Bots, K. Nylund, M. Löwenberg, K. Gecse, G. D'Haens

*Journal of Crohn's and Colitis 2021;15(8):1264-1271*

## Abstract

### Introduction

Intestinal ultrasound (IUS) is useful to assess inflammation in ulcerative colitis (UC) patients. We aimed to develop an ultrasonographic activity index using endoscopy as reference standard.

### Methods

Patients were included consecutively. IUS was performed within 3 weeks from endoscopy. IUS parameters and endoscopy were compared for each colonic segment (except the rectum). The best parameters were used to construct a UC-IUS index, which was correlated with endoscopic disease activity using the Spearman's rank test.

### Results

In 60 patients, 207 colonic segments were evaluated endoscopically. BWT >2.1 mm was optimal to discriminate between Mayo 0 and Mayo 1-3 (sensitivity 82.6%; specificity 93.0%; AUC 0.910), a cut-off of 3.2 mm was optimal to discriminate between Mayo 0-1 and Mayo 2-3 (sensitivity 89.1%; specificity 92.3%; AUC 0.946) and BWT >3.9 mm was optimal for detection of Mayo 3 (sensitivity 80.6%; specificity 84.1%; AUC 0.909). Presence of CDS predicted active disease, stretches of CDS were associated with Mayo 2-3, lack of haustrations predicted active disease and fat wrapping was associated with severe disease. Inter- and intra-rater ICC for BWT was substantial. Inter-rater agreement for CDS was substantial and ranged from slight to substantial for haustrations. Intra-rater agreement for CDS was substantial and ranged from moderate to almost perfect for haustrations. The index showed strong correlation with endoscopic disease activity (Mayo: $\varrho$0.830; $p<0.001$, UCEIS: $\varrho$ 0.759; $p<0.001$).

### Conclusion

We developed an UC-IUS index which showed strong correlation with endoscopic disease activity using internal validation. It is currently being validated in prospective studies.

## Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by relapsing and remitting episodes of inflammation usually limited to the mucosal layer of the colon. Treatment targets for UC patients nowadays include patient-reported as well as endoscopic remission. Recently, endoscopy is increasingly being used to guide treatment, since evidence suggests that mucosal healing (e.g. Mayo 0-1 activity) is associated with improved long-term outcomes.[1, 2] However, it is challenging to repeatedly perform colonoscopies to assess mucosal disease activity due to the high cost and burden for the patient.[3] Hence, alternative and reliable non-invasive methods to assess disease activity are needed.

Blood tests such as the measurement of serum C-reactive protein, albumin and platelet counts have been evaluated, but these tests are not sufficiently sensitive or specific to reflect disease activity.[4-7] Repeated measurement of faecal calprotectin (FCP) has been shown to accurately reflect the presence of disease activity.[4, 8] However, disease location, extent and severity cannot be adequately assessed with this technique. Intestinal ultrasound (IUS) is a rapid, efficient, non-invasive and relatively cheap imaging technique, which can also be performed in a point-of-care settings. IUS has been reported to be accurate in diagnosis of UC and can also be applied to determine the extent, severity and location of inflammation.[9-12]

Therefore, IUS is an attractive tool for the assessment of disease activity in UC patients. So far, few studies have been performed to compare IUS with endoscopy.[11, 13-16] Additionally, studies evaluating responsiveness of IUS to a medication with known efficacy, validation to endoscopy and evaluating reliability are scarce.[11] In a previous systematic review, we showed that, although several IUS indices have been developed for the assessment of disease activity in UC patients, the methodology was suboptimal in most studies.[9]

Therefore, we aimed to develop an ultrasound activity index for the assessment of disease activity in patients with UC, using endoscopy as the reference standard.

## Methods

*Patient population*

Adult UC patients undergoing endoscopy for evaluation of disease activity or surveillance were eligible for inclusion. Patient were consecutively included based on clinical mayo score and SCCAI (i.e. 20 quiescent disease, 40 active disease). Patients underwent endoscopy and IUS along with FCP and serum CRP measurement within the shortest period possible with a maximum window of 3 weeks. If there was a change in treatment or symptoms between IUS and endoscopy, patients were excluded. IUS and endoscopy were not performed on the same day. At the time of endoscopy, the performing endoscopist was unaware of the IUS results and vice versa. Endoscopists and ultrasonographists were not blinded for clinical symptoms as would also be the case in a real-life clinical setting.

*Ultrasound examinations*

All the IUS examinations were performed by one of two investigators experienced in IUS (SB 3 years and KN 9 years of experience), with a Philips Epiq 5 ultrasound device using the C5-1 convex transducer and L12-5 linear transducer. Frequency, focus and gain settings were optimised to get the best images. The examination was performed after at least 4 hours of fasting with the patient in supine position. The large intestine was scanned beginning at the terminal ileum and further following its course to the rectum. The 9 regions of the abdomen were also systematically scanned for the detection of enlarged lymph nodes and other possible pathology. Each colon segment scanned in B-mode was also examined with colour Doppler. The colour Doppler measurements were performed with standardised pre-sets with optimized wall filter, pulse repetition frequency , frequency, and velocity scale for registration of the slow flow in the GI wall. Cine loops were video-recorded of each segment in longitudinal sections in B-mode and colour Doppler mode.

*Ultrasound parameters and measurements*

The following IUS parameters were recorded during the procedure: bowel wall thickness (BWT), Colour Doppler Signal (CDS), image quality, normal or abnormal colonic haustrations, presence of fat wrapping (hyperechoic fat around the bowel), and presence of enlarged lymph nodes (short axis > 5mm). BWT was measured from, but not including, the central hyperechoic line of the lumen to the end of the outer hypoechoic margin of the wall (representing the muscularis propria). All BWT measurements were performed in duplicate on longitudinal sections since it is easiest to notice the thickest wall section in longitudinal direction. CDS was divided in 3 categories: absent/single vessel (categorized as absent), spots or stretches of CDS. Image quality was categorized as good, average, low or uninterpretable. Assessment of image quality was based on the opinion of the ultrasonographer, since there is no validated index for this purpose. Normal colonic haustrations were defined as clearly visible haustrations or collapsed colonic folds with BWT <2 mm. Abnormal colonic haustrations were defined as a

clearly disrupted or tube-like appearance. All measurements and image interpretations were performed by two observers (SB and KN) on the same cine loops to assess inter-rater variability. Additionally, 20 cases (5 for each severity category) were randomly selected, re-anonymized and scrambled for a second interpretation by both observers to assess intra-rater variability. The time between first and second read was at least 3 months.

*Endoscopy*

Colonoscopy or sigmoidoscopy were performed according to standard procedures at our clinic by IBD experts. Endoscopic disease activity was scored using the ulcerative colitis endoscopic index of severity (UCEIS) and the Mayo endoscopic sub-score for each segment.[17, 18] A Mayo endoscopic subscore of 1, 2 or 3 was considered as mild, moderate or severe disease, respectively. A UCEIS score of 4-5, 6-8 and 9-11 was considered as mild, moderate or severe disease, respectively.

*Biomarkers*

Blood samples were collected and analysed for C-reactive protein (mg/L) and stool samples were and analysed for FCP (μg/g) (Bühlmann fCal® ELISA). The upper limit of detection of the FCP test was 1800 μg/g. Samples were collected within 3 weeks before or after IUS as long as there was no change in treatment or clinical symptoms.

*Clinical assessments*

Medical history was assessed and information on the duration of ulcerative colitis, medical treatment, age, gender, weight, height and BMI were collected. At the IUS visit, symptom severity was scored using the simple clinical colitis activity index (SCCAI) and Mayo score.[17, 19]

*Sample size calculation*

The sample size calculation was based on mean BWT in 2 patient groups, representing UC patients in remission (group 1) and UC patients with active endoscopic disease (group 2). Based on literature data we assumed a colon wall thickness in healthy controls of a mean 1.1 mm (SD 0.3) and the cut-off of between normal and abnormal thickness of 2.0 mm.[9, 20] The colon wall in a heterogeneous group of UC patients with active disease was assumed to have a mean thickness of 4.5 mm (SD1.3).[9, 11, 15] For the sample size calculation this resulted in a sample size of 20 in each group, which would offer 80% power to detect a difference in means of 0.9mm assuming that the Group 1 standard deviation is 0.3 and the Group 2 standard deviation is 1.3 using a two group Satterthwaite t-test with a 0.05 two-sided significance level. Since we intended to study patients in multiple categories of disease activity we intended to include 20 patients with quiescent disease and 40 with active disease.

*Statistical analysis*

Descriptive statistics were used to characterize the population. BWT, CDS, fat wrapping, WLS, haustration pattern and enlarged lymph nodes were compared with endoscopic findings for each segment except for the rectum. Normally distributed parameters were compared with unpaired T-tests. Categorical parameters were compared with logistic regression. ROC analysis was performed for BWT to determine optimal cut-offs. The most predictive parameters and cut-off values were used to construct a point-based UC-US index. The results obtained from the person actually performing IUS were used for this purpose. The index was calculated for each patient and compared with the Mayo score and UCEIS score for each segment using the Spearman's rank correlation test. A value of 0.00–0.10 was considered as negligible correlation, 0.10–0.39 as weak correlation, 0.40–0.69 as moderate correlation, 0.70–0.89 as strong correlation and 0.90–1.00 as very strong correlation.[21] Inter-rater and intra-rater agreement for categorical data was tested with Cohen's kappa statistics. A value of 0.0-0.20 was considered as slight agreement, 0.21-0.4 as fair agreement, 0.41-0.60 as moderate agreement, 0.61-0.80 as substantial agreement and 0.81-1.0 as almost perfectagreement.[22, 23] Inter-rater and intra-rater agreement for continuous BWT measurements was tested using intra class correlation (ICC) statistics for average measurements. An ICC value of less than 0.50 was considered as poor agreement, a value of 0.50-0.75 as moderate agreement, a value of 0.75-0.90 as substantial agreement and a value of 0.90-1.00 as almost perfect agreement.[24] A p-value of $<0.05$ was considered to be statistically significant. All statistical analyses were performed using SPSS 25.0 software (IBM Corporation, Armonk, NY, USA).

*Ethical approval and patient consent*

This study was approved by the ethical committee of the Academic University Medical Center Amsterdam. All patients provided written informed consent prior to participation in this study.

## Results

*Patient population*

A total of 60 UC patients were included. Patient characteristics are shown in table 1. Sixteen patients were in complete endoscopic remission (Mayo 0) and 44 patients had active endoscopic disease (13 Mayo 1, 15 Mayo 2 and 18 Mayo 3). Six patients had active proctitis only. In total, 207 colonic segments were explored at endoscopy (60, 58, 49 and 40 in sigmoid, descending, transverse and ascending colon, respectively). IUS was performed within a median of 7 days (IQR 5-11 days) from endoscopy, without change in treatment or symptoms in between. FCP samples were collected within a median of 2 days (IQR 0-4 days) from IUS.

**Table 1** *Patient characteristics.*

| **Characteristic** | n=60 |
|---|---|
| Male gender | 28 (47%) |
| Age (median, IQR) | 44 (30-54) |
| Height cm (mean, SD) | 176.4 (10.0) |
| BMI (mean, SD) | 24.1 (3.2) |
| Medication use | |
| - 5-ASA | 36 (60%) |
| - Corticosteroids (oral/topical) | 25 (42%) |
| - Thiopurines | 7 (12%) |
| - Anti-TNF | 8 (13%) |
| - Vedolizumab | 1 (2%) |
| - Tofacitinib | 3 (5%) |
| - Tacrolimus (topical) | 1 (2%) |
| Endoscopy results | |
| - Mayo 0 | 16 (27%) |
| - Mayo 1 | 11 (18%) |
| - Mayo 2 | 15 (25%) |
| - Mayo 3 | 18 (30%) |
| - UCEIS <4 | 15 (25%) |
| - UCEIS 4-5 | 13 (22%) |
| - UCEIS 6-8 | 15 (25%) |
| - UCEIS 9-11 | 17 (28%) |
| Segments endoscopically explored | |
| - Rectum | 60 (100%) |
| - Sigmoid | 60 (100%) |
| - Descending | 58 (97%) |
| - Transverse | 49 (82%) |
| - Ascending | 40 (67%) |
| - Total segments explored (excl. rectum) | 207 |
| - Proctitis only | 6 (10%) |

*Ultrasound*

*Image quality*

Image quality for different colonic segments is shown in table 2. Image quality in the rectum was average or higher in only 48.3% of patients. In 38.3% of patients image quality was low and in 13.3% the images were considered uninterpretable. Image quality was considered average or higher in 98.3% in the sigmoid and descending colon and 96.7% in the transverse and ascending colon.

**Table 2.** *US image quality per segment*

| | Good | Average | Low | Uninterpretable |
|---|---|---|---|---|
| **Rectum** | 12 (20.0%) | 17 (28.3%) | 23 (38.3%) | 8 (13.3%) |
| **Sigmoid** | 49 (81.7%) | 10 (16.7%) | 0 (0%) | 1 (1.7%) |
| **Descending** | 48 (80.0%) | 11 (18.3%) | 0 (0%) | 1 (1.7%) |
| **Transverse** | 45 (75.0%) | 13 (21.7%) | 2 (3.3%) | 0 (0%) |
| **Ascending** | 43 (71.7%) | 15 (25.0%) | 2 (3.3%) | 0 (0%) |

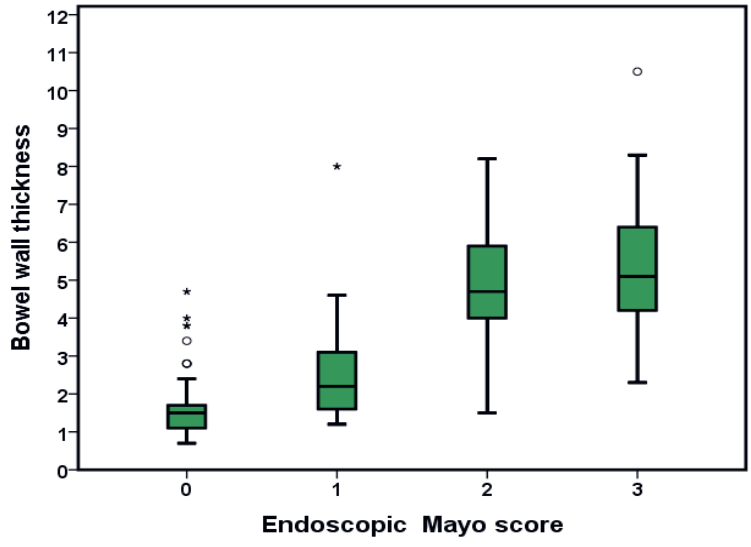


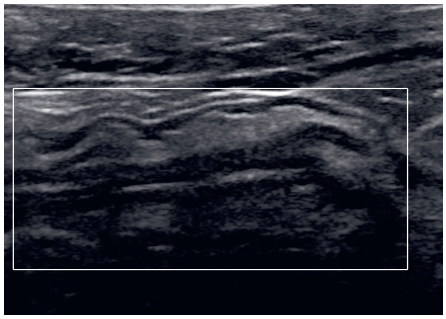

**Figure 1.** *Mean bowel wall thickness for different Mayo scores*

*Bowel wall thickness*

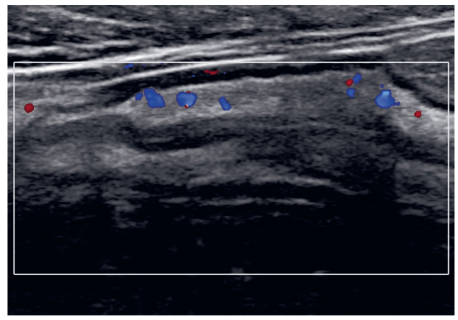
Mean BWT was statistically different between Mayo 0 and Mayo 1 endoscopic activity (p<0.001) and between Mayo 1 and Mayo 2 endoscopic activity (p<0.001), but not between Mayo 2 and Mayo 3 (p=0.548) (figure 1). A BWT cut-off of 2.1mm was best to discriminate between inactive and active endoscopic disease activity (Mayo 0 vs Mayo 1-3) (sensitivity 82.6%; specificity 93.0%; AUC 0.910). A BWT cut-off of 3.2mm, was best to discriminate between Mayo 0-1 and Mayo 2-3 endoscopic disease activity (sensitivity 89.1%; specificity 92.3%; AUC 0.946). A BWT cut-off of 3.9mm was best to discriminate between Mayo 0-2 and Mayo 3 endoscopic disease activity (sensitivity 80.6%; specificity 84.1%; AUC 0.909). ROC curves are shown in Figure 3. For individual segments, a BWT cut-off of 2.1mm was best to discriminate between Mayo 0 vs Mayo 1-3 in the sigmoid (sensitivity 88.6%; specificity 88.0%; AUC 0.913), 2.5mm in the descending (sensitivity 85.2%; specificity 87.1%; AUC 0.907), 1.75mm in the transverse (sensitivity 88.9%; specificity 90.3%; AUC 0.944) and 2.6mm in the ascending colon (sensitivity 75%%; specificity 100%%; AUC 0.903). The mean difference in BWT in all segments for the 2 observers was 0.4mm (SD 0.9; p<0.001). The inter-rater agreement for continuous BWT measurements was almost perfect (ICC 0.917; 95%CI 0.853-0.948; p< 0.001). The intra-rater agreement for continuous BWT measurements was substantial (ICC 0.802; 95%CI 0.729-0.855; p<0.001). Based on the ROC cut-off points, the following categories for BWT were made: < 2 mm, 2.0-2.9 mm, 3.0–3.9 mm and ≥ 4 mm. Sensitivity and specificity values were 82.6% and 90% for 2mm, 89.1% and 90.9% for 3mm and 77.4% and 85.0%% for 4mm. Inter-rater agreement for these categories was moderate for the sigmoid (κ 0.53; p<0.001), descending (κ 0.58; p<0.001), transverse (κ 0.55 ; p<0.001) and ascending colon (κ 0.43; p<0.001). Intra-rater agreement was substantialfor the sigmoid (κ 0.68; p<0.001), transverse (κ 0.63; p<0.001) and ascending (κ 0.68; p<0.001) colon and moderate for the descending colon (κ 0.59; p<0.001). The rectum was excluded from this analysis.

*Colour Doppler signal*

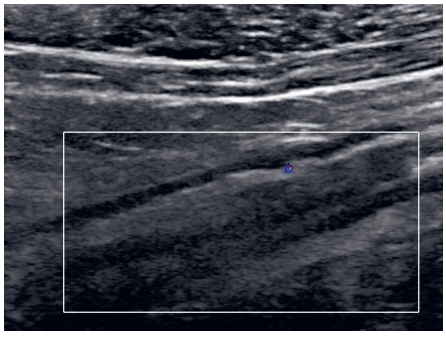
Examples of different CDS categories are shown in figure 2. Presence of any CDS was associated with presence of endoscopic disease activity (OR 14.0; 95% CI 6.8-28.7; p<0.001). Presence of any CDS was also associated with moderate to severe endoscopic activity as compared to mild or quiescent endoscopic activity (OR 14.9; 95% CI 7.3-30.4; p < 0.001) and stretches of CDS was more strongly associated with moderate-severe endoscopic activity (OR 22.3; 95% CI 7.3-67.8; p<0.001). Presence of stretches of CDS did also discriminate between moderate and severe endoscopic activity (OR 7.2; 95% CI 3.0-17.4; p<0.001). Inter-rater agreement was substantialfor the sigmoid (κ 0.79; p<0.001), descending (κ 0.78; p<0.001), transverse (κ 0.75; p<0.001) and ascending (κ 0.60; p<0.001) colon. Intra-rater agreement was substantial for the sigmoid (κ 0.78; p<0.001), descending (κ 0.69; p<0.001), transverse (κ 0.60; p<0.001) and ascending (κ 0.65; p<0.001) colon.



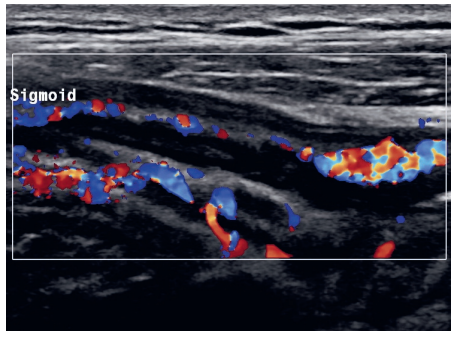
*No CDS*



*Spots of CDS*



*Single vessel (categorized as absent)*




*Stretches of CDS*

**Figure 2.** *Categories of CDS*

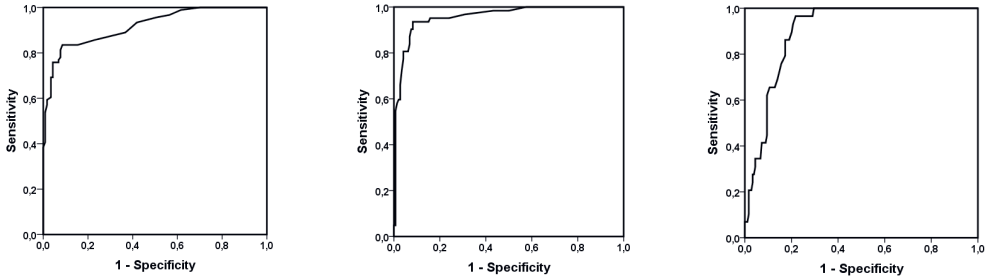
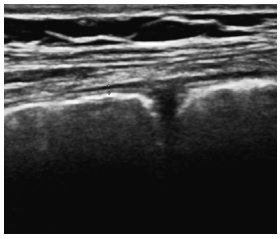


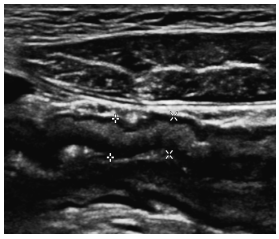

***Figure 3. A.*** *ROC curve for BWT in Mayo 0 vs Mayo 1-3 segments.* ***B.*** *ROC curve for BWT in Mayo 0-1 vs Mayo 2-3 segments.* ***C.*** *ROC curve for BWT in Mayo 0-2 vs Mayo 3 segments.*

*Haustrations*

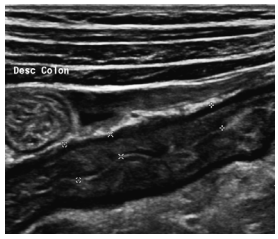
Examples of normal and abnormal haustrations are shown in figure 4. An abnormal haustration pattern was strongly associated with active endoscopic disease (OR 126.2; 95% CI 36.3-438.7; p<0.001). It was also associated, albeit to a lesser extent, with moderate-severe endoscopic disease (OR 100.7; 95% 35.0-290.1; p<0.001). Inter-rater agreement was substantial for the sigmoid (κ 0.69; p<0.001) and descending colon (κ 0.61; p<0.001), fair for the transverse colon (κ 0.36; p=0.004) and slight for the ascending colon (κ 0.17; p<0.001). Intra-rater agreement was substantial for the sigmoid colon (κ 0.65; p<0.001), moderate for the descending (κ 0.59; p<0.001) and transverse (κ 0.52; p<0.001) colon and substantial for the ascending colon (κ 0.80; p<0.001).



*Normal haustration pattern*



*Partially disrupted haustration pattern (categorized as abnormal)*




*Completely disrupted haustration pattern (categorized as abnormal)*

**Figure 4.** *Haustration pattern*

*Fat wrapping*

Fat wrapping was observed in 14/60 (23.3%) patients. Presence of fat wrapping was strongly associated with severe endoscopic disease (OR 34; 95% CI 6.0-191.8; p <0.001). Inter and intra-rater agreement for fat wrapping was not assessed since this could not be properly assessed using the available cine-loops.

*Wall layer stratification*

WLS was classified as normal in 55/60 (92%) patients in the sigmoid colon and the descending colon and in 56/60 (93%) patients in the transverse and the ascending colon. Since WLS was normal in most cases, association between endoscopic disease activity was not assessed.

*Lymph nodes*

Presence of enlarged lymph nodes was observed in only 3/60 (5%) patients. Association between presence of lymph nodes and disease activity could therefore not be assessed.

*Biomarkers*

In the patients without endoscopic activity the median FCP level was 48 μg/g (IQR 33-180) and the median CRP level was 1.7 mg/L (IQR 0.6-3.0). In the patients with endoscopic activity the median FCP level was 878 μg/g (IQR 274-1800) and the median CRP level was 3.5 mg/L (IQR 1.6-10.4). An FCP cut-off of 212 μg/g (sensitivity 81.8%; specificity 81.2%; AUC 0.870) most accurately predicted endoscopic disease activity (Mayo 0 vs Mayo 1-3). An FCP cut-off of 391 μg/g (sensitivity 81.8%; specificity 81.5%; AUC 0.878) most accurately predicted moderate to severe endoscopic disease activity (Mayo 0-1 vs Mayo 2-3). An FCP cut-off of 878 most accurately predicted severe endoscopic disease activity (sensitivity 83.3%; specificity 78.6%; AUC 0.867).

*Combination of CDS and BWT for detection of disease activity*

Sensitivity and specificity for detection of disease activity was tested for two combinations of CDS and BWT cut-offs. A combination of BWT > 2mm or presence of DS resulted in a sensitivity of 88% and a specificity of 84.3% for detection of disease activity in any colon segment but the rectum. A combination of BWT > 3mm or presence of CDS with BWT < 3mm resulted in a sensitivity of 81.5% and specificity of 87.8% for detection of disease activity in any colon segment. A combination of BWT > 2mm and CDS resulted in a sensitivity of 58.7% and specificity of 96.5% and a combination of BWT > 3mm and presence of CDS resulted in a sensitivity of 54.3% and specificity of 97.4%.

*Combination of BWT and FCP for detection of disease activity*

Sensitivity and specificity for detection of disease activity was tested for a combination of BWT and FCP cut-offs in 54 patients (table 3). Patients with proctitis only were excluded from this analysis. A combination of BWT >2mm or FCP > 200 μg/g resulted in a sensitivity of 94.9% and specificity of 66.7%% for detection of active disease (i.e. >Mayo 0)

**Table 3.** *Sensitivity and specificity for different combinations of BWT and FCP cut-offs*

| Combination | Sensitivity | Specificity |
|---|---|---|
| BWT > 2mm or FCP > 200 μg/g | 94.9% | 66.7% |
| BWT > 2mm and FCP > 100 μg/g | 86.7% | 87.2% |
| BWT > 2mm and FCP > 200 μg/g | 76.9% | 93.3% |
| BWT > 2mm and FCP > 300 μg/g | 71.8% | 93.3% |
| BWT > 2mm and FCP > 400 μg/g | 69.2% | 93.3% |

*UC-IUS index*

Based on the most predictive cut-offs and categories that were identified in the analysis, a point-based index was constructed. The index is shown in table 3. The score was calculated and compared per colon segment, excluding the rectum. Subsequently, the final scores were analysed for correlation with the UCEIS and endoscopic Mayo score. The IUS index showed strong correlation with the endoscopic mayo score ($\varrho = 0.830$; $p<0.001$). The index also showed strong correlation with the UCEIS index ($\varrho = 0.759$; $p<0.001$). The final IUS score was also calculated for the second observer and compared with the other IUS score. The mean difference between observers for the final IUS score was 0.28 (SD 1.1; p=0.08) The IUS score showed strong correlation between observers ($\varrho = 0.877$; $p<0.001$).

**Table 4.** *UC-IUS index*

| **UC-IUS score** | |
|---|---|
| **Parameters** | Points (0-7) |
| **Bowel wall thickness** | |
| - > 2mm | 1 |
| - > 3mm | 2 |
| - > 4mm | 3 |
| **Doppler signal** | |
| - Spots | 1 |
| - Stretches | 2 |
| **Abnormal haustrations** | 1 |
| **Fat wrapping** | 1 |

## Discussion

In this study, we developed a new IUS index for the grading of disease activity in UC patients. Endoscopy was used as the reference standard. It showed strong correlation with endoscopic disease activity through internal validation in this cohort. The index is currently being validated and tested for sensitivity to change in UC patients receiving medical treatment.

Several other IUS indices have been suggested for the assessment of disease activity in UC patients.[9, 11, 13, 14, 16, 25] The methodology used in these earlier studies were different as in most studies, the index parameters and cut-off values were defined before comparison with the reference standard.[13, 15, 25] We based the inclusion of parameters and determination of cut-off values on comparison with the endoscopic results, since it has been postulated that such an approach is optimal for the development of reliable diagnostic instruments.[26]

Despite the methodological differences, there are obvious similarities between our novel index and other IUS indices for assessing disease activity in UC patients. Evidently, BWT and the presence of CDS are used as parameters in most indices. However, the cut-off values for BWT, CDS categories and other included parameters tend to differ between studies. Parente et al. used a predefined cut-off of 4mm for BWT and categorized CDS as present or absent.[11] Pascu et al. used a BWT cut-off of 3mm and added increased CDS, loss of compressibility and loss of WLS as parameters.[25] Allocca et al. developed an index with BWT, CDS, WLS and presence of reactive lymph nodes as parameters.[13] The cut-off values and included parameters were predefined but the index showed good correlation with the Mayo endoscopic subscore. To our knowledge, the only index that determined cut-off values and parameters based on endoscopy as the reference standard, was developed by Civitelli et al. for assessing disease activity in pediatric UC patients.[14] These authors developed an index with BWT, CDS, loss of WLS, and presence of haustrations as parameters.

The variability in cut-off values and parameters included in different IUS indices shows that it is currently debatable which are best for the assessment of disease activity in UC patients. Additionally, it suggests that IUS is prone to variability in interpretation, as is the case with many diagnostic modalities. Therefore, we chose to construct a point-based score that is easy to use and thus less prone to variation. We did not mathematically weigh the included factors since in our opinion this will make the score unnecessarily complicated. The amount a factor is weighed would be different in every cohort and one would probably need hundreds of patients to be able to accurately weigh factors in a heterogenous population.

Since diagnostic modalities are prone to variability in interpretation, it is important to assess inter-rater agreement. To our knowledge, only Allocca et al. investigated inter-rater agreement of IUS examinations in UC patients.[13] In this study, all IUS examinations were performed by two ultrasonographers and the agreement between examiners for the overall IUS score was excellent. However, inter- and intra-rater agreement for the individual IUS parameters was

not assessed. In our study, we investigated inter- and intra-rater agreement for individual IUS parameters by reading cine loops by two investigators (SB & KN). To our knowledge, this has not been reported before. For continuous BWT values, inter-rater agreement was excellent and intra-rater agreement was good, showing that BWT measurements are reproducible between and within observers. For the constructed categories of BWT, inter-rater agreement was good and intra-rater agreement ranged from moderate to good. The lower agreement in the BWT categories is probably a result of the fact that small differences in the continuous measurements could mean a difference in categories, thus potentially leading to lower agreement. Inter-rater and intra-rater agreement for CDS assessment was good, but fair or poor with regard to haustrations in the transverse and ascending colon. Another recent study showed poor inter- and intra-rater agreement for haustrations and moderate to good agreement for the other parameters.[27] This shows that assessing haustrations is probably the most difficult of the included parameters. Since abnormal haustrations were clearly associated with disease activity we decided to include it in the index. An ongoing validation study will have to show if this parameter should remain part of the index. We were unable to assess inter- and intra-rater agreement for fat wrapping since the recorded cineloops were too short and stationary for this purpose. To properly assess fat wrapping you would need sweeping movement over a large area, which was only performed in live scanning. Inter- and intra-rater agreement could probably be improved with more experience and optimisation of measurement definitions in the future. However, it is important to note that we used multiple categories for most parameters which likely resulted in lower agreement. Another important factor could be that it is more difficult to assess certain parameters using cineloops. It is to be expected that inter-rater agreement will decline when more investigators are involved in image interpretation. Nevertheless, correlation of the final score was strong between observers. This shows that a combination of parameters results in a more accurate overall assessment. It could also be that IUS interpretation may be more reliable when performing the examination than when only interpreting cineloops. This is important to take into consideration, especially when considering the use of IUS in clinical trials that rely on central reading. Reliability of central reading of IUS examinations should therefore be investigated in future studies.

There are different technical aspects that are of importance when interpreting the results of this work and other comparable studies. For instance, IUS examinations are usually performed with a single US device in most studies. This is of importance for consistency when assessing parameters, such as CDS in the bowel wall. However, it is likely that there are differences in sensitivity of CDS measurements between US machines and US vendors. To our knowledge, a comparison of different US machines for measuring CDS in the bowel wall has never been conducted. Such a study would be of particular interest. Another potential issue when performing CDS measurements is the distance between the bowel wall and the US probe. Due to physical limitations of ultrasound, high frequency colour Doppler does not penetrate as deep into the body as lower frequency colour Doppler due to attenuation, while low frequency

Doppler has lower spatial resolution. This could reduce the number of vessels detected in deeper lying bowel segments and resulting in undervaluation of disease activity.[28] Another factor that could potentially influence the presence of CDS is fibrosis of the bowel wall in patients with longstanding UC. To our knowledge, no studies exist that have looked at the relationship between CDS and fibrosis in UC. However, two prior studies have indicated that fibrosis can result in reduced bowel wall vascularisation and less CDS in Crohn's disease patients.[29, 30]

We also assessed sensitivity and specificity for detection of disease activity when combining BWT with FCP measurements. Here, we show that sensitivity and specificity increases when combining these two parameters. Addition of FCP could therefore be of additive value in patients with minor findings on IUS, in order to better discriminate between quiescent and mild disease. FCP could also be useful for detection of proctitis in patients that have normal IUS findings in all colonic segments. Since FCP is already widely used, we believe that combining FCP and IUS for monitoring of disease activity should be of particular interest in clinical practice and in future studies.

Our study has several strengths. Firstly, we used a systematic approach for determination of cut-off values and selection of IUS parameters with endoscopy as the reference standard. Secondly, ultrasonographers and endoscopists were blinded for the results of the other examination. Thirdly, we assessed inter- and intra-rater agreement of the IUS parameters using cine-loop. Finally, the UC-IUS score was correlated with 2 different endoscopic scores. Our study also has some limitations. Firstly, IUS examinations were not performed twice by different ultrasonographers. Secondly, there was no central reading of the endoscopy and thirdly, we could not assess inter- and intra-rater agreement for all parameters (i.e. fat wrapping).

In conclusion, we developed an UC-IUS index that showed strong correlation with endoscopic disease activity through internal validation in the same cohort. Addition of FCP increased the accuracy of detection of disease activity. We showed that IUS could be a reliable substitute for endoscopy for assessing disease activity in UC patients, except in patients with proctitis. Broad implementation of IUS could therefore reduce the need for endoscopy and may especially be useful for rapid (on the spot) detection of flares and for monitoring of treatment outcomes. Since this is a pilot study, the UC-IUS index should be validated in future studies and tested for sensitivity to change after medical treatment.

## References


1. Schnitzler F, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, et al. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. Inflammatory bowel diseases. 2009;15(9):1295-301.

2. Colombel JF, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. Gastroenterology. 2011;141(4):1194-201.

3. Sharara AI, El Reda ZD, Harb AH, Abou Fadel CG, Sarkis FS, Chalhoub JM, et al. The burden of bowel preparations in patients undergoing elective colonoscopy. United European gastroenterology journal. 2016;4(2):314-8.

4. 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. Inflammatory bowel diseases. 2013;19(10):2111-7.

5. Travis S, Satsangi J, Lemann M. Predicting the need for colectomy in severe ulcerative colitis: a critical appraisal of clinical parameters and currently available biomarkers. Gut. 2011;60(1):3-9.

6. Dignass A, Eliakim R, Magro F, Maaser C, Chowers Y, Geboes K, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. Journal of Crohn's & colitis. 2012;6(10):965-90.

7. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al. Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. Journal of Crohn's & colitis. 2017;11(6):649-70.

8. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflammatory bowel diseases. 2012;18(12):2218-24.

9. Bots S, Nylund K, Lowenberg M, Gecse K, Gilja OH, D'Haens G. Ultrasound for Assessing Disease Activity in IBD Patients: A Systematic Review of Activity Scores. Journal of Crohn's & colitis. 2018;12(8):920-9.

10. Antonelli E, Giuliano V, Casella G, Villanacci V, Baldini V, Baldoni M, et al. Ultrasonographic assessment of colonic wall in moderate-severe ulcerative colitis: comparison with endoscopic findings. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2011;43(9):703-6.

11. Parente F, Molteni M, Marino B, Colli A, Ardizzone S, Greco S, et al. Are colonoscopy and bowel ultrasound useful for assessing response to short-term therapy and predicting disease outcome of moderate-to-severe forms of ulcerative colitis?: a prospective study. The American journal of gastroenterology. 2010;105(5):1150-7.

12. Maconi G, Ardizzone S, Parente F, Bianchi Porro G. Ultrasonography in the evaluation of extension, activity, and follow-up of ulcerative colitis. Scandinavian journal of gastroenterology. 1999;34(11):1103-7.

13. Allocca M, Fiorino G, Bonovas S, Furfaro F, Gilardi D, Argollo M, et al. Accuracy of Humanitas Ultrasound Criteria in Assessing Disease Activity and Severity in Ulcerative Colitis: A Prospective Study. Journal of Crohn's & colitis. 2018;12(12):1385-91.

14. Civitelli F, Di Nardo G, Oliva S, Nuti F, Ferrari F, Dilillo A, et al. Ultrasonography of the colon in pediatric ulcerative colitis: a prospective, blind, comparative study with colonoscopy. The Journal of pediatrics. 2014;165(1):78-84.e2.

15. Parente F, Molteni M, Marino B, Colli A, Ardizzone S, Greco S, et al. Bowel ultrasound and mucosal healing in ulcerative colitis. Digestive diseases (Basel, Switzerland). 2009;27(3):285-90.

16. Ishikawa D, Ando T, Watanabe O, Ishiguro K, Maeda O, Miyake N, et al. Images of colonic real-time tissue sonoelastography correlate with those of colonoscopy and may predict response to therapy in patients with ulcerative colitis. BMC gastroenterology. 2011;11:29.

17. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. The New England journal of medicine. 1987;317(26):1625-9.

18. Travis SP, Schnell D, Krzeski P, Abreu MT, Altman DG, Colombel JF, et al. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Gut. 2012;61(4):535-42.

19. Walmsley RS, Ayres RC, Pounder RE, Allan RN. A simple clinical colitis activity index. Gut. 1998;43(1):29-32.

20. Nylund K, Hausken T, Odegaard S, Eide GE, Gilja OH. Gastrointestinal wall thickness measured with transabdominal ultrasonography and its relationship to demographic factors in healthy subjects. Ultraschall in der Medizin (Stuttgart, Germany : 1980). 2012;33(7):E225-32.

21. Schober P, Boer C, Schwarte LA. Correlation Coefficients: Appropriate Use and Interpretation. Anesth Analg. 2018;126(5):1763-8.

22. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb). 2012;22(3):276-82.

23. Cohen J. A coefficient of agreement for nominal scales Educational and psychological measurement. 1960;20.1:37–46.

24. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med. 2016;15(2):155-63.

25. Pascu M, Roznowski AB, Muller HP, Adler A, Wiedenmann B, Dignass AU. Clinical relevance of transabdominal ultrasonography and magnetic resonance imaging in patients with inflammatory bowel disease of the terminal ileum and large bowel. Inflammatory bowel diseases. 2004;10(4):373-82.

26. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Annals of internal medicine. 2011;155(8):529-36.

27. de Voogd F WR, Gecse K, Allocca M, Novak K, Lu C, D'Haens G, Maaser C. Inter-observer agreement of an expert panel for gastrointestinal ultrasound in ulcerative colitis. Journal of Crohn's and Colitis. 2020;14(Supplement 1):S486–S7.

28. Postema MK, S.; Jenderka, K.V. . Principles of medical ultrasound. In: Dietrich CF, editor. EFSUMB course book on ultrasound 2nd edition. London: Latimer Trend & Company Ltd; 2018.

29. Sasaki T, Kunisaki R, Kinoshita H, Yamamoto H, Kimura H, Hanzawa A, et al. Use of color Doppler ultrasonography for evaluating vascularity of small intestinal lesions in Crohn's disease: correlation with endoscopic and surgical macroscopic findings. Scandinavian journal of gastroenterology. 2014;49(3):295-301.

30. Ripolles T, Rausell N, Paredes JM, Grau E, Martinez MJ, Vizuete J. Effectiveness of contrast-enhanced ultrasound for characterisation of intestinal inflammation in Crohn's disease: a comparison with surgical histopathology analysis. Journal of Crohn's & colitis. 2013;7(2):120-8.

# Chapter 4

## Point-of-care intestinal ultrasound in IBD patients: disease management and diagnostic yield in a real-world cohort and proposal of a point-of-care algorithm

S. Bots*, F. de Voogd*, M. de Jong, V. Ligtvoet, M. Löwenberg, M. Duijvestein, C.Y. Ponsioen, G. D'Haens, K.B. Gecse

*Contributed equally

*Journal of Crohn's and Colitis 2021 (Online ahead of print)*

## Abstract

### Introduction

Intestinal ultrasound (IUS) is useful for assessment of inflammation, complications and treatment follow-up in inflammatory bowel disease (IBD) patients. We aimed to study outcomes and impact on disease management for point-of-care (POC) IUS in IBD patients.

### Methods

Two patient cohorts undergoing POC IUS (January 2016 - July 2018 and October 2019 - December 2019) were included retrospectively. Disease management after IUS was analysed and IUS outcomes were compared with symptoms, biomarkers and additional imaging within 8 weeks from IUS. To study differences in use of IUS over time, cohorts were compared.

### Results

In total, 345 examinations (280 in Crohn's disease (CD)/65 in Ulcerative Colitis (UC)) were performed. Present inflammation on IUS was comparable between symptomatic and asymptomatic CD (67.6% vs 60.5%; p=0.291). In 60%, IUS had impact on disease management with change in medication in 47.8%. Additional endoscopy/MRI was planned after 32.8% of examinations showing good correlation with IUS in 86.3% ($\varrho$=0.70, p<0.0001) and 80.0% ($\varrho$=0.75, p<0.0001) of cases, respectively. Fecal calprotectin was higher in active versus inactive disease on IUS (664 μg/g vs 79 μg/g; p<0.001). Over the years IUS was performed more frequently to monitor treatment response and the use of MRI was reduced within the cohort.

### Conclusions

POC IUS affects clinical decision making and could detect pre-clinical relapse in CD patients with potential to reduce additional endoscopy or MRI. In addition, the paradigm expands towards monitoring treatment and close follow-up for IUS. Based on our results we propose a POC IUS algorithm for follow-up of IBD patients.

## Introduction

Inflammatory bowel disease (IBD) is a common denominator for the chronic inflammatory conditions ulcerative colitis (UC) and Crohn's disease (CD). The chronic and relapsing pattern causes long-term bowel damage and complications such as stenosis and perforating disease in CD patients.[1] Therefore, complete and objective control of inflammation is the preferred treatment target leading to superior long-term outcomes.[2, 3] Currently, this 'treat to target' concept is the ultimate strategy in the treatment of IBD patients.[1, 4]

To adequately control inflammation, close monitoring of the disease is crucial.[5] Clinical symptoms such as abdominal pain, diarrhoea, rectal blood loss as well as non-invasive biomarkers such as C-reactive protein (CRP) and fecal calprotectin (FCP) are useful to guide clinical decision making, but are not always sensitive and accurate and lack information on disease severity and extent.[6-8]



Diagnostic modalities for monitoring IBD patients include endoscopy, magnetic resonance imaging (MRI), computed tomography (CT) and intestinal ultrasound (IUS). Endoscopy is considered the reference standard for assessment of mucosal disease activity [9]. However, it is impossible to frequently implement this technique due to burden for the patient, costs and waiting lists.[9, 10] MRI is useful for the assessment of complications and small bowel inflammation. However, implementation is also limited by costs and waiting lists [11]. CT-scans are quick but generally only recommended in the acute setting due to radiation exposure. Since IUS is non-invasive, accurate, reliable and cheap, it is a suitable tool for frequent assessment of the bowel, especially in a point-of-care (POC) setting.[12-17] Additionally, it has been shown that patients prefer IUS over other modalities.[18]

POC medicine is defined as medical testing at or near the site of patient care with fast results which facilitate rapid clinical decision making. POC tests for CRP and FCP are already widely available but lack the ability to objectify complications, location, severity and extent of disease activity.[19, 20] On the contrary, IUS has the potential to identify these and thereby guide immediate decision making. Whereas studies have shown good accuracy and reliability of IUS, there is limited data on the impact of IUS outcomes on daily clinical decision making.[21, 22]

In this retrospective study, we studied a large cohort of IBD patients that were evaluated with POC IUS in a real-world outpatient setting and aimed to provide insight on the impact of POC IUS in daily clinical practice. Additionally, we compared IUS outcomes with symptoms, biomarkers and additional imaging or endoscopy. Furthermore, we highlight the potential of POC IUS to reduce the need and cost of additional imaging and to avoid treatment delay. Our findings may serve as a basis for future prospective studies and for the optimal implementation of POC IUS in IBD patients.

## Methods

*Patient population and study design*

IBD patients that were evaluated with POC IUS at the outpatient IBD clinic of the Amsterdam University Medical Centre were included consecutively from implementation in our clinic (January 2016) up to reaching 250 patients in July 2018. To compare evolution of POC IUS in clinical practice over time we collected a second cohort retrospectively before the COVID pandemic between October 2019 and December 2019. All patients were identified using the IUS outpatient lists in the electronic health cohort. All data were retrieved from electronic patient records. Exclusion criteria were: no formal IUS report available, under 18 years of age or no confirmed IBD diagnosis at the moment of data collection.

*Patient, biomarker and treatment data*

The following baseline data were collected: age at IUS, gender, age at diagnosis, disease phenotype (Montreal classification), medication use, previous surgery, clinical symptoms and biochemical markers (FCP and CRP). Data on clinical symptoms were collected and scored at data collection as follows: General well-being  good vs bad, presence/absence of abdominal pain, diarrhoea, rectal blood loss, urgency, bloating, loss of appetite, and extra intestinal manifestations. Patients were considered symptomatic when they had at least one symptom. FCP and CRP values were collected and compared with IUS when they were available within 4 weeks from IUS. FCP values >50 μg/g and CRP values >5 mg/L were considered as elevated, reflecting active inflammation. The following data on disease management after IUS were collected: medication started, stopped or adjusted, additional endoscopy or imaging planned, endoscopic dilation planned, surgery planned, continuation without change.

*IUS examinations*

All examinations were performed by investigators specifically trained in IUS (S.B.[>200 IUS in 2016], F.V. [>500 IUS in October 2019], K.G. [>500 IUS in October 2019], M.J. [>200 IUS in October 2019]). All IUS examinations at our clinic are performed by systematically scanning the bowel from the ileum through all segments of the colon and the rectum. Additionally, a sweep of the remaining small bowel is performed. Patients were not fastening. IUS parameters as mentioned below were noted in a standardized report. All the examinations were performed with a Philips EPIQ 5G machine with C5-1, L12-5 and L18-4 transducers or with a Hitachi Noblus machine with C5 and L13 transducers. At Colour Doppler the velocity was adjusted for slow flow detection with a maximum velocity scale of 5-7 cm/s.

*IUS parameters*

IUS data were collected by assessing IUS reports and reason for IUS was documented. Bowel wall thickness (BWT), colour Doppler signal (CDS), presence of fatty-wrapping, loss of colonic haustrations, loss of wall layer stratification, presence of reactive lymph nodes and absence of

small bowel motility were scored per segment (terminal ileum, ascending colon, transverse colon, descending colon, sigmoid and rectum). Presence of disease activity was scored per segment and confirmed when BWT > 2.0 and 3.0 mm for the small bowel and colon, respectively and a second parameter was pathologic (Supplementary Table 1). In addition, presence of complications was documented. Quality of the images was also documented and scored as good (proper evaluation), moderate (sufficient but incomplete evaluation), poor (hard to draw conclusions) and very poor (no conclusions possible). Furthermore, reasons for poor acquisition were collected (i.e. artefacts, reduced quality due to bowel gas, abdominal fat, complex anatomy due to surgery etc.). Uncertainty was scored by evaluation of the IUS report stating that ultrasonographer was uncertain due bowel gas, minor findings, abdominal fat or complex anatomy. Inconclusive was scored as poor image quality. The IUS results were considered adequate when a maximum of 1 bowel segment (excluding the rectum) could not be assessed.



*Additional imaging*

The following data on endoscopy and MRI performed after IUS were collected by assessing reports: presence of inflammation, location of inflammation, presence of complications and location of complications per segment. When additional imaging was performed within 8 weeks of IUS and the results of IUS and additional imaging were considered adequate, the outcomes were compared. All endoscopies were performed by an accredited gastroenterologist, according to local protocol. Endoscopic disease activity was defined as an eMayo score ≥ 1 and a SES-CD score ≥ 3 in at least one segment. Findings were considered comparable when disease activity, complications or normal findings were detected in the same locations in the bowel. Since this was a retrospective study, we did not compare disease severity between IUS, endoscopy and MRI.

*Statistical analysis*

Descriptive statistics were used to study the population. Differences in proportions were tested with the Chi-square test. Differences in not normally distributed continuous variables were tested using a Mann-Whitney U test and correlation was computed using Spearman's correlation coefficient. . A value of 0.00–0.10 was considered as negligible correlation, 0.10–0.39 as weak correlation, 0.40–0.69 as moderate correlation, 0.70–0.89 as strong correlation and 0.90–1.00 as very strong correlation.[23] Agreement between dichotomous variables was also tested with Cohen's kappa statistics. A value of 0.0-0.20 was considered as slight agreement, 0.21-0.4 as fair agreement, 0.41-0.60 as moderate agreement, 0.61-0.80 as substantial agreement and 0.81-1.0 as almost perfect agreement.[24, 25] A p-value < 0.05 was considered statistically significant. All analyses were performed with SPSS 25.0 software (IBM Corporation, Armonk, NY, USA).

*Ethical approval and patient consent*

This study was approved by the ethical committee of the Academic Medical Center Amsterdam. All data were anonymously extracted from the patient record and retrieving informed consent was therefore not necessary.

## Results

*Patient population*

We studied 301 patients with confirmed IBD in whom 345 IUS examinations were performed. Of these, 242 had CD and 59 UC (280 CD and 65 UC examinations, respectively). Cohort characteristics are described in Table 1.

**Table 1.** *Cohort characteristics.*

| **IUS examinations** | **n = 345** |
|---|---|
| | |
| **CD patients** | **n = 242** |
| Examinations in CD patients; n | n = 280 |
| Male; n (%) | 92 (38.0%) |
| Age at IUS; median (IQR), years | 37 (27-52) |
| Disease duration at time of IUS in median years (IQR) | 10 (4-19) |
| Montreal classification in CD patients | |
| A1 (<16 years) | 45 (18.6%) |
| A2 (17-40 years) | 164 (67.8%) |
| A3 (>40 years) | 33 (13.6%) |
| L1 (ileum) | 106 (43.8%) |
| L2 (colon) | 36 (14.9%) |
| L3 (ileocolonic) | 99 (40.9%) |
| + L4 (upper GI) | 1 (0.4%) |
| L4 only | 1 (0.4%) |
| B1 (non stricturing, non-penetrating) | 138 (57.0%) |
| B2 (stricturing) | 58 (24.0%) |
| B3 (penetrating) | 46 (19.0%) |
| P (Perianal disease) | 66 (27.3%) |
| Previous surgical resection at time of IUS | |
| ICR and ileal re-resections | 113 (40.4%) |
| (partial) colonic resection | 31 (11.1%) |
| Medication use at time of IUS | |
| Biologicals (infliximab, adalimumab, vedolizumab, | 112 (40.0%) |
| ustekinumab) | 64 (22.9%) |
| Immunomodulators (thiopurines/methotrexate) | 48 (17.1%) |
| Corticosteroids (oral/topical) | 10 (3.6%) |
| 5-ASA (oral/topical | |
| **UC patients** | **n = 59** |
| IUS examinations in UC patients; n | 65 |
| Male; n(%) | 22 (37.3%) |
| Age at IUS; median (range), years | 40 (27-51) |
| Disease duration at time of IUS in median years (IQR) | 7 (5-13) |

**Table 1. (***Continued).*

| | |
|---|---|
| Disease extent | |
| E1 (proctitis) | 5 (8.5%) |
| E2 (left-sided) | 19 (32.2%) |
| E3 (pancolitis) | 35 (59.3%) |
| Previous surgical resection at IUS | 0 (0%) |
| Medication use at time of IUS | |
| Biologicals (infliximab, adalimumab, golimumab, vedolizumab) | 25 (38.5%) |
| Immunomodulators | 17 (26.2%) |
| Corticosteroids (oral/topical) | 13 (20.0%) |
| 5-ASA | 38 (58.5%) |
| Tofacitinib | 3 (4.6%) |

*IUS=intestinal ultrasound, CD=Crohn's disease, IQR=interquartile range, ICR=ileocecal resection, ASA=aminosalicylate, UC=ulcerative colitis*

*Intestinal ultrasound*

The indications for IUS are shown in Supplementary Table 2, the commonest being symptoms of active disease and/or elevated FCP. Of 345 IUS examinations, 190 (55.1%) showed active disease and 113 (32.8%) showed no signs of inflammation. In 37 (10.7%) examinations, presence of inflammation was uncertain and in 5 (1.4%) the examinations were inconclusive. A total of 73 complications were detected in CD patients (i.e. strictures, abscesses, phlegmones and fistulas). The results of the IUS examinations are summarized in Table 2. Uncertainty and low image quality were explained by a variety of reasons (i.e. minor findings, bowel gas, abdominal fat, complex surgical history), as shown in Supplementary Table 3.

*Disease management after IUS*

Disease management after IUS is shown in Table 3. In 207/345 (60%) cases the treatment plan was changed (i.e. medication, imaging, surgery). Medication use was changed in 99/207 (47.7%) cases and in 122/207 (58.9%) cases additional imaging or endoscopy was planned after IUS. In 77/207 (37.2%) cases, additional evaluation was performed because this was considered necessary by the treating gastroenterologist (i.e. more information needed or IUS insufficient). Surgery was performed 16 times after IUS. Reasons for additional evaluation are shown in Supplementary Table 4.

**Table 2.** *Summary of IUS findings*

| **2a. Disease activity** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **CD (n=280)** | Inflammation | | No inflammation | | Uncertain | | Inconclusive | |
| Overall | 161 (57.5%) | | 83 (29.6%) | | 31 (11.1%) | | 5 (1.8%) | |
| Terminal ileum | 118 (42.1%) | | 128 (45.7%) | | 23 (8.2%) | | 11 (3.9%) | |
| Ascending colon | 33 (11.8%) | | 233 (83.2%) | | 3 (1.1%) | | 11 (3.9%) | |
| Transverse colon | 26 (9.3%) | | 239 (85.4%) | | 3 (1.1%) | | 12 (4.3%) | |
| Descending colon | 32 (11.4%) | | 235 (83.9%) | | 6 (2.1%) | | 7 (2.5%) | |
| Sigmoid | 34 (12.1%) | | 226 (80.7%) | | 12 (4.3%) | | 8 (2.9%) | |
| Rectum | 7 (2.5%) | | 124 (44.3%) | | 8 (2.9%) | | 141 (50.4%) | |
| Ileocolonic anastomosis (n=113) | 48 (42.5%) | | 55 (48.7%) | | 8 (7.1%) | | 3 (2.7%) | |
| Proximal small bowel* | 9 (3.2%) | | 265 (94.6%) | | 0 (0%) | | 6 (2.1%) | |
| **Ileum affected length** | | | | | | | | |
| Length cm | 0-5 | 5-10 | 10-15 | 15-20 | 20-25 | 25-30 | >30 | Unknown |
| N = 118 | 14 | 28 | 22 | 8 | 8 | 8 | 3 | 27** |
| % | 11.9% | 23.7% | 18.6% | 6.8% | 6.8% | 6.8% | 2.5% | 22.9% |
| **UC (n=65)** | | | | | | | | |
| Overall | 29 (44.6%) | | 30 (46.2%) | | 6 (9.2%) | | 0 (0%) | |
| Ascending colon | 6 (9.2%) | | 58 (89.2%) | | 1 (1.5%) | | 0 (0%) | |
| Transverse colon | 7 (10.8%) | | 58 (89.2%) | | 0 (0%) | | 0 (0%) | |
| Descending colon | 22 (33.8%) | | 42 (64.6%) | | 1 (1.5%) | | 0 (0%) | |
| Sigmoid | 28 (43.1%) | | 33 (50.8%) | | 3 (4.6%) | | 1 (1.5%) | |
| Rectum | 25 (38.5%) | | 19 (29.2%) | | 4 (6.2%) | | 17 (26.2%) | |
| **2b. Complications in CD** | | | | | | | | |
| | Present | | Absent | | Uncertain | | Inconclusive | |
| Stricture | 48 (17.1%) | | 206 (73.6%) | | 20 (7.2%) | | 6 (2.1%) | |
| Pre-stenotic dilation | 29 (10.4%) | | 236 (84.3%) | | 8 (2.9%) | | 7 (2.5%) | |
| Phlegmone | 12 (4.3%) | | 260 (92.9%) | | 1 (0.4%) | | 7 (2.5%) | |
| Abscess | 6 (2.1%) | | 264 (94.3%) | | 3 (1.1%) | | 7 (2.5%) | |
| Fistula | 7 (2.5%) | | 261 (93.3%) | | 6 (1.8%) | | 6 (1.8%) | |

**8 had disease activity in the proximal (terminal ileum), 1 had disease activity in the proximal jejunum/duodenum*

*** Reasons for unknown length not shown.*

*Uncertain = doubt regarding disease activity due to various reasons (i.e. suboptimal images, minor findings)*

*Inconclusive = no conclusions possible due to poor image quality*

*CD=Crohn's disease, UC=ulcerative colitis*

**Table 3.** *Disease management after IUS.*

| | CD examinations (n=280) | UC examinations (n=65) |
|---|---|---|
| **No change n=138 (40%)** | 106 (37.9%) | 32 (49.2%) |
| **Imaging n=122 (35.4%)** | 104 (37.1%) | 18 (27.7%) |
| Endoscopy | | |
| -Total | 73(26.1%) | 16 (24.6%) |
| -Dilation stricture | 13 (17.8%) | - |
| MRI | 23 (8.6%) | 1 (3.1%) |
| CT-scan | 8 (3.5%) | 1 (3.1%) |
| **Medication change n=99 (28.7%)** | 75 (26.8%) | 24 (36.9%) |
| Biologicals | | |
| -Start | 25 (8.9%) | 3 (4.6%) |
| -Dose intensification | 4 (1.4%) | 3 (4.6%) |
| -Dose de-escalation | 1 (0.4%) | - |
| -Stop | 1 (0.4%) | - |
| Immunomodulators | | |
| -Start | 23 (8.2%) | 3 (4.6%) |
| -Stop | 2 (0.7%) | 1 (1.5%) |
| Tofacitinib | | |
| -Stop | - | 1 (1.5%) |
| Corticosteroids (oral/topical) | | |
| -Start | 9 (3.2%) | 4 (6.2%) |
| -Stop | 1 (0.5%) | - |
| Budesonide | | |
| -Start | 8 (2.9%) | 1 (1.5%) |
| -Stop | - | 1 (1.5%) |
| 5-ASA | 0 (0%) | 6 (9.2%) |
| Inclusion in clinical trial | 1 (0.5%) | 1 (1.5%) |
| **Surgical resection** | | |
| Total | 16 (5.7%) | - |

*CD=Crohn's disease, UC=ulcerative colitis, MRI=magnetic resonance imaging, CT=computed tomography, ASA=aminosalicylate , >1 management decision was possible per patient*

*IUS versus clinical symptoms*

In total, 254/345 (73.6%) patients that received IUS were symptomatic. IUS examinations with uncertain or inconclusive outcome were excluded from comparison with clinical symptoms (n=42). IUS showed inflammation and/or complications in 145/222 (65.2%) symptomatic patients. In comparison, IUS showed inflammation and/or complications in 44/81 (54.3%) asymptomatic patients (p=0.080). In 117/173 (67.6%) symptomatic CD patients, IUS showed inflammation and/or complications. Conversely, IUS showed inflammation and/or complications in 43/71 (60.5%) asymptomatic CD patients (p=0.291). In 28/49 (57.1%)

symptomatic UC patients, IUS showed active disease. In comparison, IUS showed active disease in 1/10 (10.0%) asymptomatic UC patients (p=0.007).

*IUS versus biomarkers*

FCP measurements were available within 1 month of IUS in 229/345 (66.4%) cases and the median time between FCP measurement and IUS was 7 days (IQR 1-16). FCP levels were compared with IUS examinations with certain outcome (n=195). The median FCP level was 664 μg/g (IQR 278-1800) and 75 μg/g (IQR 22-351) in all IBD patients who had IUS examinations showing active or inactive disease, respectively (p<0.001). In CD patients, the median FCP level was 517 μg/g (IQR 224-1706) versus 79 μg/g (IQR 25-276) (p<0.001) and for UC patients the median FCP level was 1720 μg/g (IQR 400-3304) versus 75 μg/g (IQR 18-772) in IUS examinations showing active or inactive disease (p<0.001). IUS showed active disease in 110/155 (71.0%) cases with FCP > 50 μg/g versus 6/40 (15.0%) cases with FCP < 50 μg/g (P<0.001). The same comparisons were made for a FCP cut-off of 150 μg/g and 250 μg/g based on a previous work[26, 27]. IUS showed active disease in 101/137 (73.7%) cases with FCP > 150 μg/g versus 14/58 (24.1%) cases with FCP < 150 μg/g (p<0.001) and IUS showed active disease in 90/113 (79.6%%) cases with FCP > 250 μg/g versus 26/82 (31.7%) cases with FCP < 250 μg/g (p<0.001).

CRP measurements were available within 1 month of IUS in 275 (79.7%) cases and the median time between CRP measurement and IUS was 5 days (IQR 0-17). CRP levels were compared with IUS examinations with certain outcome (n=259). When comparing active disease or complications versus inactive disease on IUS, the median CRP level in was 5.5 mg/L (IQR 1.9-20.1) versus 2.1 mg/L (IQR 0.8-5.5) (p<0.001). In CD patients, the median CRP level was 6.7 mg/L (IQR 1.8-20.5) versus 1.9 mg/L (IQR 0.7-3.7) (p< 0.001) and in UC patients the median CRP level was 3.6 mg/L(IQR 1.4-20.8) versus 1.8 (IQR 0.6-6.4) (P=0.076). IUS showed disease activity or complications in 86/111 (77.5% ) cases with CRP level >5mg/L versus 76/148 (51.4%) in cases with CRP level <5mg/L (p<0.001).

*Endoscopy after IUS*

Endoscopy was planned following IUS in 89 cases and was performed within 8 weeks after IUS in 65 cases. The median time between IUS and endoscopy was 4 weeks (IQR 1-6). Of these, 51 IUS examinations and endoscopies had a certain outcome, which were analysed further. Overall, presence or absence of disease activity was comparable between IUS and endoscopy 44 out of 51 times (86.3%) (p<0.001) and showed strong correlation (ρ=0.70, p<0.0001). The Kappa agreement was substantial (κ= 0.61; p<0.001). In 36/41 (87.8%) cases, both IUS and endoscopy showed active disease. In 5/13 (38.5%) cases, endoscopy showed active disease while IUS did not. Of these, 2/5 had rectal disease and 1/5 had minor findings on IUS, not considered as active disease. Of the remaining 2 with normal IUS, 1 had ileitis and 1 had left sided Crohn's colitis (SES-CD 4) on endoscopy. Fecal calprotectin levels were elevated in all these 5 patients( 4/5 FCP >150 μg/g and 1/5 FCP > 50 μg/g). In 2/10 (20.0%) cases IUS showed active colonic disease while endoscopy did not. Both patients (1 CD and 1 UC) were treated

with corticosteroids for several weeks before endoscopy was performed. In 12/12 cases with a stricture on IUS, this stricture was also seen on endoscopy. However, endoscopy identified a stricture which was not seen on IUS in 4 cases. Full comparison between IUS and endoscopy is shown in Table 4.

*MRI after IUS*

MRI was planned after IUS in 24 cases of which 19 were conducted within 8 weeks. The median time between MRI was 4 weeks (IQR 3-7). In 15 cases, the IUS results were considered adequate (see below), which were analysed further. Overall, assessment of disease activity and strictures was comparable between IUS and MRI in 12/15 (80.0%) ($P<0.001$) cases and showed strong correlation ($\varrho=0.75$, $p<0.0001$). The agreement was moderate ($\kappa= 0.47$; $p=0.032$). In 15 IUS and MRI examinations with certain outcome (i.e. adequate image quality and no doubt) and MRI performed within 8 weeks, 9/12 (75.0%) IUS examinations showed active disease comparable to MRI versus 3/12 (25.0%) IUS examinations that did not show active disease of which MRI showed active disease ($p=0.018$). Full comparison between IUS and MRI is shown in Table 4.

*IUS from 2016-2018 versus IUS in 2019*

Characteristics of the two cohorts were compared. A total of 250 and 95 IUS examinations were performed in the first and second cohort. For the same period in 2016 (October-December) and 2019 (October-December) 40 and 95 IUS examinations were performed, respectively. Distribution of age, gender, disease duration, age at disease onset and Montreal classification were not different between cohorts. In addition, presence of clinical symptoms were equally distributed in both cohorts. In the first cohort, FCP was more frequently ≥50 μg/g (83.7% vs 53.7%, $p<0.0001$) and ≥250 μg/g (57.1% vs 40.0%, $p=0.004$) compared to the second cohort. Other biochemical parameters were not significantly different between cohorts.

In the first cohort, confirming active inflammation (63% vs 43%, $p=0.001$) and complications (30.2% vs 11.1%, $p<0.0001$) were more often indications to request IUS than in the second cohort. In addition, IUS showed active disease more often in the first cohort as opposed to the second cohort (70% vs 55.8%, $p=0.017$). In the second cohort, monitoring treatment response was more often an indication to perform IUS when compared to the first cohort (25.0% vs 6.4%, $p<0.0001$). Furthermore, patients in the first cohort were treated less with corticosteroids (9.6% vs 39%, $p<0.0001$) and biologicals (35.2% vs 51.6%, $p=0.004$) while use of thiopurines and methotrexate was similar between the cohorts. Disease management after IUS was comparable, except for a more frequent decision to continue current treatment in the second cohort (36.4% vs 49.5%, $p=0.019$).

There were no differences in certainty of the IUS conclusion, visibility of segments and detection of complications. Furthermore, the results of subsequent endoscopy or MRI and their correlation with IUS did not differ between the groups. However, we did perform MRI

more frequently in the first cohort when compared to the second cohort (8.4% vs 2.1%, p=0.024). There was no difference in amount of performed endoscopies between the cohorts.

**Table 4.** *Comparison of IUS findings versus endoscopy and when IUS and additional imaging were adequate and performed within 8 weeks.*

| IUS versus Endoscopy | | | | | |
|---|---|---|---|---|---|
| **N=51** | **Comparable findings** | **IUS active** | **IUS inconclusive** | **Endoscopy active** | **Endoscopy inconclusive** |
| Overall | 44/51 (86.3%) | 38/51 (74.5%) | - | 41/51 (80.4%) | - |
| Terminal ileum | 37/38 (97.4%) | 19/51 (37.3%) | 2/51 (3.9%) | 20/51 (39.2%) | 11/51 (21.6%) |
| Ascending colon | 38/43 (88.4%) | 3/51 (5.9%) | - | 4/51 (7.8%) | 8/51 (15/7%) |
| Transverse colon | 36/45 (80.0%) | 10/51 (19.6%) | - | 9/51 (17.6%) | 6/51 (11.8%) |
| Descending colon | 44/51 (86.3%) | 15/51 (29.4%) | - | 14/51 (27.5%) | - |
| Sigmoid colon | 44/50 (88.0%) | 17/51 (33.3%) | - | 16/51 (31.4%) | - |
| Rectum | 26/40 (65.0%) | 10/51 (19.6%) | 11/51 (21.6%) | 21/51 (41.2%) | - |
| Stricture | 47/51 (92.2%) | 12/51 (23.5%) | - | 16/51 (31.4%) | - |

| IUS versus MRI | | | |
|---|---|---|---|
| **n=15** | **Comparable findings** | **IUS present** | **MRI present** |
| **Disease activity** | | | |
| Overall | 12 (80.0%) | 9 (60%) | 12 (80.0%) |
| Ileal disease | 12 (80.0%) | 8 (53.3%) | 11 (73.3%) |
| Proximal small bowel disease | 9 (60.0%) | 1 (6.7%) | 4 (26.7%) |
| **Complications** | | | |
| Stricture | 12 (80.0%) | 5 (33.3%) | 8 (53.3%) |
| Intra-abdominal abscess | 15 (100%) | 0 (0%) | 0 (0%) |
| Intra-abdominal fistula | 12 (80.0%) | 1 (6.7%) | 1 (6.7%) |

*Overall = presence/absence of disease activity for endoscopy and presence/absence of disease activity and/or complications for MRI*

*IUS inconclusive= segment not investigated due to various reasons*

*Endoscopy inconclusive = not investigated due to various reasons (i.e. sigmoidoscopy, technical difficulties etc.)*

*IUS=intestinal ultrasound, MRI=magnetic resonance imaging*

*Proposal of a POC IUS algorithm*

In Figure 1 we propose a POC IUS algorithm, based on the results of this study and previous studies.

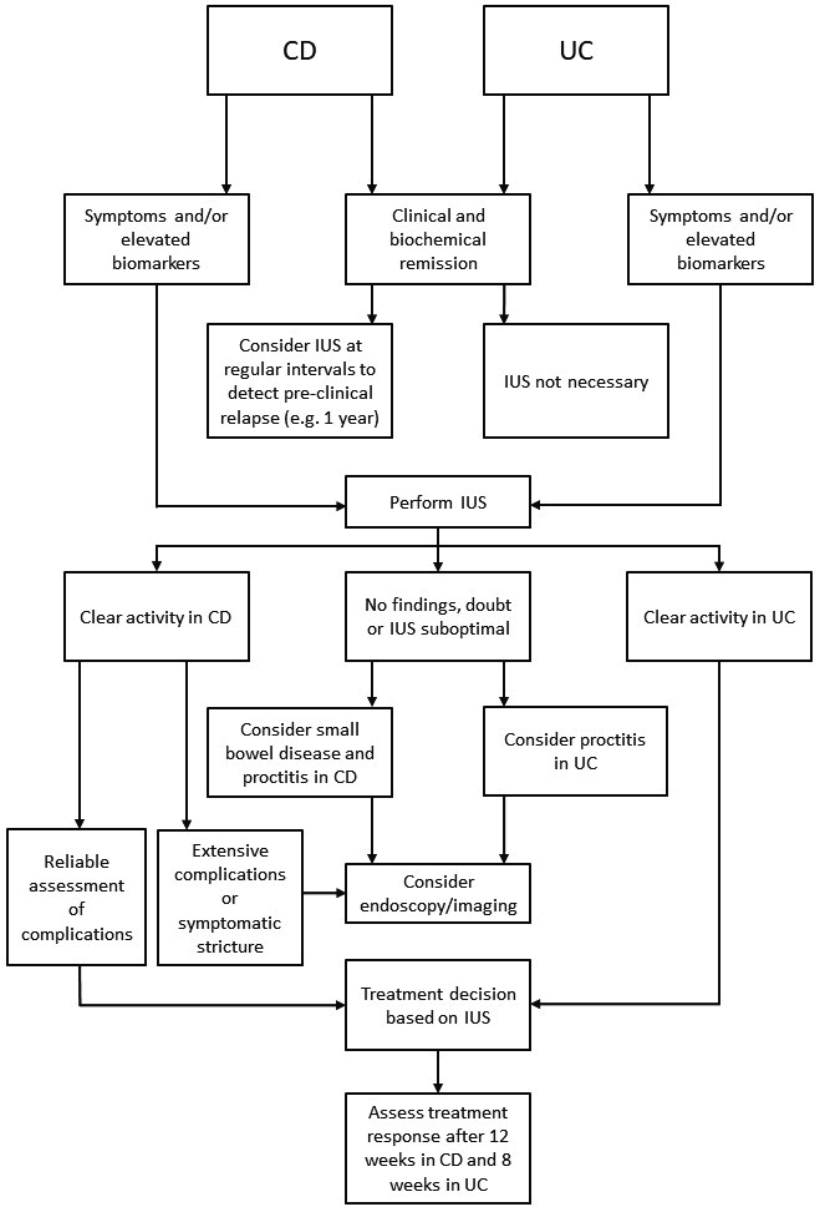



**Figure 1.** *Proposal of a point-of-care intestinal ultrasound algorithm*

*CD=Crohn's disease, UC=ulcerative colitis, IUS=intestinal ultrasound*

## Discussion

In this study we describe the validity of POC IUS and its impact on disease management in a large real-world cohort of IBD patients. In our cohort, IUS revealed presence of disease activity in more than half of patients leading to change in medication in almost half of these patients. This is in line with previous work.[21] In most cases treatment was initiated or upscaled as opposed to downscaling or stopping treatment.

When comparing IUS findings with clinical symptoms we observed a large proportion of asymptomatic CD patients with signs of inflammation and/or complications on IUS. In CD patients, symptoms show poor correlation with biochemical or endoscopic disease activity and complications.[21, 28, 29] Therefore, IUS had an important role in the detection of pre-clinical or subclinical relapse in CD patients, especially when combined with biochemical markers. Conversely, clinical symptoms are more reliable in UC patients, as shown by our data and in previous studies.[30-32] Therefore, presence of disease activity on IUS in UC patients together with clinical disease activity or elevated biochemical markers could be sufficient to guide disease management. Additionally, IUS is sufficient in most UC patients with symptoms and/or elevated biomarkers to confirm disease activity and to determine disease extent, as also suggested previously.[33]

Endoscopy did not provide additional information regarding presence of inflammation in 86.3% of cases when the IUS results were considered adequate. As such, IUS has significant cost, time and burden-saving potential. Several studies, have shown high accuracy, sensitivity and specificity for IUS when compared to other modalities in both CD and UC patients.[17, 33-40] In our cohort, we also showed comparable detection of inflammation for IUS and endoscopy. However, it is important to note that we did not compare disease severity between the two modalities and that we did not include IUS examinations with uncertain outcome in the comparison.

In our cohort, we found endoscopically active disease in 5/13 patients despite 'normal' IUS. Two of these patients had rectal disease and in the other three IUS did not detect ileal or colonic disease. Additionally, in 7/10 (70%) patients with uncertain outcome on IUS, active disease was observed with endoscopy. Hence, a certain proportion of patients will benefit from additional investigation even when IUS does not indicate disease activity. Clinical disease activity and/or elevated biomarkers could guide the decision to perform additional evaluation in these cases.

In approximately 25% of CD patients a complication was found, most often a stricture with or without prestenotic dilation. Since many CD patients develop strictures over time, POC IUS could play an important role in early detection of strictures and guiding decision making in these patients. In our cohort, IUS guided disease management such as referring patient for endoscopic dilation and/or surgery. Although data is lacking on identifying strictures with

IUS that are most suitable for endoscopic dilation, anti-inflammatory treatment or surgery, IUS has the potential to guide disease management and ongoing studies are focusing on this (Netherlands Trial Register: NL9105).

IUS is less accurate when assessing the proximal small bowel or the rectum.[33] In our study, MRI showed small bowel disease more often than IUS in a small proportion of patients. Taylor et al. found similar results when MRI and IUS were compared in small bowel CD.[38] Furthermore, proctitis was more frequently shown with endoscopy than with IUS. This is in line with previous work, showing that IUS is generally not feasible for assessment of the rectum [33]. However, since IUS was comparable with endoscopy for assessment of the rectum in 65% of cases, it could be useful in some cases, especially in patients with established proctitis and in combination with FCP. However, a recent study found perineal ultrasound to be a more accurate but non-invasive alternative to assess the rectum when compared to endoscopy.[41]

Furthermore, we have studied the implementation of IUS over time in clinical practice. When we started utilizing IUS in our clinic in 2016, all examinations were performed by one physician, predominantly to confirm active disease or diagnose complications in patients with clinical symptoms or elevated biomarkers. In recent years, the paradigm for IUS has expanded towards monitoring treatment response and reassuring quiescent disease. This has resulted in an increased demand in IUS examinations and more physicians who were trained at our clinic are now performing IUS. Concurrently, MRI was performed less frequently in the second cohort. In this cohort more patients received therapy with probably milder disease activity and hence less MRI requests. However, the increase of IUS in the second cohort might also be a valid reason for the decline in MRI Although future research should confirm this statement, we show that with sufficient expertise, IUS could be used as first non-invasive choice in a POC setting.

Overall, our findings indicate that POC IUS has the potential to reduce the need for endoscopy and MRI with the latter already occurring in clinical practice. It seems that additional evaluation should mainly be considered when the results of IUS are uncertain and in case of suspicion of small bowel disease, proctitis or in cases of extensive complications such as multiple strictures and complex fistulising disease. Indeed, in our cohort endoscopy was more often planned when the IUS outcome was uncertain. Other indications for endoscopy include stricture dilation or screening for malignancy. It has been postulated that endoscopy or MRI should also be considered for decisions such as starting and monitoring treatment with biologicals. This too may be subject for debate since studies have shown that IUS can be reliably used for follow-up of biologic treatment.[1, 12, 14, 42-44] More studies on this topic are expected in the future.

Other studies that investigated the implementation of POC IUS are limited. Novak et al. studied POC IUS in 49 CD patients by comparing POC IUS with regular care in a blinded study.[21] They found that POC IUS changed clinical management in 60% of patients, which is

similar to our findings. Additionally, they showed that many asymptomatic CD patients had signs of active disease on IUS, also in concordance with our findings. Shatanantan et al. compared POC IUS in 74 IBD patients with ileocolonoscopy in a blinded study and found high sensitivity and specificity of POC IUS for detection of disease activity in both UC and CD.[22] A third study found also a high correlation for IUS and endoscopic disease activity in CD patients.[45] In our cohort we found similar findings. We studied a large real-world cohort and further demonstrated the impact of IUS on clinical decision making. Furthermore, we performed a detailed analysis of IUS outcomes and reasons for uncertainty in daily clinical practice. With regards to uncertainty, bowel gas, obesity and mild inflammation all contributed to poor image quality or uncertainty. In these patients, endoscopy or other cross-sectional imaging techniques are more suitable to detect inflammation. However, endoscopy was performed only in a small number of patients with uncertain IUS outcome thus controlled studies are needed to elucidate the role of additional endoscopy in patients with uncertain outcomes at IUS as inflammation might be limited or absent. On the contrary, an unsuccessful endoscopy could be an additional reason for IUS to objectify disease activity, predominantly in the TI or proximal small bowel.[45]



To illustrate the use of POC IUS in daily clinical practice we propose an algorithm which may have the potential to reduce unnecessary additional evaluation in the future and to reduce delay in disease management. In a recent review, Allocca et al. also proposed a POC IUS algorithm based on the available literature.[46] This algorithm is mostly comparable with our suggestion. However, we defined a somewhat different strategy between UC and CD patients, since absence of symptoms is more reliable in UC patients. Additionally, we propose when additional imaging should be performed after IUS, such as when the IUS outcome is uncertain. We also propose to assess treatment response at different time points in CD and UC patients since recent studies suggest that UC patients respond to treatment earlier than CD patients.[14, 47] However, prospective studies are needed to optimize the implementation of POC IUS for the monitoring of IBD patients. Especially the best timing for assessment of treatment response and IUS evaluation in patients without symptoms and normal biomarkers is unknown. From a logistic point of view it would also be challenging to frequently perform scheduled IUS in every IBD patient in a large clinic with a large cohort of patients. This emphasizes the need for proper risk assessment. In our algorithm we propose to perform POC IUS once a year in CD patients. However, it is plausible that predicted disease progression should also be taken into account when determining the frequency of scheduled IUS.

Our study has several limitations. Firstly, this was a retrospective observational cohort study. Therefore, comparisons with symptoms, biochemical markers and other imaging modalities were probably less reliable than in a prospective controlled setting. We could not properly account for time, change of symptoms and change of medication between IUS and additional imaging. Additionally, we did not compare severity between IUS and endoscopy or MRI as endoscopy/MRI might show already improvement after starting any treatment based on IUS

findings. Also, there is a considerable risk for selection bias between the first and second cohort as IUS became more standard care over time. Hence, reason for IUS could have changed accordingly. In addition, we did not have a control group receiving no IUS and we could therefore not determine the absolute effect on disease management for IUS. However, we were able to study a large cohort of patients. Additionally, real-world data are more representative of the clinical situation, which could also be considered a strength. For instance, our data may reliably show the incidence of problems that may arise when performing IUS, such as poor image quality and unreliable results due to bowel gas, abdominal fat or mild disease activity. Regardless, we show a clear impact of IUS in clinical disease management in a real-world cohort.

In conclusion, POC IUS significantly impacts disease management in the follow-up of IBD patients and has the potential to reduce the need for additional endoscopy and MRI. We proposed an algorithm for implementation of POC IUS. Prospective studies are needed to study the optimal implementation and timing of POC IUS in close-monitoring and treatment follow-up in daily clinical care.

## References


1. Maaser C, Sturm A, Vavricka SR, Kucharzik T, Fiorino G, Annese V, et al. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. Journal of Crohn's & colitis. 2019;13(2):144-64.

2. Schnitzler F, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, et al. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. Inflammatory bowel diseases. 2009;15(9):1295-301.

3. Colombel JF, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. Gastroenterology. 2011;141(4):1194-201.

4. Peyrin-Biroulet L, Sandborn W, Sands BE, Reinisch W, Bemelman W, Bryant RV, et al. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. The American journal of gastroenterology. 2015;110(9):1324-38.

5. Maaser C, Sturm A, Vavricka SR, Kucharzik T, Fiorino G, Annese V, et al. ECCO-ESGAR Guideline for Diagnostic Assessment in Inflammatory Bowel Disease. Journal of Crohn's & colitis. 2018.

6. Schoepfer AM, Vavricka S, Zahnd-Straumann N, Straumann A, Beglinger C. Monitoring inflammatory bowel disease activity: clinical activity is judged to be more relevant than endoscopic severity or biomarkers. Journal of Crohn's & colitis. 2012;6(4):412-8.

7. Halpin SJ, Ford AC. Prevalence of symptoms meeting criteria for irritable bowel syndrome in inflammatory bowel disease: systematic review and meta-analysis. The American journal of gastroenterology. 2012;107(10):1474-82.

8. Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. Gastroenterology. 2000;119(1):15-22.

9. Spiceland CM, Lodhia N. Endoscopy in inflammatory bowel disease: Role in diagnosis, management, and treatment. World journal of gastroenterology : WJG. 2018;24(35):4014-20.

10. Sharara AI, El Reda ZD, Harb AH, Abou Fadel CG, Sarkis FS, Chalhoub JM, et al. The burden of bowel preparations in patients undergoing elective colonoscopy. United European gastroenterology journal. 2016;4(2):314-8.

11. Ordas I, Rimola J, Rodriguez S, Paredes JM, Martinez-Perez MJ, Blanc E, et al. Accuracy of magnetic resonance enterography in assessing response to therapy and mucosal healing in patients with Crohn's disease. Gastroenterology. 2014;146(2):374-82.e1.

12. Kucharzik T, Wittig BM, Helwig U, Borner N, Rossler A, Rath S, et al. Use of Intestinal Ultrasound to Monitor Crohn's Disease Activity. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2016.

13. Novak KL, Nylund K, Maaser C, Petersen F, Kucharzik T, Lu C, et al. Expert consensus on optimal acquisition and development of the International Bowel Ultrasound Segmental Activity Score (IBUS-SAS):

a reliability and inter-rater variability study on intestinal ultrasonography in Crohn's Disease. Journal of Crohn's & colitis. 2020.

14. Maaser C, Petersen F, Helwig U, Fischer I, Roessler A, Rath S, et al. Intestinal ultrasound for monitoring therapeutic response in patients with ulcerative colitis: results from the TRUST&UC study. Gut. 2020;69(9):1629-36.

15. De Voogd F, Wilkens R, Gecse K, Allocca M, Novak K, Lu C, et al. A reliability study - strong inter-observer agreement of an expert panel for intestinal ultrasound in ulcerative colitis. Journal of Crohn's & colitis. 2021.

16. de Voogd FAE, Verstockt B, Maaser C, Gecse KB. Point-of-care intestinal ultrasonography in inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2021.

17. Panes J, Bouzas R, Chaparro M, Garcia-Sanchez V, Gisbert JP, Martinez de Guerenu B, et al. Systematic review: the use of ultrasonography, computed tomography and magnetic resonance imaging for the diagnosis, assessment of activity and abdominal complications of Crohn's disease. Alimentary pharmacology & therapeutics. 2011;34(2):125-45.

18. Miles A, Bhatnagar G, Halligan S, Gupta A, Tolan D, Zealley I, et al. Magnetic resonance enterography, small bowel ultrasound and colonoscopy to diagnose and stage Crohn's disease: patient acceptability and perceived burden. European radiology. 2019;29(3):1083-93.

19. Delefortrie Q, Schatt P, Grimmelprez A, Gohy P, Deltour D, Collard G, et al. Comparison of the Liaison(R) Calprotectin kit with a well established point of care test (Quantum Blue - Buhlmann-Alere(R)) in terms of analytical performances and ability to detect relapses amongst a Crohn population in follow-up. Clinical biochemistry. 2016;49(3):268-73.

20. Rogler G, Aldeguer X, Kruis W, Lasson A, Mittmann U, Nally K, et al. Concept for a rapid point-of-care calprotectin diagnostic test for diagnosis and disease activity monitoring in patients with inflammatory bowel disease: expert clinical opinion. Journal of Crohn's & colitis. 2013;7(8):670-7.

21. Novak K, Tanyingoh D, Petersen F, Kucharzik T, Panaccione R, Ghosh S, et al. Clinic-based Point of Care Transabdominal Ultrasound for Monitoring Crohn's Disease: Impact on Clinical Decision Making. Journal of Crohn's & colitis. 2015;9(9):795-801.

22. Sathananthan D, Rajagopalan A, Van De Ven L, Martin S, Fon J, Costello S, et al. Point-of-care gastrointestinal ultrasound in inflammatory bowel disease: An accurate alternative for disease monitoring. JGH Open. 2020;4(2):273-9.

23. Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. Anesthesia & Analgesia. 2018;126(5):1763-8.

24. Cohen J. A coefficient of agreement for nominal scales. Educational and psychological measurement. 1960;20(1):37-46.

25. McHugh ML. Interrater reliability: the kappa statistic. Biochemia medica. 2012;22(3):276-82.

26. Turner D, Ricciuto A, Lewis A, D'Amico F, Dhaliwal J, Griffiths AM, et al. STRIDE-II: an update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) initiative of the International Organization for the Study of IBD (IOIBD): determining therapeutic goals for treat-to-target strategies in IBD. Gastroenterology. 2021;160(5):1570-83.

27. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflammatory bowel diseases. 2012;18(12):2218-24.

28. Zittan E, Kabakchiev B, Kelly OB, Milgrom R, Nguyen GC, Croitoru K, et al. Development of the Harvey-Bradshaw Index-pro (HBI-PRO) Score to Assess Endoscopic Disease Activity in Crohn's Disease. Journal of Crohn's & colitis. 2017;11(5):543-8.

29. Sandborn WJ, Feagan BG, Hanauer SB, Lochs H, Löfberg R, Modigliani R, et al. A review of activity indices and efficacy endpoints for clinical trials of medical therapy in adults with Crohn's disease. Gastroenterology. 2002;122(2):512-30.

30. D'Haens G, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. Gastroenterology. 2007;132(2):763-86.

31. Walmsley RS, Ayres RC, Pounder RE, Allan RN. A simple clinical colitis activity index. Gut. 1998;43(1):29-32.

32. Lewis JD, Chuai S, Nessel L, Lichtenstein GR, Aberra FN, Ellenberg JH. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. Inflammatory bowel diseases. 2008;14(12):1660-6.

33. Smith RL, Taylor KM, Friedman AB, Gibson RN, Gibson PR. Systematic Review: Clinical Utility of Gastrointestinal Ultrasound in the Diagnosis, Assessment and Management of Patients With Ulcerative Colitis. Journal of Crohn's and Colitis. 2019;14(4):465-79.

34. Bots S, Nylund K, Lowenberg M, Gecse K, Gilja OH, D'Haens G. Ultrasound for Assessing Disease Activity in IBD Patients: A Systematic Review of Activity Scores. Journal of Crohn's & colitis. 2018;12(8):920-9.

35. Goodsall TM, Nguyen TM, Parker CE, Ma C, Andrews JM, Jairath V, et al. Systematic Review: Gastrointestinal Ultrasound Scoring Indices for Inflammatory Bowel Disease. Journal of Crohn's & colitis. 2021;15(1):125-42.

36. Allocca M, Fiorino G, Bonovas S, Furfaro F, Gilardi D, Argollo M, et al. Accuracy of Humanitas Ultrasound Criteria in Assessing Disease Activity and Severity in Ulcerative Colitis: A Prospective Study. Journal of Crohn's & colitis. 2018;12(12):1385-91.

37. Bots S, Nylund K, Löwenberg M, Gecse K, D'Haens G. Intestinal Ultrasound to Assess Disease Activity in Ulcerative Colitis: Development of a novel UC-Ultrasound index. Journal of Crohn's & colitis. 2021.

38. Taylor SA, Mallett S, Bhatnagar G, Baldwin-Cleland R, Bloom S, Gupta A, et al. Diagnostic accuracy of magnetic resonance enterography and small bowel ultrasound for the extent and activity of newly diagnosed and relapsed Crohn's disease (METRIC): a multicentre trial. Lancet Gastroenterol Hepatol. 2018;3(8):548-58.

39. Sasaki T, Kunisaki R, Kinoshita H, Yamamoto H, Kimura H, Hanzawa A, et al. Use of color Doppler ultrasonography for evaluating vascularity of small intestinal lesions in Crohn's disease: correlation with endoscopic and surgical macroscopic findings. Scandinavian journal of gastroenterology. 2014;49(3):295-301.

40. Allocca M, Fiorino G, Bonifacio C, Furfaro F, Gilardi D, Argollo M, et al. Comparative Accuracy of Bowel Ultrasound Versus Magnetic Resonance Enterography in Combination With Colonoscopy in Assessing Crohn's Disease and Guiding Clinical Decision-making. Journal of Crohn's & colitis. 2018;12(11):1280-7.

41. Sagami S, Kobayashi T, Aihara K, Umeda M, Morikubo H, Matsubayashi M, et al. Transperineal ultrasound predicts endoscopic and histological healing in ulcerative colitis. Alimentary pharmacology & therapeutics. 2020;51(12):1373-83.

42. Moreno N, Ripollés T, Paredes JM, Ortiz I, Martínez MJ, López A, et al. Usefulness of abdominal ultrasonography in the analysis of endoscopic activity in patients with Crohn's disease: changes following treatment with immunomodulators and/or anti-TNF antibodies. Journal of Crohn's & colitis. 2014;8(9):1079-87.

43. Albshesh A, Ungar B, Ben-Horin S, Eliakim R, Kopylov U, Carter D. Terminal Ileum Thickness During Maintenance Therapy Is a Predictive Marker of the Outcome of Infliximab Therapy in Crohn Disease. Inflammatory bowel diseases. 2020;26(10):1619-25.

44. Parente F, Molteni M, Marino B, Colli A, Ardizzone S, Greco S, et al. Bowel ultrasound and mucosal healing in ulcerative colitis. Digestive diseases (Basel, Switzerland). 2009;27(3):285-90.

45. Wilkens R, Novak KL, Lebeuf-Taylor E, Wilson SR. Impact of intestinal ultrasound on classification and management of Crohn's disease patients with inconclusive colonoscopy. Canadian Journal of Gastroenterology and Hepatology. 2016;2016.

46. Allocca M, Furfaro F, Fiorino G, Peyrin-Biroulet L, Danese S. Point-of-Care Ultrasound in Inflammatory Bowel Disease. Journal of Crohn's & colitis. 2021;15(1):143-51.

47. Kucharzik T, Wittig BM, Helwig U, Börner N, Rössler A, Rath S, et al. Use of Intestinal Ultrasound to Monitor Crohn's Disease Activity. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2017;15(4):535-42.e2.

## Supplementary material

**Supplementary Table 1.** *Intestinal ultrasound parameters.*

| IUS parameter | Measurement technique | Pathologic |
|---|---|---|
| Bowel Wall Thickness | (Thickness in longitudinal plane + Thickness in cross-sectional plane) / 2 | BWT > 3.0 mm for colonic segments; BWT > 2.0 mm for TI |
| Colour Doppler Signal | 0: absent<br>1: small spots (single vessels) visible within the wall<br>2: long stretches visible within the wall<br>3: long stretches visible extending into the mesentery | > Grade 1 |
| Loss of wall layer stratification | 0: preserved<br>1: not preserved | Not preserved |
| Loss of haustration | 0: preserved<br>1: not preserved | Not preserved |
| Fatty wrapping | 0: absent<br>1: present | Present |

*IUS=intestinal ultrasound, BWT=bowel wall thickness, TI=terminal ileum*

**Supplementary Table 2.** *Indications for IUS (patients could have more than one indication).*

| Indication | CD (n=280) | UC (n=65) | Total (n=365) |
|---|---|---|---|
| Symptoms of active disease | 142 (50.7%) | 56 (86.2%) | 198 (57.4%) |
| Suspicion of stricture/abscess | 83 (29.6%) | 2 (3.1%) | 85 (24.6%) |
| Elevated FCP | 107 (38.2%) | 23 (35.4%) | 130 (37.7%) |
| Elevated CRP | 99 (35.4%) | 16 (24.6%) | 115 (33.3%) |
| Monitoring treatment response | 27 (9.6%) | 13 (20.0%) | 40 (11.6%) |

*CD = Crohn's disease, UC = ulcerative colitis, FCP=fecal calprotectin, CRP=C-reactive protein*

**Supplementary Table 3.** *Image quality and reasons for uncertainty.*

| **Image quality** | |
|---|---|
| Good | 243 (70.4%) |
| Moderate | 68 (19.7%) |
| Poor | 29 (8.4%) |
| Very poor | 5 (1.4%) |
| **Reasons for uncertainty (patients could have more than 1 reason)** | |
| Minor findings | 28 (8.1%) |
| Bowel gas | 31 (9.0%) |
| Abdominal fat | 34 (9.9%) |
| Complex surgical history | 8 (2.3%) |

**Supplementary Table 4.** *Reasons for additional endoscopy, MRI or CT.*

| | N=122 |
|---|---|
| IUS insufficient/additional evaluation deemed necessary | 77 |
| Baseline evaluation before starting or follow-up of biological treatment | 13 |
| Stricture dilation | 14 |
| Evaluation of treatment response | 3 |
| Extensive complications | 4 |
| Inclusion in clinical trial | 8 |
| Melena | 1 |
| Suspicion of malignancy | 2 |

*IUS=intestinal ultrasound*

# Chapter 5

## Early intestinal ultrasound predicts endoscopic response on anti-TNF-$\alpha$ treatment in Crohn's Disease

F. de Voogd*, S. Bots*, K. Gecse, O.H. Gilja3, G. D'Haens, K. Nylund

*contributed equally

*Submitted*

## Abstract

### Background and Aims

Objective measures are superior to clinical improvement in Crohn's Disease (CD) treatment response assessment. In this perspective, intestinal ultrasound (IUS) is emerging with recent studies demonstrating high accuracy for IUS to detect CD flares. However, less is known for IUS in treatment follow-up and early transmural changes. Therefore, we investigate conventional IUS parameters and contrast-enhanced ultrasound (CEUS) parameters to predict (early) endoscopic treatment response.

### Methods

Consecutive patients with endoscopically active CD starting anti-TNF$\alpha$ therapy were included. In addition, clinical, biochemical, IUS and CEUS parameters at baseline (T0), after 4-8 weeks (T1) and 12-34 weeks (T2) were collected. The most severely inflamed segment at endoscopy (highest segmental SES-CD) and IUS (highest segmental bowel wall thickness (BWT)) was identified. At T2, endoscopic response (decrease SES-CD≥50%) and endoscopic remission (SES-CD=0) was scored for this most severe segment.

### Results

40 patients were included, 14 reached endoscopic remission and 17 endoscopic response. At T1 (3.1 mm [1.9-4.2] vs 5.3 mm [3.8-6.9], p=0.005) and T2 (2.0 mm [1.8-3.1] vs 5.1 [3.0-6.3] mm, p=0.002) BWT was lower in patients with endoscopic remission. 18% BWT decrease at T1 (OR: 10.80, 95%CI: 1.69-68.94, p=0.012) and 29% at T2 (OR: 37.50, 95%CI: 2.77-507.48, p=0.006) predicted endoscopic response. 3.2 mm was most accurate to determine endoscopic remission (OR: 39.42, 95%CI: 7.67-202.57, p<0.0001). In addition to BWT, Colour Doppler Signal (OR: 13.76, p=0.03) and the CEUS parameter Wash-out Rate (OR: 0.76, p=0.019) improved the prediction model.

### Conclusion

Both (early) IUS and CEUS parameters predicted endoscopic response and remission. Furthermore, we provide accurate cut-off values for BWT reflecting endoscopic response and remission at different time points.

## Introduction

Crohn's disease (CD) is a chronic inflammatory disease, which can affect the complete gastrointestinal (GI) tract. It is characterized by a relapse-remitting pattern with often an onset in young adulthood.[1] In the treatment of CD, close-monitoring in a treat-to-target setting is a key principle to prevent relapse and complications.[2] Although the presence of clinical symptoms might reflect active inflammation, clinical scoring indices show poor correlation with the true state of disease activity; hence, other objective measures are needed.[2]

Endoscopy has become the gold standard to objectify active inflammation [1, 2]. However, it is invasive, expensive and not without risks.[3, 4] Consequently, it is an unattractive tool for frequent monitoring. Alternatively, non-invasive biochemical markers such as C-reactive protein (CRP) and fecal calprotectin (FCP) are being used and theoretically attractive. However, they lack ability to determine disease location, severity and extent of disease activity and are not always accurate.[5, 6]

Intestinal ultrasound (IUS) is a promising non-invasive, cross-sectional imaging technique that has a low cost and high accessibility. Previous studies showed a high accuracy of IUS to detect disease activity, severity and extent when compared to endoscopy or MRI.[7-9] Furthermore, reliability is high among different operators.[10] Predominantly bowel wall thickness (BWT), combined with colour Doppler signal (CDS) indicate presence of disease activity in most patients. Multiple cross-sectional studies have confirmed these findings.[8, 11] So far, studies assessing the capability of IUS to measure change (i.e. responsiveness) after initiation of treatment in CD using IUS are limited, particularly with endoscopy as the reference standard.

In addition B-mode and Doppler parameters, contrast-enhanced ultrasound (CEUS) has been investigated.[12-15] Inflammation in CD leads to increased micro-vessel density and a local dysregulation of blood flow in the GI-wall.[16-18] This causes changes in the bowel wall perfusion, which can be quantified with CEUS. Previous studies have shown a role for CEUS in determining disease activity at endoscopy and furthermore in predicting endoscopic response and remission in an early phase.[12-14] However, data are conflicting and limited.

In this study, we aimed to investigate conventional IUS parameters and CEUS parameters to predict endoscopic treatment response and remission early after treatment initiation. If IUS shows treatment response in an early phase, this could be utilized as an early surrogate marker for endoscopic assessment and this could allow for tight monitoring. In addition, we aimed to determine cut-off values for IUS and CEUS parameters to reflect endoscopic treatment response and remission.

## Materials and Methods

*Study design*

This was a single center, longitudinal, prospective cohort study. Patients ≥18 years of age with active CD at endoscopy (simple endoscopic score for Crohn's disease (SES-CD)≥3 in at least one segment) starting treatment with TNF$\alpha$ inhibitors (adalimumab or infliximab) were eligible for inclusion.

Patients were excluded when there was no endoscopy performed at the start of treatment and treatment was changed between baseline endoscopy and IUS examination. Previous TNF-$\alpha$ inhibitor use, pregnancy, obesity (BMI>35 kg/m2), chronic obstructive lung disease, unstable heart disease, ongoing gastroenteritis or a previous allergic reaction to SonoVue or its components were also exclusion criteria. In addition, patients were excluded when there was no thickened bowel segment at IUS or endoscopy did not show at least aphthous ulcers. All patients were informed and gave informed consent. This study was approved by the medical ethical committee of the Amsterdam University Medical Center (MEC2015_359).

*Procedures*

Medical history and demographics were collected at baseline. At start of treatment (T0), after 4-8 weeks (T1) and after 12-34 weeks (T2) the Harvey-Bradshaw Index (HBI) score, C-reactive protein (CRP), albumin, haemoglobin, leukocyte count, thrombocyte count and fecal calprotectin (FCP) levels were collected and IUS with CEUS was performed. At T0 and T2 a complete ileocolonoscopy was performed.

*Intestinal ultrasound measurements*

All the IUS examinations were performed by one of 3 trained ultrasonographers (K.N., S.B. and F.V) using an Epiq 5G ultrasound scanner (Philips, the Netherlands) with a C5-1 convex and L12-5 linear probe. Frequency, focus and gain settings were optimised to get the best images in the patient. For CDS the L12-5 transducer was used with a velocity scale of 5 cm/s for registration of the slow flow in the GI wall. The terminal ileum (TI) and large intestines were scanned by following its course from the TI in the right lower quadrant to the rectum while the small intestine was examined by scanning systematically through the nine sectors of the abdomen. Images and cine loops of pathological segments were stored per segment. At the time of IUS, the sonographer was blinded to biochemical and endoscopic disease activity information.

At least 3 months after IUS examination and blinded to all other patient data and each other's data, two raters (S.B. (5 years of IUS experience) and F.V. (3 years of IUS experience)) independently scored all IUS parameters per segment (Table 1) using a DICOM-viewer (RadiAnt DICOM Viewer [Software]. Version 2016). As there is a current lack of consensus per

parameter, measurements and definitions were based on current literature and were discussed in a study team consensus meeting before the scoring procedures started (10, 34). Next to individual parameters, presence or absence of disease activity was scored per rater. In addition, the most severely affected segment was defined as the segment with the highest BWT and was also independently determined by the two raters.

*Contrast-enhanced ultrasound measurements*

The L12-5 transducer was used together with contrast specific presets on an Epiq 5G ultrasound scanner that were equal for all patients. The most affected (i.e. thickest) bowel segment was chosen for CEUS measurements. At T1 and T2 CEUS measurements were performed in the same segment, also when BWT normalized. The mechanical index (MI) was set as close to 0.05 as possible by adjusting power and depth, and the focus region was set just below the area of interest. The gain was kept constant during the study. 2.4 mL of contrast agent (Sonovue, Bracco, Milan, Italy) with 10 mL 0.9% saline was administered via a venous catheter with a diameter of at least 1.1 mm in the left elbow vein. Immediately after administration, a cine-loop was recorded for 90 seconds. This procedure and recording was performed twice in the same segment.



At post-processing all CEUS cine-loops were analysed by one ultrasonographer (F.V.) with VueBox (Bracco, Milan, Italy). The complete bowel with surrounding mesentery was delineated using the peritoneum as delineation border. Then, motion compensation was applied utilizing the peak-enhancement (PE) slide as reference standard. Subsequently, images with >1 cm motion out of the delineation area were omitted. The cine-loop with least omitted images was used in further processing and analysis. Then, four region of interests (ROI) were drawn with ROI1 encompassing the complete anterior wall, ROI2 the submucosa, ROI3 a single vessel in mucosa or submucosa and ROI 4 mucosa and submucosa (Supplementary Figure 1a-b). All ROIs (except ROI3) had to be over 0.05 cm2 surface and at least one ROI had to reach a quality of fit ≥ 85%.[19]

Subsequently, data on peak enhancement (PE), wash-in area under the curve (WiAUC), rise time (RT), mean transit time (MTT), time to peak (TTP), wash-in rate (WiR), wash-in perfusion index (WiPi), wash-out area under the curve (WoAUC), wash-in and wash-out area under the curve (WiWoAUC), fall time (FT) and wash-out rate (WoR) were collected both as linear and log converted data (decibel) or seconds (s). To assess inter-observer agreement for CEUS measurements 30 CEUS cine-loops were randomly selected and similarly rated by a second reader (K.N.).

*Ileocolonoscopy*

The patients were also scheduled for ileocolonoscopy at T=0 and T=2. All examinations were performed by trained gastroenterologists. The interval between the first ileocolonoscopy and IUS was always shorter than 12 weeks without changes in treatment between the procedures. The ileocolonoscopies were directly scored per segment for SES-CD. In addition, the most affected segment was determined as the segment with the highest SES-CD score. Segmental endoscopic remission was defined as SES-CD=0, segmental endoscopic treatment response was defined as a decrease of SES-CD≥50% and complete endoscopic remission was defined as SES-CD=0 in all segments. The performing gastroenterologist was blinded for the results of IUS.

*Statistics*

Statistical analysis was performed with SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA). All normally distributed data were reported in mean ± SD and non-normally distributed in median and interquartile range (IQR). Mann-Whitney-U tests were used to compare continuous non-parametric variables, Chi-square tests for dichotomous variables and Wilcoxon rank tests or McNemar tests for paired samples. Area under the curve (AUROC) was used to determine accuracy, sensitivity and specificity. Logistic regression was used to determine odds ratios and for univariable and multivariable analysis. Spearman correlation coefficient was used to determine correlation with 0.00–0.09, 0.10–0.39, 0.40–0.69, 0.70–0.89, 0.90–1.00 considered as negligible, weak, moderate, strong, very strong correlation, respectively. Inter-observer agreement was assessed with intra-class correlation coefficient (ICC), weighted kappa (κ) and Cohen's kappa (κ) for continuous, ordinal and dichotomous outcomes.[20, 21] For ICC, a value below 0.50 was considered as poor, 0.50-0.75 as moderate, 0.75-0.90 as substantial and 0.90-1.00 as strong agreement. Kappa statistics 0.0-0.20, 0.21-0.40, 0.41-0.60, 0.61-0.80 and 0.81-1.00 were considered as slight, fair, moderate, substantial and perfect agreement, respectively. For ICC a p-value of 0.05 was used to determine significance.

**Table 1.** *Intestinal ultrasound parameters.*

| IUS parameter | Technique/categories | Pathologic |
|---|---|---|
| Bowel wall thickness | (2x longitudinal plane + 2 x cross-sectional plane)/4 | ≥3.0 mm |
| Colour Doppler Signal | 1: absent;<br>2: small spots (single vessels) within the wall;<br>3: long stretches within the wall;<br>4: long stretches extending into the mesentery<br>5: measurement failed | Category 3 or 4 |
| Wall layer stratification | 1: preserved;<br>2: focal loss (< 3 cm extent)<br>3: extensive loss (≥ 3 cm extent)<br>4: measurement failed | Category 2 or 3 |
| Presence of inflammatory fat | 1: absent;<br>2: uncertain;<br>3: present | Category 3 |
| Presence of enlarged lymph nodes (≥5 mm in shortest axis) | 1: absent;<br>2: uncertain;<br>3: present | Category 3 |
| Motility in terminal ileum | 1: present;<br>2: uncertain;<br>3: absent | Category 3 |
| Colonic haustrations | 1: preserved;<br>2: uncertain<br>3: loss | Category 3 |

## Results

*Baseline and follow-up characteristics*

From April 2016 to March 2020 we included 40 consecutive CD patients with active disease starting anti-TNF$\alpha$ treatment. Baseline characteristics are shown in Table 2. Follow-up is demonstrated in Supplementary figure 2. In total, 23 patients underwent a second endoscopy at T2 (median: 22 weeks [IQR: 19-26.5]) to evaluate treatment response. In further analysis, all patients that underwent surgery between T0 and T2 were considered as non-responders.

At baseline endoscopy the worst segment was the TI in 26/40 and a colonic segment in 14/40 patients, respectively. At baseline IUS the TI was the worst segment in 25/40 patients and a colonic segment in 15/40 patients (Table 2). Per segment analysis (sigmoid, descending, transverse, ascending colon and TI) showed strong correlation between endoscopic and IUS presence or absence of disease ($\rho$=0.81, p<0.0001). BWT was higher in patients with disease activity in the TI than in the colon (5.8 ± 1.5 mm vs 4.9 ± 1.2 mm, p=0.04). There was a mean of 38 ± 38 days between baseline ileocolonoscopy and IUS without change in treatment.

At T2, 14/40 (35.0%) [14/23 (60.9%)] patients had complete endoscopic remission in all segments and 17/40 (42.5%) [17/23 (73.9%)] had endoscopic response. Patients with a colonic segment as the most severely affected at T0, were more likely to have endoscopic response (OR: 2.59 (95%CI: 1.43-4.70), p=0.004) and remission (OR: 3.07 (95%CI: 1.44-6.52), p=0.006) at T2 as compared to patients with disease activity in the TI at T0. There was a mean of 39 ± 21 days between ileocolonoscopy and IUS at T2.

**Table 2.** *Baseline characteristics.*

| **Baseline** | **n = 40** |
|---|---|
| Female; n (%) | 20 (50.0%) |
| Age at inclusion; median (range), years | 33 (18-68) |
| Disease duration in median years (IQR) | 3.88 (1-14.25) |
| Montreal classification in CD patients | |
| A1 (<16 years) | 5 (12.5%) |
| A2 (17-40 years) | 27 (67.5%) |
| A3 (>40 years) | 8 (20.0%) |
| L1 (ileum) | 17 (42.5%) |
| L2 (colon) | 9 (22.5%) |
| L3 (ileocolonic) | 14 (35.0%) |
| B1 (non stricturing, non-penetrating) | 16 (40.0%) |
| B2 (stricturing) | 12 (30.0%) |
| B3 (penetrating) | 12 (30.0%) |
| P (Perianal disease) | 11 (27.5%) |
| Previous surgical resection at time of IUS | |
| ICR and ileal re-resections | 15 (37.5%) |
| (partial) colonic resection | 5 (12.5%) |
| Medication use in medical history | |
| Biologicals (infliximab, adalimumab, vedolizumab, ustekinumab) | 15 (37.5%) |
| Immunomodulators (thiopurines/methotrexate) | 20 (50.0%) |
| Corticosteroids | 30 (75.0%) |
| Aminosalicylates | 8 (20.0%) |
| Medication use at inclusion | |
| Corticosteroids | 6 (15.0%) |
| Aminosalicylates | 1 (2.5%) |
| Immunomodulators (thiopurines/methotrexate) | 25 (62.5%) |
| Medication after inclusion | |
| Infliximab | 28 (70.0%) |
| Adalimumab | 12 (30.0%) |
| Clinical and biochemical parameters in median (IQR) | |
| Harvey-Bradshaw Index | 5.0 (3.0-8.0) |
| C-reactive protein in mg/L | 8.25 (2.43-30.08) |
| Hemoglobin in mmol/L | 8.10 (7.40-8.88) |
| Leukocyte count in 109/L | 7.65 (5.95-10.75) |
| Platelet count 1012/L | 340.0 (269.25-405.25) |
| Albumin in g/L | 41.0 (37.75-44.25) |
| Fecal calprotectin in µg/g | 688.0 (382.0-1810.50) |

**Table 2.** *(Continued).*

| | |
|---|---|
| Intestinal ultrasound parameters | |
| Most severe affected segment | |
| -Sigmoid colon | 5 (12.5%) |
| -Descending colon | 4 (10.0%) |
| -Transverse colon | 2 (5.0%) |
| -Ascending colon | 4 (10.0%) |
| -Terminal ileum | 25 (62.5%) |
| Bowel wall thickness in mm (median and IQR) | 5.21 (4.60-6.84) |
| Colour Doppler Signal | |
| -No signal | 1 (2.5%) |
| -Single vessel | 5 (12.5%) |
| -Stretches within the wall | 21 (52.5%) |
| -Stretches in the wall and mesentery | 10 (25.0%) |
| -Measurement failed | 3 (7.5%) |
| Loss of stratification | |
| -Preserved | 19 (47.5%) |
| -Focal loss (< 3 cm) | 11 (27.5%) |
| -Extensive loss (≥ 3 cm) | 7 (17.5%) |
| -Measurement failed | 3 (7.5%) |
| Presence of inflammatory fat | |
| -Not present | 8 (20.0%) |
| -Uncertain | 6 (15.0%) |
| -Present | 26 (65.0%) |
| Presence of lymph nodes (> 5 mm in shortest axis) | 8 (20.0%) |
| -Present | 3 (7.5%) |
| -Uncertain | 29 (72.5%) |
| -Not present | |
| Motility in terminal ileum (n=25) | |
| -Present | 3 (12.5%) |
| -Uncertain | 3 (12.5%) |
| -Absent | 19 (79.2%) |
| Colonic haustrations (n=15) | |
| -Loss | 11 (73.3%) |
| -Preserved | 4 (26.7%) |

**Table 2.** *(Continued).*

| | |
|---|---|
| Endoscopic parameters | |
| Most severe affected segment | |
| -Rectum | 1 (2.5%) |
| -Sigmoid colon | 6 (15.0%) |
| -Descending colon | 2 (5.0%) |
| -Transverse colon | 2 (5.0%) |
| -Ascending colon | 3 (7.5%) |
| -Terminal ileum | 26 (65.0%) |
| Total SES-CD score (median and IQR) | 9.0 (5.25-15.00) |
| SES-CD of most affected segment (median and IQR) | 6.50 (3.25-8.00) |

*IQR= interquartile range, ICR= ileocecal resection*



*IUS parameters*

*Correlation with SES-CD*

There was moderate to strong correlation for BWT (ϱ=0.61, p<0.0001) (Figure 1), CDS (ϱ=0.73, p<0.0001), loss of motility (ϱ=0.50, p=0.001), presence of inflammatory fat (ϱ=0.58, p<0.0001) and loss of haustrations (ϱ=0.48, p=0.031) with presence of disease activity at endoscopy (≥SES-CD 1) both at T0 and T2. In addition, there was weak correlation for loss of wall layer stratification (WLS) (ϱ=0.34, p=0.007). ΔBWT at T1 (ϱ=0.54, p=0.003) and T2 (ϱ=0.47, p=0.025) correlated moderately with ΔSES-CD for the most severe segment (Figure 2).

*Bowel wall thickness*

Patients with endoscopic response had a significantly lower BWT at T1 and T2 compared to patients without endoscopic response (Figure 3). Similarly, for endoscopic remission, BWT was lower at T1 (3.1 mm [1.9-4.2] vs 5.3 mm [3.8-6.9], p=0.005) and T2 (2.0 mm [1.8-3.1] vs 5.1 [3.0-6.3] mm, p=0.002). In addition, decrease in BWT at T1 was significantly different between endoscopic responders versus non-responders (ΔBWT: -1.7 mm [-2.6- -0.2] vs -0.1 mm [-1.1-0.7], p=0.012), respectively. At T2, BWT decreased further compared to T0 and was significantly different between endoscopic responders and non-responders (ΔBWT: -2.5 mm [-3.3- -1.4] vs -0.7 [-1.5- -0.2], p=0.035), respectively.

At T2, a BWT cut-off value of 3.2 mm was most accurate to predict endoscopic remission (AUROC: 0.940, 95%CI: 0.862-1.000, p<0.0001) with 70% sensitivity and 85% specificity. For prediction of endoscopic response a decrease in BWT from baseline expressed in percentage was most accurate. At T1, a 18% decrease in BWT predicted endoscopic response (AUROC:

0.765, 95%CI: 0.580-0.949, p=0.02) with 82% sensitivity and 71% specificity. A 29% decrease in BWT at T2 predicted endoscopic response (AUROC: 0.833, 95%CI: 0.626-1.000, p=0.017) with 83% sensitivity and 88% specificity

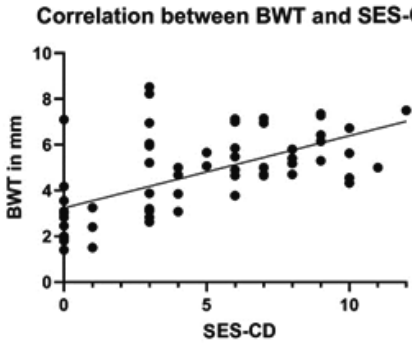



**Figure 1.** *Correlation between SES-CD score and BWT for the most severe affected segment (ρ=0.61, p<0.0001).*

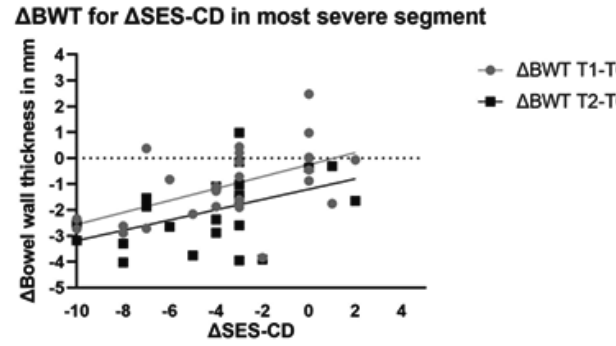
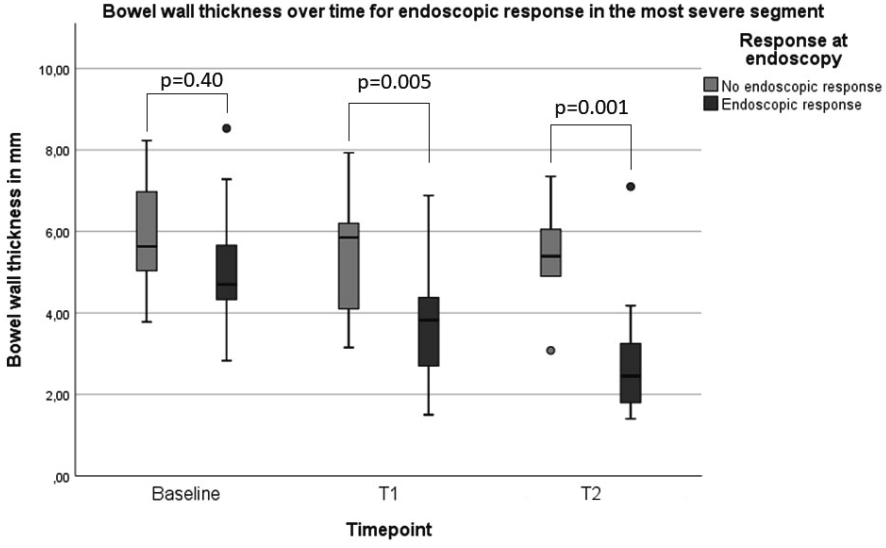



**Figure 2.** *Correlation between ΔBWT (T1 (ρ=0.54, p=0.003) and T2 (ρ=0.47, p=0.025)) and ΔSES-CD for the most severe affected segment.*




| Time-point | Endoscopic response | Endoscopic non-response | p-value |
|---|---|---|---|
| Median BWT [IQR] at T0 | 4.7 mm [4.2-6.8] | 5.4 mm [4.7-7.0] | 0.40 |
| Median BWT [IQR] at T1 | 3.9 mm [2.5-4.8] | 5.4 mm [3.4-6.4] | 0.005 |
| Median BWT [IQR] at T2 | 2.5 mm [1.8-3.3] | 5.3 mm [4.0-6.4] | 0.001 |

**Figure 3.** *Decrease in BWT in the most severe affected segment at IUS according to endoscopic response and non-response at T1 and T2.*

*Other parameters*

When there was endoscopic response, presence of hyperemia (CDS 3 or 4) decreased significantly at T1 (T0: 87% vs T1: 35.5%, p=0.004) and T2 (T0: 87% vs T2 6%, p<0.0001) (Supplementary Figure 3a). A decrease of 1-point in CDS score at T1 (OR: 2.89, 95%CI: 1.054-7.907, p=0.039) and T2 (OR: 5.44, 95%CI: 1.258-23.478, p=0.023) was associated with endoscopic response. WLS normalized more frequently at T2 when there was endoscopic response (T0: 55% vs T2: 12%, p=0.016) but not at T1 (T0: 53% vs T1: 29%, p=0.289). However, a normalization of WLS at T1 (OR: 4.91, 95%CI: 0.496-48.622, p=0.174) or T2 (OR: 3.56, 95%CI: 0.326-38.777, p=0.30) respectively could not predict endoscopic response at T2. Presence of lymph nodes did not decrease significantly when there was endoscopic response at T1 (T0: 19% vs T1: 19%, p=1.00) or at T2 (T0: 19% vs T2: 6.3%, p=0.08). Presence of inflammatory fat did decrease with a trend towards significance at T1 (T0: 82% vs T1: 44%, p=0.07) and significantly at T2 (T0: 82% vs T2: 6%, p<0.0001), respectively (Supplementary Figure 3b). There was no significant change in presence of inflammatory fat when there was no endoscopic response. In patients with disease activity in the TI, motility returned at T2 (absence of motility T0: 88% vs T2: 10%, p=0.03) but not at T1 (absence of motility T0: 88% vs T1: 45%, p=0.25). When there was no endoscopic response at T2, there was no significant change in motility (data not shown). When there was endoscopic response, colonic haustrations normalized significantly at T1 (absence of haustrations T0: 78% vs absence of haustrations at T1: 14%, p=0.031) and at T2 (absence of haustrations T0: 78% vs absence of haustrations at T2: 0%, p=0.016). When there was no endoscopic response, there was no normalization of colonic haustrations (data not shown).

*Contrast-enhanced ultrasound*

40, 32 and 23 patients underwent CEUS at T0, T1 and T2, respectively. In 3 patients at T1 and 6 patients at T2 CEUS measurements were of low quality of fit due to a normal BWT (n=7) or present motility in the TI (n=2) and were excluded from further analysis. For the 17 patients at T2 with a second endoscopy and valid CEUS measurements available, 12 (71%) patients had endoscopic response and 8 (47%) patients were in endoscopic remission.

CEUS was analyzed for ROI 1, 2 and 3. ROI 4 measurements were omitted from further analysis as air in the lumen was often within the ROI resulting in uninterpretable data or a ROI matching ROI 2. Quality of fit (QoF) at baseline was high for ROI1 (median: 93.81% [84.93-96.17]), ROI2 (median: 88.21% [80.68-91.97]) and ROI3 (median: 93.06% [80.19-95.16]). At T1 and T2 QoF for all ROIs did not significantly differ with regards to baseline (data not shown). At T1 QoF for ROI 3 was lower when there was endoscopic response compared to no endoscopic response at T2 (median QoF ROI 3: 73.11% [47.28-84.94] vs 86.38% [81.59-93.47], p=0.012).

Supplementary Table 1a-c demonstrates the logarithmic differences in CEUS parameters for the three ROIs between intestinal segments with and without endoscopic remission at T2. The corresponding linear CEUS data is demonstrated in Supplementary Table 2a-c. At T1, none of the logarithmic or linear data could predict endoscopic remission (data not shown). For ROI1, percentage decrease of WoR at T1 was significantly different when there was endoscopic response compared to non-response at T2 (median ΔT0-T1: -32.55% [-44.74- -1.90] vs -1.28% [-12.58-49.53], p=0.04). At T2, decrease in percentage for PE, WiR, WiPI and WoR was significantly more pronounced in endoscopic responders (Supplementary Figure 4). For ROI2, ROI3 and the other CEUS parameters there was no significant change distinguishing endoscopic responders from non-responders at T1 or T2. Also, for the linear data at T1 or T2 there was no significant change with regards to baseline for all three ROI's (data not shown).

*Clinical and biochemical response*

HBI, ΔHBI, FCP and ΔFCP values were significantly different between endoscopic responders and non-responders at T1 and T2 (Supplementary Table 3). Corresponding AUROC and cut-off values for FCP are demonstrated in Supplementary Table 4. All other (change in) biochemical parameters were not significantly different between endoscopic responders and non-responders.



*Multivariable regression for endoscopic remission and response with conventional IUS and CEUS parameters, HBI and FCP*

When BWT was dichotomized with a cut-off value of 3.2 mm endoscopic remission was shown (OR for BWT≤3.2 mm: 39.42, 95%CI: 7.67-202.57, p<0.0001) and normalization of CDS (CDS<3) significantly improved the model (OR: 13.76, 95%CI: 1.28-147.78, p=0.03). Adding the other IUS parameters, FCP or HBI did not significantly improve the model. In addition, BWT decrease of 18% and 29% at T1 (OR: 10.80, 95%CI: 1.69-68.94, p=0.012) and T2 (OR: 37.50, 95%CI: 2.77-507.48, p=0.006) predicted endoscopic response, respectively. The other IUS parameters, FCP and HBI did not significantly improve the model to predict endoscopic response. When combined with BWT, WoR significantly improved the model to predict endoscopic remission at T2 (WoR per 1 dB increase: OR: 0.76, 95%CI: 0.60-0.96, p=0.019). All other CEUS parameters did not improve the model. In addition, at T1, none of the absolute CEUS values nor change in CEUS parameters at T1 or T2 contributed to the model with BWT to predict endoscopic response.

*Inter-observer agreement per segment*

There was perfect agreement on the most affected segment between the two raters (κ=0.81, 95%CI: 0.68-0.93, p<0.0001). Per segment agreement on presence of disease activity is demonstrated in Supplementary Table 5. For BWT, there was strong agreement for sigmoid colon (ICC: 0.979, 95%CI: 0.938-0.993, p<0.0001), ascending colon (ICC: 0.971, 95%CI: 0.855-

0.994, p<0.0001) and TI (ICC: 0.953, 95%CI: 0.917-0.973, p<0.0001). There was substantial and moderate agreement for descending (ICC: 0.884, 95%CI: 0.669-0.960, p<0.0001) and transverse colon (ICC: 0.697, 95%CI: 0.006-0.907, p=0.024), respectively. Agreement per parameter and per segment is demonstrated in Supplementary Table 6.

*Inter-observer agreement for CEUS*

For CEUS measurements there was moderate to strong agreement (Supplementary Table 7). Particularly, WoR had strong agreement (ICC: 0.943 (0.875-0.974, p<0.0001). For the other ROIs (single vessel and submucosa) there was moderate to strong agreement (data not shown).

## Discussion

IUS, particularly BWT and CDS, showed moderate to strong correlation with the SES-CD in the most severe affected segment. In addition, BWT and CDS were subjective to response and decreased in patients with endoscopic response (Figure 4). After 4-8 weeks after treatment initiation BWT showed already a significant decrease with 18% thereby predicting endoscopic response with high accuracy (OR: 10.80, 95%CI: 1.69-68.94, p=0.012). Accuracy increases after 12-34 weeks with a BWT decrease of 29% being most accurate to determine endoscopic response (OR: 37.50, 95%CI: 2.77-507.48, p=0.006) and a cut-off value of 3.2 mm most accurate to reflect endoscopic remission (OR: 39.42, 95%CI: 7.67-202.57, p<0.0001). Although other IUS parameters and CEUS parameters also decrease when there is endoscopic response they are of limited merit in predicting and determining endoscopic outcomes in addition to BWT and CDS. Furthermore, inter-observer agreement for both conventional IUS parameters and CEUS parameters was good to perfect indicating a high reliability for IUS and CEUS in clinical practice.



In this study, we have demonstrated high accuracy for IUS, and specifically BWT to predict endoscopic response and remission early after initiation of anti-TNF$\alpha$ treatment. Previous studies have demonstrated the value of IUS to measure treatment response.[12, 22-31] The largest study was conducted by Kucharzik et al and showed IUS to detect response to treatment in a large cohort of CD patients clinically responding to anti-inflammatory treatment.[28] Most IUS parameters normalized within the first 3 months of treatment, which is in concordance with our findings. However, a robust reference standard was not present. In our study, predominantly BWT, CDS and inflammatory fat were discriminative in an early phase and are perhaps the most responsive to improvement and healing of the wall which has also been demonstrated in recent studies.[12, 22, 24-26, 29, 31] Moreover, these parameters were often pathologic at baseline in most patients whereas WLS and presence of lymph nodes were less frequently seen and, accordingly, also less responsive to change during treatment.

Previous studies have investigated IUS response according to endoscopic response and remission.[22-26, 29, 31, 32] Although these studies found favorable outcomes for patients reaching transmural healing in addition to mucosal healing, definitions for transmural healing varied and were not validated. Therefore, in our cohort, we decided to investigate which IUS parameters best reflect endoscopic response and remission. To our knowledge, this is first study presenting accurate cut-off values and decrease in percentages for BWT indicating endoscopic remission and response in the corresponding segments, respectively. Intriguingly, a BWT cut-off value of 3.2 mm reflected endoscopic remission both in the TI and colon accurately. Whereas in diagnosing CD a cut-off value of 2.0 mm (especially in the TI) or 3.0 mm, is not uncommon, this might be too stringent to determine endoscopic remission after anti-inflammatory treatment.[8, 11] Moreover, fibrosis or scar tissue might results in a thickened bowel wall.[33] However, a certain proportion of patients reached a cut-off value <2.0 mm or 3.0

mm. Consequently, even in patients with a BWT<3.2 mm, there might be room for improvement. Future research should elucidate this.

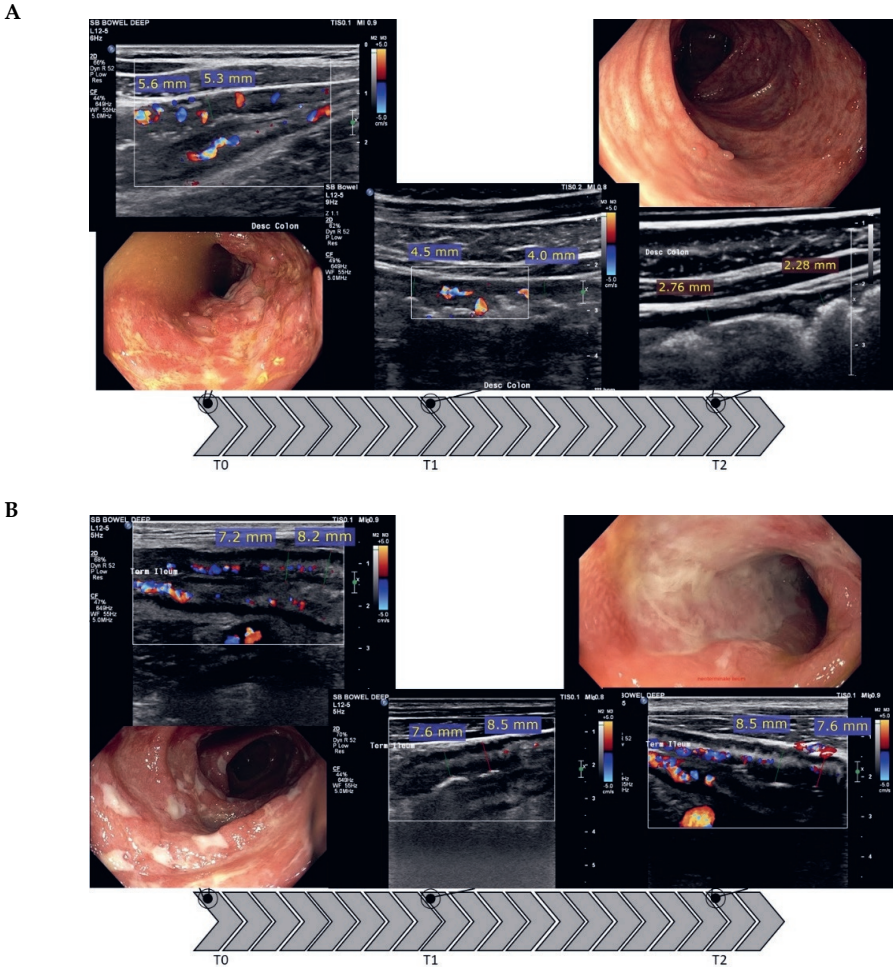



**Figure 4.** (*A) Response on IUS and paired colonoscopy in the most severe affected segment (descending colon). BWT decreases with 22% at T1 from 5.5 mm (T0) to 4.3 mm (T1). At T2 BWT decreased further to 2.6 mm or 53% with regards to baseline. Also Colour Doppler Signal shows improvement over time and colonic haustrations have returned. SES-CD=7 at baseline with deep ulcerations present. At T2, SES-CD=0 with no ulcers present, presence of pseudopolyps and mucosal scar tissue. (B) No response on IUS and paired colonoscopy in the most severe affected segment (terminal ileum). BWT shows no improvement with 7.7 mm at baseline, 8.1 mm at T1 and 8.1 mm at T2. Colour Doppler Signal improves at T1 but deteriorates at T2 and is similar to baseline. SES-CD=10 in the neoterminal ileum at baseline. At T2, SES-CD=9, no endoscopic response.*

IUS is also sensitive enough to demonstrate endoscopic response. A 29% decrease of BWT at T2 reflected endoscopic treatment response. A recent study defining treatment response according to SES-CD score and the Crohn's Disease Activity Index found similar findings after 12 weeks of treatment with anti-TNF$\alpha$.[34] Another study defined IUS response as a decrease of 25% in BWT and found moderate correlation with endoscopic response after 16 weeks of treatment with ustekinumab according to the SES-CD score in the corresponding segment.[27] In our cohort, a decrease of 18% at T1 could already reflect endoscopic treatment response in an early phase. However, future research should confirm this statement in prospective designs with larger patient cohorts.

Most CEUS parameters significantly reflected endoscopic disease activity, in line with previous studies.[12, 30, 35, 36] In our cohort only percentage change in WoR and FT could significantly predict endoscopic response and non-response whereas previous studies also showed other parameters to reflect endoscopic response.[12, 34] Quaia et al. found a significant percentage change in most CEUS parameters after 6 weeks of treatment between endoscopic responders and non-responders after 14 weeks of treatment. Similarly, Laterza et al. found a percentage change for almost all evaluated CEUS parameters already after 2 to 6 weeks of treatment in endoscopic responders after 12 weeks.[34] In contrast to these previous studies, we had to exclude patients due to normalization of the bowel wall or return of motility resulting in poor CEUS cine-loops while most of these patients were endoscopic responders. Consequently, change for CEUS parameters during treatment within the endoscopic responding group might be underestimated. Although we reached high inter-observer agreements for most CEUS parameters in a subset of randomly selected patients, CEUS might become less feasible when BWT normalizes. Secondly, we included multiple CD phenotypes whereas other studies included only patients with terminal ileitis. Since there is emerging evidence that TI and colonic CD respond differently to treatment, CEUS might be more suitable for the TI to detect early changes.[37, 38] However, this is not supported by actual data as we had limited amount of patients and separate analyses for colon and TI were not feasible. Thirdly, not all patients had complete follow-up or stopped treatment for various reasons. This resulted in fewer follow-up endoscopies, but it also reflects real-world data.

While BWT is the most important parameter indicating endoscopic remission, CDS or WoR added significantly to the model reflecting endoscopic remission. Two recent studies using consensus panels found a combination of BWT with CDS accurately to reflect endoscopic disease activity and endoscopic response.[10, 39] In addition, a recent and partly validated scoring index with endoscopy as reference standard incorporated both BWT and CDS.[40] In clinical practice, patients with a normal BWT but increased CDS are less likely to have reached complete endoscopic remission for the specific segment. Whether this should lead to dose escalation or change of treatment is unclear and is subject for future studies. Potentially, WoR could fulfill a similar role as CDS. While promising and reproducible, measuring WoR is more difficult and time-consuming than CDS as a patient needs contrast administration and CEUS

cine-loops need post-processing. Especially in a point-of-care setting this is less attractive. In addition, a recent study found similar accuracy for a model with BWT and CDS compared to a model with BWT, CDS and CEUS parameters.[30] Since CDS is a reliable parameter to score, CDS deserves recommendation over WoR to be incorporated in future scoring indices and definitions for transmural response or healing.[10] Consequently, the role for CEUS in treatment follow-up and response assessment is limited.

In addition, we have shown that next to BWT, decrease in FCP accurately detects endoscopic response in an early phase. However, early absolute measurements for FCP could not predict endoscopic response or remission. Moreover, FCP is subjective to other circumstances which could lead to false-positive or negative results when compared to endoscopic outcomes.[41, 42] In our cohort, we have demonstrated that both absolute measurements and change in BWT measurements reflects endoscopic disease activity at a later stage. Consequently, BWT is not inferior to FCP and additionally informs on disease extent. Incorporating FCP in an IUS parameter based prediction model did not significantly improve the model to predict endoscopic response or remission.

Our study has a few limitations. Some patients did not reach T2 because of surgery, worsening disease or loss to follow-up. Although this is suboptimal for the analysis, our results truly reflect clinical practice which might therefore also be a strength of this study. Also, time between IUS and endoscopy was in some patients suboptimal. We generally performed IUS and CEUS with the first anti-TNF$\alpha$ administration and reflects a real-world and point of care setting. Furthermore, we scored IUS and CEUS cine-loops and images per segment after the patient visit which might have resulted in a certain bias for inter-observer agreement. IUS is operator-dependent and ideally inter-observer agreement is scored in a real-time setting with blinded operators. However, our scoring methods approaches a clinical trial setting with central reading and we have demonstrated a feasible and reliable process using still images and cine-loops to score IUS and CEUS parameters.

Our study also has several strengths. Sonographer and gastroenterologists were blinded to the other examinations. In addition, we used a validated and robust endoscopic reference standard and have shown good correlation with IUS and CEUS parameters. Also, we did not predefine IUS response or remission but showed changes on IUS and demonstrated cut-off values for BWT according to endoscopic changes.

In conclusion, we have demonstrated IUS response to anti-TNF$\alpha$ treatment according to endoscopic treatment response and remission. As endoscopy is still the gold standard but invasive, IUS, and especially decrease of BWT in percentages, has potential to determine endoscopic response in most patients for both the TI and colon. In addition, this is feasible in an early phase. The additional value of performing (early) CEUS in this perspective was limited in our study. Defining, standardization and validation of transmural healing and

transmural response should be a next step in incorporation of IUS in research and clinical practice.

## References


1. Maaser C, Sturm A, Vavricka SR, Kucharzik T, Fiorino G, Annese V, et al. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. Journal of Crohn's and Colitis. 2019;13(2):144-64K.

2. Turner D, Ricciuto A, Lewis A, D'Amico F, Dhaliwal J, Griffiths AM, et al. STRIDE-II: an update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) initiative of the International Organization for the Study of IBD (IOIBD): determining therapeutic goals for treat-to-target strategies in IBD. Gastroenterology. 2021;160(5):1570-83.

3. Buisson A, Gonzalez F, Poullenot F, Nancey S, Sollellis E, Fumery M, et al. Comparative acceptability and perceived clinical utility of monitoring tools: a nationwide survey of patients with inflammatory bowel disease. Inflammatory bowel diseases. 2017;23(8):1425-33.

4. Terheggen G, Lanyi B, Schanz S, Hoffmann R, Böhm S, Leifeld L, et al. Safety, feasibility, and tolerability of ileocolonoscopy in inflammatory bowel disease. Endoscopy. 2008;40(08):656-63.

5. Gecse KB, Brandse JF, Van Wilpe S, Löwenberg M, Ponsioen C, Van den Brink G, et al. Impact of disease location on fecal calprotectin levels in Crohn's disease. Scandinavian journal of gastroenterology. 2015;50(7):841-7.

6. Schoepfer AM, Beglinger C, Straumann A, Trummler M, Vavricka SR, Bruegger LE, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Official journal of the American College of Gastroenterology| ACG. 2010;105(1):162-9.

7. Dong J, Wang H, Zhao J, Zhu W, Zhang L, Gong J, et al. Ultrasound as a diagnostic tool in detecting active Crohn's disease: a meta-analysis of prospective studies. European radiology. 2014;24(1):26-33.

8. Goodsall TM, Nguyen TM, Parker CE, Ma C, Andrews JM, Jairath V, et al. Systematic review: gastrointestinal ultrasound scoring indices for inflammatory bowel disease. Journal of Crohn's and Colitis. 2021;15(1):125-42.

9. Panes J, Bouzas R, Chaparro M, García-Sánchez V, Gisbert J, Martínez de Guereñu B, et al. Systematic review: the use of ultrasonography, computed tomography and magnetic resonance imaging for the diagnosis, assessment of activity and abdominal complications of Crohn's disease. Alimentary pharmacology & therapeutics. 2011;34(2):125-45.

10. Novak KL, Nylund K, Maaser C, Petersen F, Kucharzik T, Lu C, et al. Expert Consensus on Optimal Acquisition and Development of the International Bowel Ultrasound Segmental Activity Score [IBUS-SAS]: A Reliability and Inter-rater Variability Study on Intestinal Ultrasonography in Crohn's Disease. Journal of Crohn's and Colitis. 2021;15(4):609-16.

11. Bots S, Nylund K, Löwenberg M, Gecse K, Gilja OH, D'Haens G. Ultrasound for assessing disease activity in IBD patients: a systematic review of activity scores. Journal of Crohn's and Colitis. 2018;12(8):920-9.

12. Quaia E, Gennari AG, Cova MA. Early Predictors of the Long-term Response to Therapy in Patients With Crohn Disease Derived From a Time-Intensity Curve Analysis After Microbubble Contrast Agent Injection. Journal of Ultrasound in Medicine. 2019;38(4):947-58.

13. Ripollés T, Martínez MJ, Paredes JM, Blanc E, Flors L, Delgado F. Crohn disease: correlation of findings at contrast-enhanced US with severity at endoscopy. Radiology. 2009;253(1):241-8.

14. Saevik F, Nylund K, Hausken T, Ødegaard S, Gilja OH. Bowel perfusion measured with dynamic contrast-enhanced ultrasound predicts treatment outcome in patients with Crohn's disease. Inflammatory bowel diseases. 2014;20(11):2029-37.

15. Piscaglia F, Nolsøe C, Dietrich Ca, Cosgrove D, Gilja O, Nielsen MB, et al. The EFSUMB Guidelines and Recommendations on the Clinical Practice of Contrast Enhanced Ultrasound (CEUS): update 2011 on non-hepatic applications. Ultraschall in der Medizin-European Journal of Ultrasound. 2012;33(01):33-59.

16. Maconi G, Parente F, Bollani S, Imbesi V, Ardizzone S, Russo A, et al. Factors affecting splanchnic haemodynamics in Crohn's disease: a prospective controlled study using Doppler ultrasound. Gut. 1998;43(5):645-50.

17. Danese S, Sans M, De La Motte C, Graziani C, West G, Phillips MH, et al. Angiogenesis as a novel component of inflammatory bowel disease pathogenesis. Gastroenterology. 2006;130(7):2060-73.

18. Hatoum OA, Binion DG, Otterson MF, Gutterman DD. Acquired microvascular dysfunction in inflammatory bowel disease: loss of nitric oxide-mediated vasodilation. Gastroenterology. 2003;125(1):58-69.

19. Wilkens R, Peters DA, Nielsen AH, Hovgaard VP, Glerup H, Krogh K. Dynamic contrast-enhanced magnetic resonance enterography and dynamic contrast-enhanced ultrasonography in Crohn's disease: an observational comparison study. Ultrasound international open. 2017;3(01):E13-E24.

20. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. Journal of chiropractic medicine. 2016;15(2):155-63.

21. Landis JR, Koch GG. The measurement of observer agreement for categorical data. biometrics. 1977:159-74.

22. Calabrese E, Rispo A, Zorzi F, De Cristofaro E, Testa A, Costantino G, et al. Ultrasonography tight control and monitoring in Crohn's disease during different biological therapies: a multicenter study. Clinical Gastroenterology and Hepatology. 2021.

23. Castiglione F, Imperatore N, Testa A, De Palma GD, Nardone OM, Pellegrini L, et al. One-year clinical outcomes with biologics in Crohn's disease: transmural healing compared with mucosal or no healing. Alimentary pharmacology & therapeutics. 2019;49(8):1026-39.

24. Castiglione F, Mainenti P, Testa A, Imperatore N, De Palma GD, Maurea S, et al. Cross-sectional evaluation of transmural healing in patients with Crohn's disease on maintenance treatment with anti-TNF alpha agents. Digestive and Liver Disease. 2017;49(5):484-9.

25. Castiglione F, Testa A, Rea M, De Palma GD, Diaferia M, Musto D, et al. Transmural healing evaluated by bowel sonography in patients with Crohn's disease on maintenance treatment with biologics. Inflammatory bowel diseases. 2013;19(9):1928-34.

26. Helwig U, Fischer I, Hammer L, Kolterer S, Rath S, Maaser C, et al. Transmural Response and Transmural Healing Defined by Intestinal Ultrasound-New Potential Therapeutic Targets? Journal of Crohn's and Colitis. 2021.

27. Kucharzik T, Wilkens R, Maconi G, Agostino M, Le Bars M, Nazar M, et al. Intestinal ultrasound response and transmural healing after ustekinumab induction in Crohn's disease: Week 16 interim analysis of the STARDUST trial substudy. Zeitschrift für Gastroenterologie. 2020;58(05):P04.

28. Kucharzik T, Wittig BM, Helwig U, Börner N, Rössler A, Rath S, et al. Use of intestinal ultrasound to monitor Crohn's disease activity. Clinical Gastroenterology and Hepatology. 2017;15(4):535-42. e2.

29. Moreno N, Ripollés T, Paredes JM, Ortiz I, Martínez MJ, López A, et al. Usefulness of abdominal ultrasonography in the analysis of endoscopic activity in patients with Crohn's disease: changes following treatment with immunomodulators and/or anti-TNF antibodies. Journal of Crohn's and Colitis. 2014;8(9):1079-87.

30. Ripollés T, Poza J, Suarez Ferrer C, Martínez-Pérez MJ, Martín-Algíbez A, de las Heras Paez B. Evaluation of Crohn's disease activity: development of an ultrasound score in a multicenter study. Inflammatory Bowel Diseases. 2021;27(1):145-54.

31. Zorzi F, Ghosh S, Chiaramonte C, Lolli E, Ventura M, Onali S, et al. Response assessed by ultrasonography as target of biological treatment for Crohn's disease. Clinical Gastroenterology and Hepatology. 2020;18(9):2030-7.

32. Chen J-M, He L-W, Yan T, Guo X-F, Hu P-J, Peng J-S, et al. Oral exclusive enteral nutrition induces mucosal and transmural healing in patients with Crohn's disease. Gastroenterology report. 2019;7(3):176-84.

33. Bettenworth D, Bokemeyer A, Baker M, Mao R, Parker CE, Nguyen T, et al. Assessment of Crohn's disease-associated small bowel strictures and fibrosis on cross-sectional imaging: a systematic review. Gut. 2019;68(6):1115-26.

34. Laterza L, Ainora ME, Garcovich M, Galasso L, Poscia A, Di Stasio E, et al. Bowel contrast-enhanced ultrasound perfusion imaging in the evaluation of Crohn's disease patients undergoing anti-TNFα therapy. Digestive and Liver Disease. 2021;53(6):729-37.

35. De Franco A, Di Veronica A, Armuzzi A, Roberto I, Marzo M, De Pascalis B, et al. Ileal Crohn disease: mural microvascularity quantified with contrast-enhanced US correlates with disease activity. Radiology. 2012;262(2):680-8.

36. Serafin Z, Białecki M, Białecka A, Sconfienza LM, Kłopocka M. Contrast-enhanced ultrasound for detection of Crohn's disease activity: systematic review and meta-analysis. Journal of Crohn's and Colitis. 2016;10(3):354-62.

37. Atreya R, Siegmund B. Location is important: differentiation between ileal and colonic Crohn's disease. Nature Reviews Gastroenterology & Hepatology. 2021:1-15.

38. Dulai PS, Singh S, Casteele NV, Boland BS, Rivera-Nieves J, Ernst PB, et al. Should we divide Crohn's disease into ileum-dominant and isolated colonic diseases? Clinical Gastroenterology and Hepatology. 2019;17(13):2634-43.

39. Goodsall TM, Jairath V, Feagan BG, Parker CE, Nguyen TM, Guizzetti L, et al. Standardisation of intestinal ultrasound scoring in clinical trials for luminal Crohn's disease. Alimentary Pharmacology & Therapeutics. 2021;53(8):873-86.

40. Sævik F, Eriksen R, Eide GE, Gilja OH, Nylund K. Development and validation of a simple ultrasound activity score for Crohn's disease. Journal of Crohn's and Colitis. 2021;15(1):115-24.

41. Cremer A, Ku J, Amininejad L, Bouvry M-R, Brohet F, Liefferinckx C, et al. Variability of faecal calprotectin in inflammatory bowel disease patients: an observational case-control study. Journal of Crohn's and Colitis. 2019;13(11):1372-9.

42. Du L, Foshaug R, Huang VW, Kroeker KI, Dieleman LA, Halloran BP, et al. Within-stool and within-day sample variability of fecal calprotectin in patients with inflammatory bowel disease. Journal of clinical gastroenterology. 2018;52(3):235-40.

## Supplementary Material

**Supplementary Table 1a.** *Logarithmic CEUS measurements for ROI1 (complete wall) for both T0 and T2 in the most severe affected segment for present endoscopic disease activity (SESCD≥1) and absent endoscopic disease activity (SESCD=0) analyzed with a non-parametric Mann-Whitney U Test.*

| | **Present endoscopic disease activity at T2 (n=9)** | **No present endoscopic disease activity at T2 (n=8)** | **p-value** |
|---|---|---|---|
| Peak enhancement in dB (median [IQR]) | 32.56 [30.51-33.65] | 25.74 [24.02-29.24] | **0.006** |
| Wash-in area under the curve in dB (median [IQR]) | 39.16 [35.97-40.21] | 34.42 [31.39-38.78] | 0.08 |
| Rise time in seconds (median [IQR]) | 7.45 [5.01-11.24] | 9.08 [7.63-12.34] | 0.24 |
| Mean transit time in seconds (median [IQR]) | 43.02 [20.12-104.42] | 58.78 [36.44-117.33] | 0.93 |
| Time to peak in seconds (median [IQR]) | 10.24 [6.21-14.92] | 11.38 [9.96-14.21] | 0.28 |
| Wash-in rate in dB (median [IQR]) | 25.60 [22.30-27.68] | 18.35 [14.60-21.10] | **0.008** |
| Wash-out area under the curve in dB (median [IQR]) | 42.74 [39.69-44.92] | 38.56 [35.23-44.90] | 0.32 |
| Wash-in and wash-out area under the curve in dB (median [IQR]) | 44.44 [41.22-46.14] | 39.99 [36.74-46.25] | 0.26 |
| Wash-in perfusion index in dB (median [IQR]) | 30.67 [28.77-31.92] | 23.84 [22.16-27.56] | **0.006** |
| Fall time in seconds (median [IQR]) | 17.13 [12.60-33.26] | 24.62 [19.33-40.67] | 0.26 |
| Wash-out rate in dB (median [IQR]) | 20.48 [15.81-21.81] | 13.30 [9.11-14.54] | **0.005** |

**Supplementary Table 1b.** *Logarithmic CEUS measurements for ROI2 (submucosa) for both T0 and T2 in the most severe affected segment for present endoscopic disease activity (SESCD≥1) and absent endoscopic disease activity (SESCD=0) analyzed with a non-parametric Mann-Whitney U Test.*

| | **Present endoscopic disease activity (n=9)** | **No present endoscopic disease activity (n=8)** | **p-value** |
|---|---|---|---|
| Peak enhancement in dB (median [IQR]) | 34.37 [32.06-36.79] | 29.83 [26.17-32.01] | **0.01** |
| Wash-in area under the curve in dB (median [IQR]) | 40.30 [37.95-42.87] | 36.88 [32.35-42.04] | 0.11 |
| Rise time in seconds (median [IQR]) | 6.31 [5.20-9.67] | 7.53 [5.80-10.28] | 0.33 |
| Mean transit time in seconds (median [IQR]) | 50.46 [22.63-83.80] | 47.57 [28.33-93.04] | 0.76 |
| Time to peak in seconds (median [IQR]) | 11.04 [6.28-14.62] | 10.44 [8.78-14.30] | 0.33 |
| Wash-in rate in dB (median [IQR]) | 28.07 [23.78-30.83] | 11.37 [19.05-24.64] | **0.01** |
| Wash-in perfusion index in dB (median [IQR]) | 32.40 [30.25-34.82] | 27.89 [24.23-30.10] | **0.01** |
| Wash-out area under the curve in dB (median [IQR]) | 43.09 [40.70-46.82] | 40.16 [35.46-45.57] | 0.28 |
| Wash-in and wash-out area under the curve in dB (median [IQR]) | 44.93 [42.56-48.40] | 41.84 [37.18-47.16] | 0.23 |
| Fall time in seconds (median [IQR]) | 16.79 [9.74-22.36] | 15.99 [12.78-23.98] | 0.51 |
| Wash-out rate in dB (median [IQR]) | 23.79 [19.28-24.23] | 17.12 [14.68-20.29] | **0.01** |

**Supplementary Table 1c.** *Logarithmic CEUS measurements for ROI3 (single vessel) for both T0 and T2 in the most severe affected segment for present endoscopic disease activity (SESCD≥1) and absent endoscopic disease activity (SESCD=0) analyzed with a non-parametric Mann-Whitney U Test.*

| | **Present endoscopic disease activity (n=9)** | **No present endoscopic disease activity (n=8)** | **p-value** |
|---|---|---|---|
| Peak enhancement in dB (median [IQR]) | 33.73 [30.99-34.50] | 27.43 [23.81-30.29] | **0.008** |
| Wash-in area under the curve in dB (median [IQR]) | 40.11 [35.98-41.02] | 34.90 [31.15-41.21] | 0.12 |
| Rise time in seconds (median [IQR]) | 8.02 [5.03-10.16] | 8.13 [7.69-10.52] | 0.23 |
| Mean transit time in seconds (median [IQR]) | 40.62 [23.80-95.82] | 53.84 [30.71-131.23] | 0.53 |
| Time to peak in seconds (median [IQR]) | 11.00 [6.01-914.35] | 10.51 [10.06-13.45] | 0.26 |
| Wash-in rate in dB (median [IQR]) | 27.30 [22.97-29.01] | 19.27 [15.47-22.07] | **0.005** |
| Wash-in perfusion index in dB (median [IQR]) | 31.86 [29.26-32.75] | 25.57 [22.03-28.47] | **0.008** |
| Wash-out area under the curve in dB (median [IQR]) | 43.55 [39.54-45.37] | 38.87 [34.97-45.36] | 0.30 |
| Wash-in and wash-out area under the curve in dB (median [IQR]) | 45.17 [41.12-46.74] | 40.34 [36.49-46.77] | 0.26 |
| Fall time in seconds (median [IQR]) | 18.43 [12.93-26.35] | 22.40 [17.57-27.38] | 0.23 |
| Wash-out rate in dB (median [IQR]) | 22.14 [16.49-22.66] | 14.48 [9.79-16.40] | **0.004** |

**Supplementary Table 2a.** *Linear CEUS measurements for ROI1 (complete wall) for both T0 and T2 in the most severe affected segment for present endoscopic disease activity (SESCD≥1) and absent endoscopic disease activity (SESCD=0), (a.u.=arbitrary units) analyzed with a non-parametric Mann-Whitney U Test.*

| | **Present endoscopic disease activity (n=9)** | **No present endoscopic disease activity (n=8)** | **p-value** |
|---|---|---|---|
| Peak enhancement in a.u. (median [IQR]) | 1800 [1142-2224] | 354 [254-843] | **0.016** |
| Wash-in area under the curve in a.u. (median IQR]) | 8250 [3945-10610] | 2865 [1375-6855] | 0.13 |
| Wash-in rate in dB (median [IQR]) | 363 [170-586] | 68 [42-129] | 0.13 |
| Wash-out area under the curve in a.u. (median [IQR]) | 18800 [9265-31200] | 4080 [3270-29100] | 0.25 |
| Wash-in and wash-out area under the curve in a.u. median [IQR]) | 27800 [13200-41450] | 5930 [4680-36900] | 0.22 |
| Wash-in perfusion index in a.u. (median [IQR]) | 1170 [766-1499] | 242 [166-574] | **0.017** |
| Wash-out rate in a.u. (median [IQR]) | 112 [38-152] | 23 [11-30] | 0.16 |

**Supplementary Table 2b.** *Linear CEUS measurements for ROI3 (submucosa) for both T0 and T2 in the most severe affected segment for present endoscopic disease activity (SESCD≥1) and absent endoscopic disease activity (SESCD=0), (a.u.=arbitrary units) analyzed with a non-parametric Mann-Whitney U Test.*

| | **Present endoscopic disease activity (n=9)** | **No present endoscopic disease activity (n=8)** | **p-value** |
|---|---|---|---|
| Peak enhancement in a.u. (median [IQR]) | 2740 [1610-44998] | 1016 [424-1600] | **0.018** |
| Wash-in area under the curve in a.u. (median [IQR]) | 10700 [5975-19600] | 4890 [1770-13926] | 0.15 |
| Wash-in rate in dB (median [IQR]) | 647 [239-1253] | 159 [105-292] | 0.065 |
| Wash-out area under the curve in a.u. (median [IQR]) | 20400 [11815-49803] | 9910 [3580-25600] | 0.13 |
| Wash-in and wash-out area under the curve in a.u. (median [IQR]) | 31100 [17800-72362] | 15300 [5340-37800] | 0.12 |
| Wash-in perfusion index in a.u. (median [IQR]) | 1740 [1061-2855] | 456 [251-845] | **0.002** |
| Wash-out rate in a.u. (median [IQR]) | 240 [84-266] | 59 [30-122] | 0.17 |

**Supplementary Table 2c. Linear** *CEUS measurements for ROI3 (single vessel) for both T0 and T2 in the most severe affected segment for present endoscopic disease activity (SESCD≥1) and absent endoscopic disease activity (SESCD=0), (a.u.=arbitrary units) analyzed with a non-parametric Mann-Whitney U Test.*

| | **Present endoscopic disease activity (n=9)** | **No present endoscopic disease activity (n=8)** | **p-value** |
|---|---|---|---|
| Peak enhancement in a.u. (median [IQR]) | 2360 [1275-2508] | 613 [258-1080] | **0.021** |
| Wash-in area under the curve in a.u. (median [IQR]) | 7010 [3486-11700] | 3565 [1365-7548] | 0.26 |
| Wash-in rate in a.u. (median [IQR]) | 538 [198-834] | 131 [50-254] | **0.05** |
| Wash-out area under the curve in a.u. (median [IQR]) | 22600 [9600-34800] | 3570 [3060-25100] | 0.18 |
| Wash-in and wash-out area under the curve in a.u. (median [IQR]) | 32900 [13680-47450] | 5310 [4300-33300] | 0.17 |
| Wash-in perfusion index in a.u. (median [IQR]) | 1530 [853-1900] | 435 [170-714] | **0.02** |
| Wash-out rate in a.u. (median [IQR]) | 151 [39-172] | 35 [11-46] | 0.14 |

**Supplementary Table 3.** *Median and Δmedian values for HBI and FCP in patients with and without endoscopic response at T1 and T2. [HBI: Harvey-Bradshaw Index, FCP: fecal calprotectin).*

| Parameter | T1 | | p-value | T2 | | p-value |
|---|---|---|---|---|---|---|
| | Endoscopic response | Endoscopic non-response | | Endoscopic response | Endoscopic non-response | |
| HBI | 1.0 [0.0-2.5] | 4.0 [3.0-7.0] | **0.002** | 1.0 [0.0-2.0] | 3.0 [0.5-8.5] | **0.049** |
| ΔHBI | -2.0 [-4.0- -1.0] | 0.00 [-5.3-2.3] | 0.22 | -3.5 [-4.8- -1.3] | -2.0 [-5.5-0.0] | 0.42 |
| FCP (μg/g) | 75 [19-250] | 239 [38-405] | 0.29 | 73 [11.50-164] | 83 [20-189] | 0.88 |
| ΔFCP (μg/g) | -1199 [-3029- -456] | -256 [-606-42] | **0.013** | -1699 [-3309- -967] | -92 [-314-19] | **0.006** |

**Supplementary Table 4.** *Area under the ROC-curve analysis for ΔFecal calprotectin at T1 and T2 to determine endoscopic response after 12-34 weeks of anti-TNFα.*

| Parameter | Cut-off value | Area under the ROC | Sensitivity | Specificity | p-value |
|---|---|---|---|---|---|
| ΔFecal calprotectin T0-T1 | -600 μg/g | 0.82 (95%CI: 0.65-0.96) | 75% | 71% | 0.014 |
| ΔFecal calprotectin T0-T2 | -676 μg/g | 0.96 (95%CI: 0.85-1.00) | 100% | 82% | 0.009 |

**Supplementary Table 5.** *Inter-observer agreement on presence of disease activity per segment at IUS.*

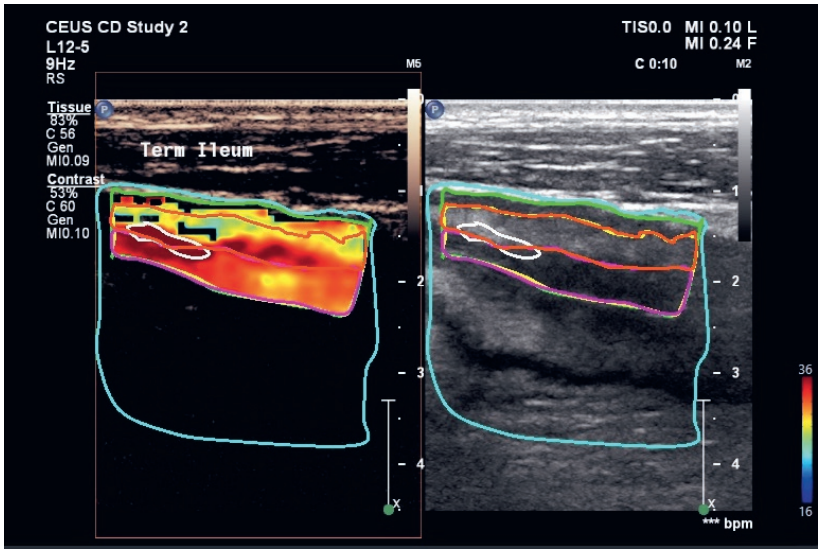
| Segment | Kappa agreement (95%CI) | Significance |
|---|---|---|
| Sigmoid colon | κ=0.72 (0.54-0.90) | p<0.0001 |
| Descending colon | κ=0.808 (0.66-0.95) | p<0.0001 |
| Transverse colon | κ=0.732 (0.56-0.91) | p<0.0001 |
| Ascending colon/cecum | κ=0.62 (0.35-0.86) | p<0.0001 |
| Terminal ileum | κ=0.72 (0.58-0.85) | p<0.0001 |
| Absence of disease activity | κ=0.60 (0.36-0.83) | p<0.0001 |

**Supplementary Table 6.** *Inter-observer agreement per IUS parameter per segment.*

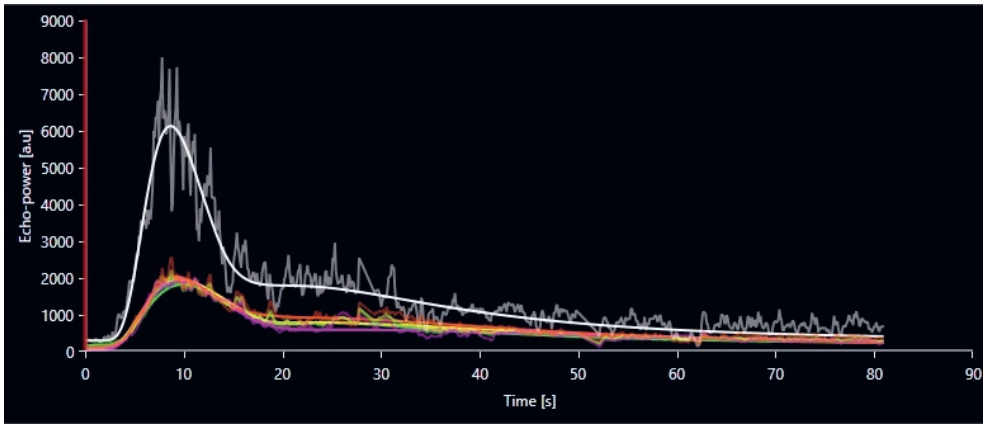
| Segment | Doppler | Loss of WLS | Fatty wrapping | Presence of lymphnodes | Loss of haustrations | Loss of motiltiy |
|---|---|---|---|---|---|---|
| Sigmoid colon | κ=0.62 (0.37-0.88), p<0.0001 | κ=0.56 (0.28-0.84), p<0.0001 | κ=0.57 (0.24-0.90), p=0.014 | κ=0.25 (-0.15-0.65), p<0.0001 | κ=0.67 (0.46-0.89), p<0.0001 | n/a |
| Descending colon | κ=0.64 (0.41-0.88), p<0.0001 | κ=0.69 (0.49-0.88), p<0.0001 | κ=0.38(0.03-0.73), p=0.041 | κ=1.00 (1.00-1.00), p<0.0001 | κ=0.70 (0.53-0.88), p<0.0001 | n/a |
| Transverse colon | κ=0.56 (0.25-0.88), p-0.002 | κ=0.61 (0.36-0.86), p<0.0001 | κ=0.39 (0.03-0.74), p=0.053 | κ=1.00 (1.00-1.00), p<0.0001 | κ=0.52 (0.29-0.75), p<0.0001 | n/a |
| Ascending colon/cecum | κ=0.51 (0.24-0.78), p=0.012 | κ=0.80 (0.54-1.07), p<0.0001 | κ=0.33 (-0.16-0.82), p=0.26 | κ=0.49 (-0.11-1.09), p<0.0001 | κ=0.65 (0.29-1.00), p<0.0001 | n/a |
| Terminal ileum | κ=0.75 (0.60-0.90), p<0.0001 | κ=0.61 (0.43-0.79), p<0.0001 | κ=0.80 (0.70-0.91), p<0.0001 | κ=0.23 (-0.04-0.49), p=0.029 | n/a | κ=0.65 (0.49-0.81), p<0.0001 |

**Supplementary Table 7.** *Intra-class correlation coefficients for the two blinded raters per CEUS parameter for ROI1 (complete wall) (n=30).*

| CEUS parameter | Intraclass correlation coefficient (95%CI) | p-value |
|---|---|---|
| Peak enhancement | 0.973 (0.944-0.987) | <0.0001 |
| Wash-in Area under the curve | 0.935 (0.864-0.969) | <0.0001 |
| Rise Time | 0.826 (0.634-0.917) | <0.0001 |
| Mean transit time | 0.632 (0.226-0.825) | 0.004 |
| Wash-in rate | 0.971 (0.940-0.986) | <0.0001 |
| Wash-in peak intensity | 0.976 (0.949-0.988) | <0.0001 |
| Wash-out Area under the curve | 0.926 (0.838-0.966) | <0.0001 |
| Wash-in wash-out Area under the curve | 0.933 (0.852-0.969) | <0.0001 |
| Fall Time | 0.671 (0.278-0.850) | 0.003 |
| Wash-out rate | 0.943 (0.875-0.974) | <0.0001 |
| Quality of fit | 0.710 (0.390-0.862) | 0.001 |




**A**




**B**

**Figure 1 (a-b).** *Region of interests for the terminal ileum in a 25 year-old Crohn's Disease patient with a: the contrast-enhanced still image for peak enhancement and b: the time-intensity curves per region of interest.* (Blue: delineation area, Green: complete wall, Yellow: mucosa and submucosa, Purple: mucosa, Orange: Submucosa, White: single vessel/hotspot).

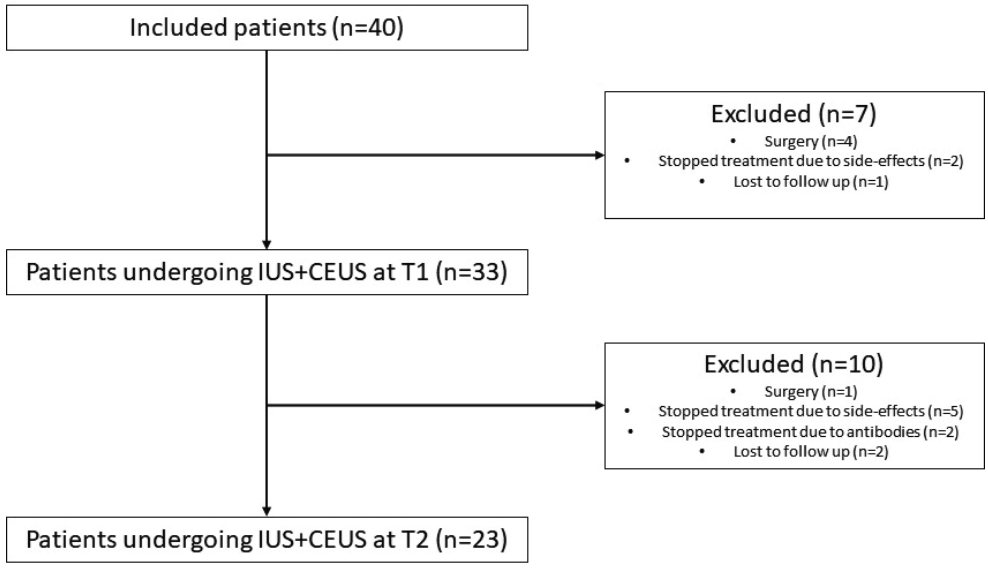
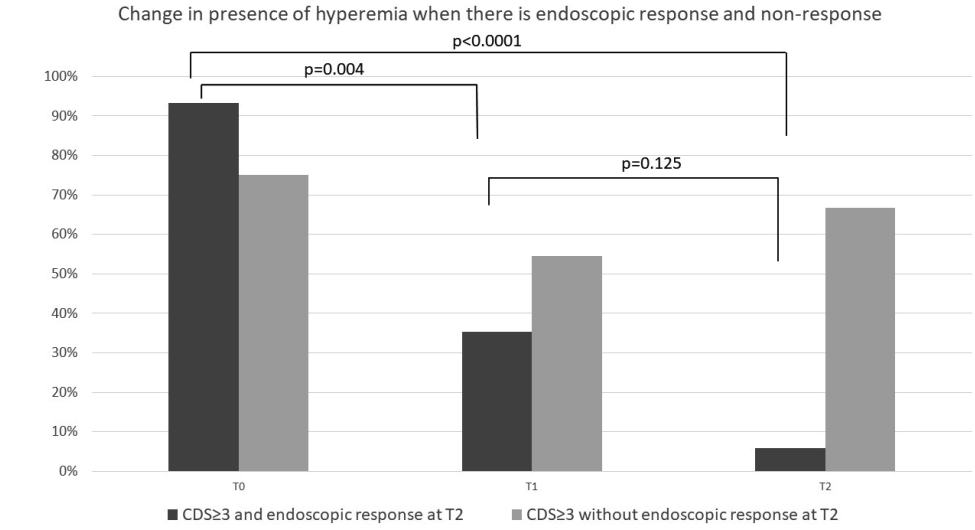
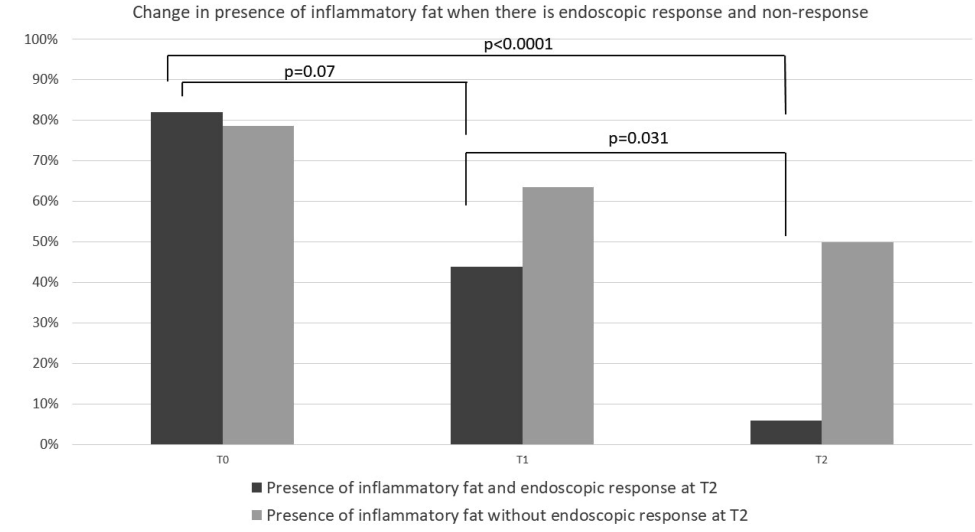



**Figure 2.** *Flow-diagram of follow-up. [IUS=intestinal ultrasound, CEUS=contrast-enhanced ultrasound].*

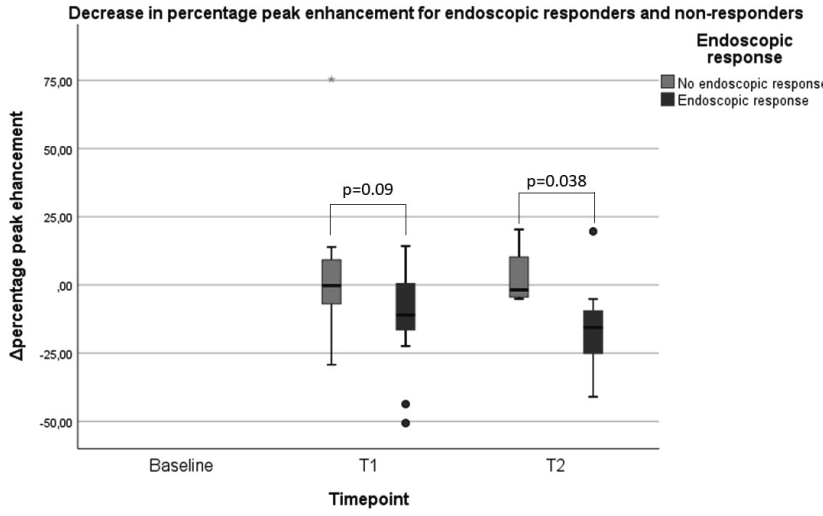



**A**

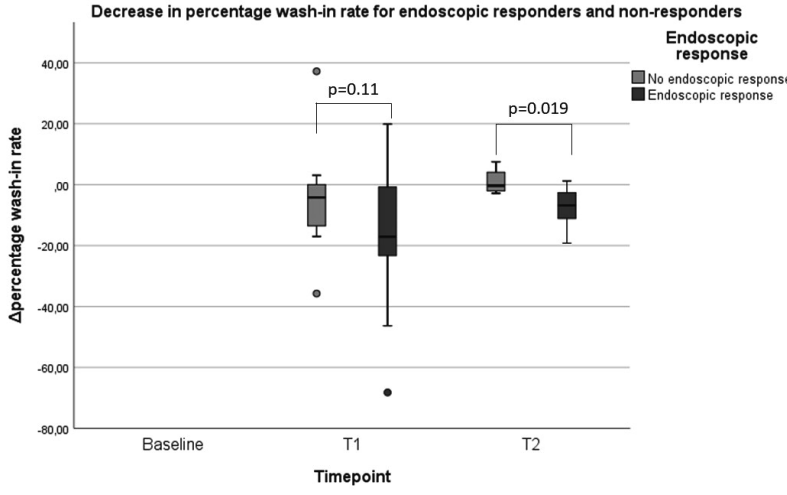



**B**

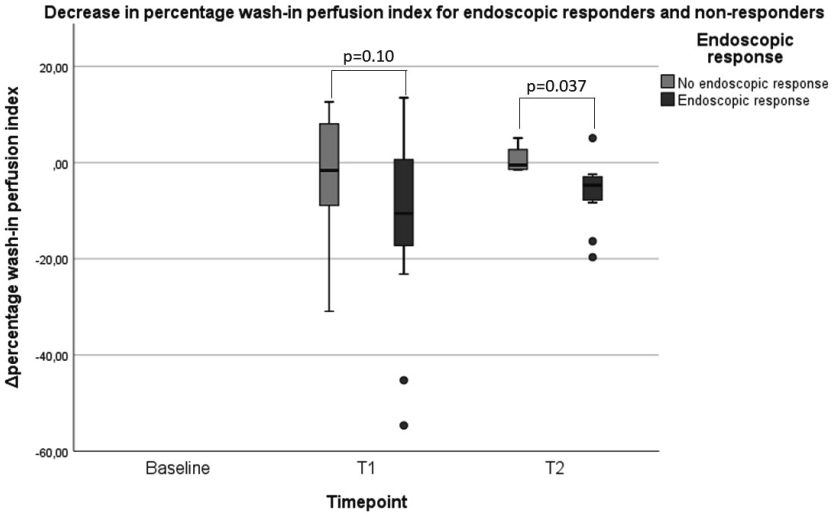
**Figure 3.** *Significant change in Colour Doppler Signal (A) and inflammatory fat (B) during anti-TNFα treatment in patients with and without endoscopic response after 12 to 34 weeks.*


Decrease in percentage peak enhancement for endoscopic responders and non-responders
Endoscopic response
No endoscopic response
Endoscopic response
Δpercentage peak ehancement
75,00
50,00
25,00
,00
-25,00
-50,00
p=0.09
p=0.038
Baseline
T1
T2
Timepoint

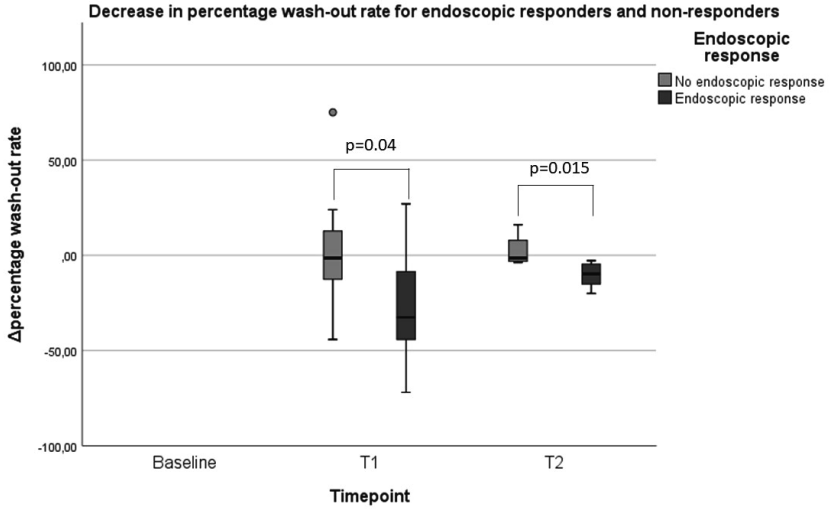
**A**


Decrease in percentage wash-in rate for endoscopic responders and non-responders
Endoscopic response
No endoscopic response
Endoscopic response
Δpercentage wash-in rate
40,00
20,00
,00
-20,00
-40,00
-60,00
-80,00
p=0.11
p=0.019
Baseline
T1
T2
Timepoint

**B**




C




D

**Figure 4 (a-d).** *Decrease in percentage according to endoscopic response per time-point for peak enhancement (A), wash-in rate (B), wash-in perfusion index (C) and wash-out rate (D).*

# Part II

## Optimal Use of Biologics

# Chapter 6

## Optimization of anti-TNF therapy in patients with Inflammatory Bowel Disease

A. Strik, S. Bots, G. D'Haens, M. Löwenberg

*Expert Review of Clinical Pharmacology 2016;9(3):429-439*

## Abstract

After the introduction of anti-tumor necrosis factor (anti-TNF) agents, the clinical outcome of patients with Inflammatory Bowel Disease (IBD) has improved significantly. However, use of anti- TNF therapy is complicated by loss of response. In order to maintain remission, adequate serum levels are required. Hence, therapeutic drug monitoring (TDM) is important in order to optimize serum drug levels, especially in patients with loss of response to these agents. Optimization of anti-TNF therapy by applying TDM enables clinicians to regain response to TNF blockers in a significant proportion of patients. It is important to use anti-TNF agents in their most optimal way, since these therapeutic agents are expensive and the medical options after failing anti-TNF therapy are limited. Here, we will discuss how to optimize treatment with anti-TNF agents in IBD patients in order to improve treatment efficacy, prevent anti-drug antibody formation, reduce side effects, discontinue unnecessary treatment and manage costs.

## Introduction

In recent years, medical treatment of patients suffering from inflammatory bowel disease (IBD) has changed significantly. Novel therapeutic agents have become available and our knowledge how to optimize treatment strategies for IBD patients has improved markedly. The need for individualized treatment regimens is becoming more and more important.

Identification of the pro-inflammatory cytokine tumor necrosis factor (TNF) as an important inflammatory mediator in several chronic inflammatory conditions paved the way for the development of TNF antagonists. These anti-TNF agents not only bind and neutralize TNF, but they also down regulate inflammation by inducing apoptosis.[1] Currently, there are four anti-TNF agents available for the treatment of IBD, namely infliximab (IFX), adalimumab (ADL), golimumab (GLM, only for ulcerative colitis (UC)) and certolizumab pegol (CZP). CZP has only been approved for Crohn's Disease (CD) in the United States and Switzerland, and will not be discussed here.[2] IFX and ADL are efficacious in inducing and maintaining clinical remission in patients with CD and UC.[3-7] Introduction of these two agents has led to a decrease in bowel-related surgery and hospitalization rates. IFX is an effective drug to treat fistulizing CD, but for ADL there are less data available.[8, 9] GLM is an effective agent to induce and maintain remission in patients with moderate-to-severe UC.[10, 11] Treatment with anti-TNF agents contributed to a better disease control with a reduction in (late) complications of the disease and improved quality of life in IBD patients.[12] Unfortunately, a considerable proportion of patients fail to respond to anti-TNF induction therapy and these patients are labeled as primary non-responders. Up to 50% of patients who initially respond to anti-TNF therapy show loss of response (LOR) over time (i.e. secondary LOR), which often leads to discontinuation of treatment.[13] An important factor that contributes to secondary LOR is the development of anti-drug antibodies (ADA). The so-called 'immunogenicity' can result in faster clearance of the drug subsequently leading to lower serum drug levels and LOR.[14-16] Formation of ADA is also associated with infusion reactions.[17, 18]

Therapeutic options for IBD patients who fail anti-TNF therapy are limited, although novel agents such as anti-interleukin-23 antibodies and Janus kinase inhibitors may offer alternatives in the foreseeable future. Nonetheless, it is important to use anti-TNF agents in their most optimal way in order to improve treatment efficacy, reduce side-effects and manage costs.[19] Here, we will discuss how to optimize treatment with anti-TNF therapy in patients with IBD.

## Development of anti-TNF treatment

Our knowledge regarding the effective use of anti-TNF agents has improved considerably during recent years. Instead of switching to other agents in the case of LOR, prevention of secondary LOR by applying the right intervention is a more common approach nowadays. Importance of adequate serum levels of anti-TNF antibodies has been well established and several factors that may influence serum drug concentrations have been identified.[20-27] Rutgeerts et al. showed that patients who received scheduled IFX treatment instead of episodic treatment had fewer hospitalizations, higher rates of mucosal healing and a reduction in the formation of Ada compared to patients who received episodic treatment.[28] Maser et al. demonstrated a positive association between detectable IFX trough levels (TLs), higher rates of clinical remission and endoscopic improvement in CD patients on maintenance treatment with IFX.[28, 29] Since serum drug levels seem to be positively associated with clinical outcome, it is essential to define appropriate cut-off levels. From ACCENT-1 it was concluded that the therapeutic threshold of IFX serum levels should be above 3.5 μg/ml.[21] It remains unclear if there is a maximum to the therapeutic range. However, recent work from our group showed that high IFX and ADL TLs (i.e. > 5.5 and 6.6 μg/ml, respectively) were associated with a lower disease-specific quality of life in IBD patients, particularly, regarding systemic symptoms and emotional status. A trend towards lower SF-36 and higher fatigue scores was observed in the higher anti-TNF TL group, but this difference was not significant.[30] Based on available literature, it appears that a TL of 3 μg/ml should be sufficient for IBD patients who are in clinical remission. Vande Casteele et al. showed in the TAXIT study that in patients with a TL > 7 ug/ml, the IFX dosage could safely be de-escalated.[31] In clinical practice, this means that IFX dosages may be reduced to achieve a TL of 3 μg/ml, which might result in a decrease in side-effects, reduction of costs and a higher proportion of patients who maintain clinical remission. The PRECISION study, a currently ongoing prospective trial at our institute, is performed to test these hypotheses. For ADL, a serum concentration at trough above 6–7 μg/ml is considered to be sufficient and a TL above 4.9 μg/ml is associated with mucosal healing.[14, 32]

One of the latest additions to the anti-TNF class of drugs is GLM, a subcutaneous human anti-TNF agent that was recently approved by the Food and Drug Administration and European Medicines Agency for the treatment of moderately to severely active UC. The therapeutic window for GLM remains to be determined in IBD patients.[10, 11] Data on the pharmacokinetic profile of GLM are based on trials in patients with ankylosing spondylitis, psoriatic arthritis and rheumatoid arthritis.[33-35] We are currently performing a pharmacokinetic study in UC patients who receive GLM treatment in order to increase our knowledge about the pharmacokinetic profile of this agent. A meta-analysis from Thorlund et al. suggested that IFX and GLM are comparable in efficacy and that both agents may statistically be superior to ADL, but more research is needed to confirm these findings.[36]

## Risk stratification

Appropriate selection of patients before initiating anti-TNF therapy is essential. Disease phenotype (severity, extent of the disease, etc.) and the medical history, such as previous (non)response to other therapeutic agents, should be taken into account. Poor prognostic factors that are associated with disabling disease can be used to select patients who benefit from early intervention with anti-TNF agents, so-called top-down therapy. Especially, adult patients with a young age at diagnosis (i.e. < 40 years), extensive small bowel involvement and/or perianal CD benefit from early introduction with anti-TNF therapy.[37] However, the majority of patients have a relatively milder disease course and show a favorable response to conventional step-up care that consists of sequential treatment with 5-aminosalicylic acid, corticosteroids, immunomodulators and biologicals (mainly anti-TNF agents). However, incorporation of an algorithm with response assessment guiding treatment escalation ('accelerated stepcare') was recently shown to be beneficial.[38] Besides correct risk stratification before starting anti-TNF therapy, the increased risk of malignancy and opportunistic infections when starting combination therapy is important to take into account.[39]

## Screening before starting anti-TNF treatment

Screening procedures that should be done before anti-TNF treatment is started are partly based on evidence (guidelines) and on expert opinion. Screening for infectious diseases should be performed at baseline, including screening for tuberculosis (using Mantoux, chest X-ray and Interferon Gamma Release Assay (IGRA)) as well as viral serology for hepatitis B, hepatitis C, cytomegalovirus (CMV), Epstein-barr virus (EBV), varicella zoster virus (VZV) and human immunodeficiency virus (the latter two only on indication). Pneumococcal vaccination and annual influenza is advised before anti-TNF therapy is started. Yellow fever (in the case of traveling to endemic areas), hepatitis B (in high-risk groups: homosexual men, those traveling to endemic areas and intravenous drug abuse) and VZV (in the case of seronegativity or increased exposure risk, e.g. young children) should be considered. Stool tests for Salmonella/Shigella/Yersinia/ Campylobacter (SSCY)/Escherichia coli and Clostridium difficile toxins should be routinely done. Screening for other infectious diseases should be performed according to geographical location and prevalence.

## Combination therapy

In addition to the implementation of scheduled treatment with anti-TNF agents, the importance of combination therapy with an immunomodulator in order to enhance anti-TNF treatment efficacy and in order to prevent immunogenicity has been recognized. The impact of combination therapy on IFX monotherapy is well understood.[40-42] For ADL, there is less evidence that combination therapy is superior to monotherapy.[43] Several randomized controlled trials showed that concomitant use of immunomodulators (azathioprine, 6-mercaptopurine or methotrexate) increases anti-TNF serum levels and reduces

immunogenicity thereby improving clinical outcome.[40-42] However, in the COMMIT trial IFX in combination with methotrexate was not more effective than IFX monotherapy, but it must be recognized that all patients received a high dose of prednisolone at the time induction treatment with IFX was started.[44] Furthermore, combination therapy might also reduce the incidence and severity of infusion reactions, which are (at least partially) related to immunogenicity.[16, 45, 46] Evidence suggests that combination treatment is important, especially within the first 12 months after starting anti-TNF therapy in order to reduce the risk of developing immunogenicity.[47] Furthermore, there is evidence to support the idea that combination therapy with IFX and azathioprine is more cost-effective compared to IFX monotherapy.[48] Hence, starting anti-TNF therapy in combination with an immunomodulator should be the preferred strategy, but this decision has to be made on an individual basis since combination treatment is associated with an increased risk of malignancies and opportunistic infections.[39, 49-55] An immunomodulator can also be introduced in patients with LOR while receiving anti-TNF monotherapy, since recapturing response after addition of immunomodulators has been described.[56, 57] In our center, a significant proportion of patients with LOR on anti-TNF monotherapy regained response after addition of an immunomodulator. In these patients, we observed an accompanied increase in serum drug concentrations and in most patients ADA levels disappeared. Further research should be performed in order to identify patient- and disease-specific characteristics that could predict which patients would benefit from combination therapy. Besides ADA at a continuous measurable level, transient ADA can also appear during treatment, but their clinical contribution seems not significant.[47, 58]

## Appropriate dosing of anti-TNF agents in patients with active IBD

When optimizing serum concentrations of anti-TNF agents in order to obtain therapeutic drug levels, it is important to take the severity of the disease into account, which can be reflected by increased C-reactive protein (CRP) and low serum albumin levels, amongst others. A low body weight and the presence of ADA also have a negative impact on the pharmacokinetic profile of these drugs by increasing their clearance.[59, 60] Recent studies by Brandse et al. and Yarur et al. investigated the role of luminal (feces) and mucosal (tissue) compartments in the pharmacokinetics of IFX.[27, 61] Brandse et al. showed that a significant proportion of IFX is lost through leakage from the gut into the feces, especially during induction treatment in patients with severe colitis and this was associated with impaired clinical outcomes. Interestingly, no correlation was found between fecal and serum concentrations of IFX, although strict quantification of fecal loss of IFX was not performed.[61] Furthermore, therapeutic antibodies can also be degraded by proteases in the mucosal compartment, in particular metalloproteinases.[62] In order to fully understand the pharmacokinetic profile of anti-TNF agents, a pharmacokinetic model is being developed consisting of three physiological compartments (i.e. tissue, serum and feces).

Induction therapy with anti-TNF agents in IBD patients using standard dosages is sufficient in the majority of patients. For IFX, the induction phase consists of 5 mg/kg infusions that are administered at week 0, 2 and 6. For ADL, usually 160 mg subcutaneously (s.c) is used as starting doses although in some countries 80 mg is the preferred starting dose. For GLM, the weight of the patient should be taken into account. Induction treatment consists of 200 mg s.c. followed by 100 mg s.c. after two weeks. In Europe, after the induction phase, patients ≥ 80 kg receive 100 mg every

4 weeks and patients < 80 kg receive maintenance treatment with 50 mg GLM every four weeks. However, there is evidence that IBD patients with severe inflammation should receive higher doses of anti-TNF agents in the acute phase of the disease.[63] The goals of administering higher doses of anti-TNF induction treatment are to neutralize circulating and tissue TNF, to correct for fecal loss and protease inactivation and to avoid occurrence of low serum drug levels and rapid ADA formation. Based on our own experience, hospitalized patients who have low albumin and high CRP levels benefit most from this approach. In patients who show a partial response with inadequate drug levels during the induction phase, treatment should be intensified by giving additional doses or by shortening the treatment interval, instead of immediately switching to another agent. Serum CRP and fecal calprotectin are valuable monitoring tools in this context.[64-66] In the case of switching between different anti-TNF agents, it is known that patients who developed ADA to IFX more often develop ADA to ADL versus anti-TNF naïve patients.[67, 68] Identification of these patients on beforehand is not possible yet, but there is evidence that immunogenicity to IFX is associated with HLA-DRB1.[69] A systematic review performed by Gisbert et al. showed that therapeutic efficacy of a second anti-TNF agent in CD patients mainly depends on the reason for switching.[70] When primary or secondary failure was the reason to switch within class, relatively low remission rates were seen (30% and 45%, respectively) compared to patients who were intolerant to the first anti-TNF agent.[70] In our opinion, a second (or third) anti-TNF agent can be efficacious in patients with prior response to an anti-TNF agent and subsequent intolerance to these agents.

## Management of loss of response

Before the era of TDM, the management of secondary LOR to anti-TNF agents consisted of empiric treatment intensification by either increasing the dose or by shortening the interval (based on patient's/physician's preference). Alternatively, patients could be switched to another drug (out or within class). This approach was largely based on trial and error rather than on evidence and many patients probably did not receive optimal treatment. Nowadays, TDM is more and more used in daily practice in order to improve treatment efficacy and to reduce side-effects. This is done by individually adjusting the dose within a relatively narrow therapeutic range, because treatment with anti-TNF agents can easily be over or under dosed.

In IBD patients who receive treatment with anti-TNF agents, TDM is usually applied in the case of suspicion of active disease and secondary LOR (figure 1). The first step is to determine

clinical and biochemical disease activity. CRP testing is relatively cheap and widely available, but an elevated serum CRP concentration is not specific for intestinal inflammation. Fecal calprotectin has a greater specificity for intestinal inflammation compared to CRP and correlates significantly with endoscopic disease activity, especially in UC.[71, 72] Combining CRP, calprotectin and additional information seen at endoscopy, in most cases allows the physician to determine disease activity.[73-76] Endoscopy and imaging modalities (such as magnetic resonance imaging) are particularly useful in order to differentiate between active inflammation and other causes of abdominal pain such as fibrostenotic strictures, gastrointestinal infections or functional pain (Irritable Bowel Syndrome). If active disease is confirmed, the next step is to perform TDM according to the LOR algorithm that we use in our clinic (figure 1).

If patients fail to respond to induction treatment with anti-TNF agents or lose response over time (primary or secondary LOR, respectively), the first question that should be answered is whether the patient may benefit from dose escalation. Depending on the combination of results, patients can be divided into three different groups: (I) patients with low or undetectable serum concentrations with ADA; (II) patients with low or undetectable serum drug levels without ADA; and (III) patients with therapeutic drug levels without ADA. In the first group, increased serum concentrations and a decline in ADA can be achieved by adding an immunomodulator.[56, 57] If patients already receive combination therapy, it seems valid to intensify anti-TNF treatment by doubling the dose or decreasing the treatment interval, but this seems only a successful strategy in patients with low ADA titers.[77-79] Katz et al. showed that in patients with CD, doubling the IFX dose was not superior to halving the treatment interval in terms of regaining response. However, dose-doubling may be the preferable choice in terms of costs.[80] When deciding which type of dose intensification should be applied, it is important to take the symptoms of the patient into account. If patients have symptoms during the entire treatment interval, increasing the dose is recommended. If symptoms appear during the treatment interval, for example, at week 6 after the last infusion, shortening the interval seems more logical. When none of these options are available, treatment should be switched to another agent (within or out of class).

In the second situation (i.e. low or undetectable serum drug levels without ADA), dose intensification may suffice to regain response.[24, 77-79] Again, two different strategies of dose intensification can be applied: increasing the dose or shortening the treatment interval. If dose intensification is not sufficient, switching to another agent is recommended.

In the third group (i.e. patients with therapeutic drug levels without ADA) anti-TNF treatment appears to be ineffective. For these patients, evaluation of disease activity is key, endoscopy being the most informative diagnostic tool. If active endoscopic disease is confirmed, anti-TNF therapy should be discontinued and switch out of class should be initiated (i.e. drug with another mode of action). It is possible that in such patients other cellular mediators than TNF play a dominant role. Vedolizumab, a monoclonal antibody which binds to $\alpha 4\beta 7$ integrins, is

a valuable option for CD and UC patients who fail anti-TNF therapy.[81, 82] In the nearby future, ustekinumab (anti-IL12/ IL23) will likely become available for patients with refractory IBD.

When assessing LOR, adherence to treatment should also be determined. A systematic review performed by Lopez et al. showed that more than three-quarters of IBD patients adhere to biologics, but when assessing secondary LOR, non-adherence to treatment should be recognized since poor adherence may undermine the therapeutic benefit of biologics.[83-86]

## Individualized treatment strategies

Personalized medicine is becoming more and more important in modern healthcare. Individualized anti-TNF treatment strategies are a major area of research and several strategies are gradually being implemented in clinical practice. We already discussed the use of TDM for the management of LOR, but TDM is also becoming increasingly important for treatment optimization. VandeCasteele et al. showed that targeting patient's serum drug levels to the therapeutic range of IFX resulted in higher clinical remission rates in CD patients as compared to conventional dosing.[31] After dose optimization, it seemed not beneficial to continue concentration-based dosing in order to maintain clinical remission. Clinical remission is an important outcome for patients, but mucosal healing (i.e. no ulcers seen at endoscopy) is the desired treatment goal, since this is associated with improved long-term outcomes and it can also be objectively measured.[87, 88] There is evidence to support the idea that TDM predicts the likelihood of achieving mucosal healing following IFX dose intensification in CD and UC.[24]



Anti-TNF agents are expensive and account for 64% and 31% of the total health costs in CD and UC in The Netherlands.[19] TDM seems to be a cost-effective approach, as it was shown that costs of individualized treatment using algorithm based interventions were significantly lower compared to costs of empiric IFX dose intensification (5 mg/kg every four weeks) in CD patients failing IFX.[89, 90] Hence, TDM perfectly fits in the personalized treatment approach in order to optimize treatment outcomes and manage costs.

Other valuable tools for personalization of anti-TNF therapy are so-called point-of-care diagnostic tests, since rapid diagnostic results might be helpful in certain clinical situations in order to make proactive treatment adjustments. Endoscopy is the gold standard for assessment of disease activity, but this cannot be implemented on a regular basis. Transabdominal ultrasound is a safe and relatively cheap technique that has proven to be valuable for follow-up of IBD patients and to monitor treatment efficacy.[91, 92] Therefore, this modality is being used in our out-patient clinic for point-of-care assessment of disease activity. Moreover, MRI technology can be very useful for assessing small bowel disease and perianal disease in CD patients.[93, 94] For optimization of anti-TNF therapy, point-of-care tests that measure serum drug concentrations in (capillary) blood seem to be a valuable tool as they would allow for rapid treatment adjustments, thereby avoiding unnecessary treatment delay. A quick test for fecal calprotectin is already available for 'on the spot' assessment of disease

activity.[95] Hence, introduction of point-of-care assays will allow for rapid treatment adjustments.

## Discontinuation and reintroduction of anti-TNF treatment

In a proportion of patients anti-TNF therapy is discontinued because of longstanding remission. Discontinuation of anti-TNF therapy may be considered for various reasons, such as safety concerns, pregnancy, preference of the patient, etc. We believe that several criteria must be met before discontinuation of anti-TNF therapy is considered. In the case of deep remission (defined by clinical, biochemical and endoscopic remission), discontinuation of anti-TNF therapy may be considered, but this should be done on an individual basis. The long-term outcome of patients previously included in de STORI trial showed that after stopping anti-TNF treatment because of sustained remission under combination therapy, 85% of the patients had to restart treatment again. In the case of extensive small bowel disease and/or symptomatic perianal fistulas, long-term treatment with anti-TNF agents is indicated, because alternative medical therapies are limited.[96-98] Retreatment of patients that experience a relapse after cessation of IFX therapy is usually well tolerated and the success rate is high, ranging from 71% to 94% in different studies.[96-98] Nevertheless, for IFX it is known that patients have an increased risk of developing (severe) infusion reactions and delayed hypersensitivity during reintroduction with IFX.[18, 99, 100] Despite limited evidence, we do recommend to always restart IFX together with corticosteroids and a H1-receptor antagonist as pre-medication in order to reduce the chance of developing an acute infusion reaction.[101, 102] There is also evidence that reintroduction of anti-TNF therapy together with an immuno- modulator decreases the risk of infusion reactions.[102]

## Expert commentary

Preventing and managing LOR remains one of the most challenging aspects in the management of IBD patients who receive treatment with anti-TNF agents. There is evidence that personalized strategies improve treatment outcomes with anti-TNF agents.[24, 31] Low serum drug concentrations and ADA are associated with LOR. Therefore, optimization of serum drug concentrations and minimizing the chance of ADA formation should be the first step in preventing LOR. We recommend to start anti-TNF therapy in combination with an immunomodulator, if possible, and to incorporate TDM in the early phase of anti-TNF therapy (i.e. during the induction phase) in order to prevent primary and secondary LOR by optimizing serum drug concentrations in adult patients. In pediatric patients, there is too little evidence especially about the safety of combination therapy. During induction therapy, doubling the anti-TNF dose in the case of severe colitis seems to be of value in order to prevent primary LOR.

Nowadays, TDM is mainly used to assess secondary LOR. But optimization of serum drug levels during induction therapy is not implemented on a regular basis yet. In our view, TDM

should be applied more often since it may result in preventing secondary LOR, especially for patients that may not have received the optimal dose in the first place. In order to ensure that patients respond to treatment with anti-TNF agents, endoscopic evaluation after starting anti-TNF therapy is mandatory in our opinion. There are no data to answer the question what the best timing is for performing

endoscopy. For CD patients who embark on anti-TNF therapy, we evaluate endoscopic disease activity after approximately 6 months. For UC patients, we evaluate endoscopic responses at week 10–18 after starting anti-TNF therapy. Moreover, fecal calprotectin is often used as a surrogate marker to assess disease activity in our outpatient clinic as it has been shown that there is a high correlation between fecal calprotectin and endoscopic disease activity in IBD patients.[72, 103] The ultimate goal of all these strategies is to discontinue ineffective or unnecessary treatment, improve treatment efficacy and reduce side-effects of anti-TNF therapy.

## Five-year view

After the recent introduction of vedolizumab, other biologicals such as ustekinumab (IL-12/IL-23 blocking antibody) will be introduced for the treatment of IBD in the nearby future. Personalized treatment with biologicals (anti-TNF treatment being at the forefront) will become more and more important and this type of treatment will be facilitated by point-of-care tests, such as on the spot measurements of serum drug concentrations, CRP and fecal calprotectin that allow for immediate treatment decisions. It is likely that disease activity will also be monitored via E-health which may lead to less hospital visits.[104] Another example of such technology is the development of so-called dash- board systems (i.e. software packages that integrate information and calculations about therapeutics from multiple components into a single interface for use in the clinical environment), which are currently being developed in order to simplify TDM of anti-TNF agents.[105] This will likely improve implementation of TDM on a larger scale and should enable gastroenterologists to make proactive treatment adjustments in order to maintain a targeted TL. TDM will also be increasingly used in order to prevent primary non-response instead of only managing secondary LOR.

Biosimilar IFX has recently entered the IBD arena. Efficacy and safety data from clinical trials in rheumatoid arthritis and spondyloarthritis have been extrapolated to IBD. We believe that ongoing clinical trials that investigate treatment efficacy and switching strategies in IBD patients with anti-TNF biosimilars will increase our knowledge and confidence in using these agents. The use of anti-TNF biosimilars will significantly reduce treatment costs which will result in the ability to treat more patients with anti-TNF therapy. In conclusion, the future of IBD treatment with anti-TNF agents will become more personalized, the number of available therapeutic agents will increase in the next five years end the use of anti-TNF biosimilars will expand.

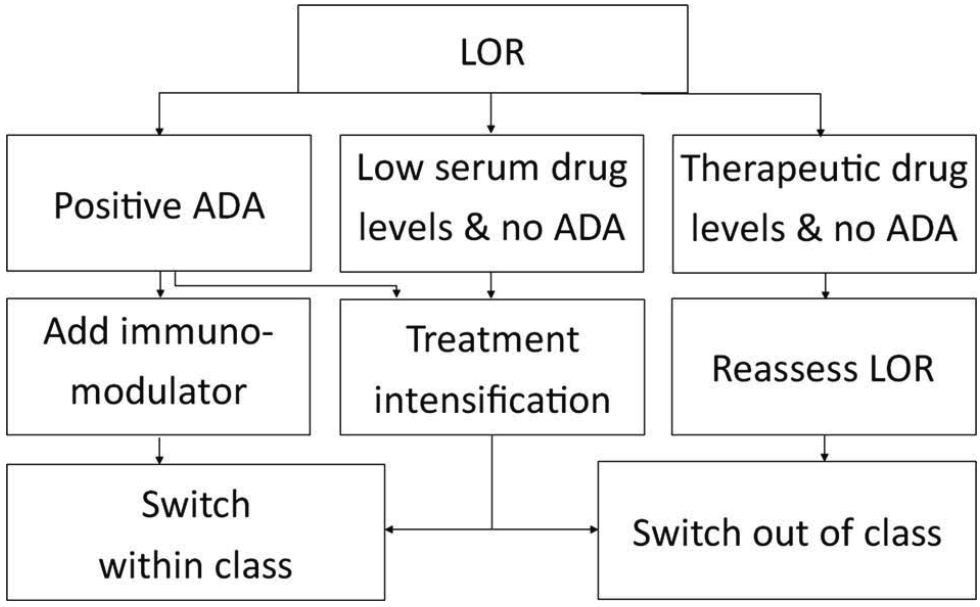



**Figure 1.** *TDM algorithm (LOR=Loss of response, ADA=anti-drug antibodies). In patients with positive ADA, an immuno modulator can be introduced or therapy intensification (increasing dose or decreasing interval) can be applied. If therapy intensification is not sufficient, switching to another agent (out or within class) is recommended.*

## References


1. Tracey D, Klareskog L, Sasso EH, Salfeld JG, Tak PP. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. Pharmacol Ther. 2008;117(2):244-79.

2. Sandborn WJ, Feagan BG, Stoinov S, Honiball PJ, Rutgeerts P, Mason D, et al. Certolizumab pegol for the treatment of Crohn's disease. The New England journal of medicine. 2007;357(3):228-38.

3. Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet. 2002;359(9317):1541-9.

4. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. The New England journal of medicine. 2005;353(23):2462-76.

5. Hanauer SB, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh D, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology. 2006;130(2):323-33; quiz 591.

6. Sandborn WJ, Hanauer SB, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh DG, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. Gut. 2007;56(9):1232-9.

7. Reinisch W, Sandborn WJ, Hommes DW, D'Haens G, Hanauer S, Schreiber S, et al. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. Gut. 2011;60(6):780-7.

8. Lichtenstein GR, Yan S, Bala M, Blank M, Sands BE. Infliximab maintenance treatment reduces hospitalizations, surgeries, and procedures in fistulizing Crohn's disease. Gastroenterology. 2005;128(4):862-9.

9. Feagan BG, Panaccione R, Sandborn WJ, D'Haens GR, Schreiber S, Rutgeerts PJ, et al. Effects of adalimumab therapy on incidence of hospitalization and surgery in Crohn's disease: results from the CHARM study. Gastroenterology. 2008;135(5):1493-9.

10. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146(1):96-109.e1.

11. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146(1):85-95; quiz e14-5.

12. Vogelaar L, Spijker AV, van der Woude CJ. The impact of biologics on health-related quality of life in patients with inflammatory bowel disease. Clinical and experimental gastroenterology. 2009;2:101-9.

13. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Alimentary pharmacology & therapeutics. 2011;33(9):987-95.

14. Karmiris K, Paintaud G, Noman M, Magdelaine-Beuzelin C, Ferrante M, Degenne D, et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. Gastroenterology. 2009;137(5):1628-40.

15. West RL, Zelinkova Z, Wolbink GJ, Kuipers EJ, Stokkers PC, van der Woude CJ. Immunogenicity negatively influences the outcome of adalimumab treatment in Crohn's disease. Alimentary pharmacology & therapeutics. 2008;28(9):1122-6.

16. Baert F, Noman M, Vermeire S, Van Assche G, G DH, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. The New England journal of medicine. 2003;348(7):601-8.

17. O'Meara S, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in patients with inflammatory bowel disease: a systematic review and meta-analysis. Inflammatory bowel diseases. 2014;20(1):1-6.

18. Steenholdt C, Svenson M, Bendtzen K, Thomsen OO, Brynskov J, Ainsworth MA. Severe infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with inflammatory bowel disease. Alimentary pharmacology & therapeutics. 2011;34(1):51-8.

19. van der Valk ME, Mangen MJ, Leenders M, Dijkstra G, van Bodegraven AA, Fidder HH, et al. Healthcare costs of inflammatory bowel disease have shifted from hospitalisation and surgery towards anti-TNFalpha therapy: results from the COIN study. Gut. 2014;63(1):72-9.

20. Seow CH, Newman A, Irwin SP, Steinhart AH, Silverberg MS, Greenberg GR. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut. 2010;59(1):49-54.

21. Cornillie F, Hanauer SB, Diamond RH, Wang J, Tang KL, Xu Z, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. Gut. 2014;63(11):1721-7.

22. Levesque BG, Greenberg GR, Zou G, Sandborn WJ, Singh S, Hauenstein S, et al. A prospective cohort study to determine the relationship between serum infliximab concentration and efficacy in patients with luminal Crohn's disease. Alimentary pharmacology & therapeutics. 2014;39(10):1126-35.

23. Bortlik M, Duricova D, Malickova K, Machkova N, Bouzkova E, Hrdlicka L, et al. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. Journal of Crohn's & colitis. 2013;7(9):736-43.

24. 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. Inflammatory bowel diseases. 2013;19(12):2568-76.

25. Chiu YL, Rubin DT, Vermeire S, Louis E, Robinson AM, Lomax KG, et al. Serum adalimumab concentration and clinical remission in patients with Crohn's disease. Inflammatory bowel diseases. 2013;19(6):1112-22.

26. Colombel JF, Sandborn WJ, Allez M, Dupas JL, Dewit O, D'Haens G, et al. Association between plasma concentrations of certolizumab pegol and endoscopic outcomes of patients with Crohn's disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2014;12(3):423-31.e1.

27. Yarur AJ, Jain A, Sussman DA, Barkin JS, Quintero MA, Princen F, et al. The association of tissue anti-TNF drug levels with serological and endoscopic disease activity in inflammatory bowel disease: the ATLAS study. Gut. 2015.

28. Rutgeerts P, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Comparison of scheduled and episodic treatment strategies of infliximab in Crohn's disease. Gastroenterology. 2004;126(2):402-13.

29. Maser EA, Villela R, Silverberg MS, Greenberg GR. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2006;4(10):1248-54.

30. Brandse JF, Vos LM, Jansen J, Schakel T, Ponsioen CI, van den Brink GR, et al. Serum Concentration of Anti-TNF Antibodies, Adverse Effects and Quality of Life in Patients with Inflammatory Bowel Disease in Remission on Maintenance Treatment. Journal of Crohn's & colitis. 2015.

31. Vande Casteele N, Ferrante M, Van Assche G, Ballet V, Compernolle G, Van Steen K, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology. 2015;148(7):1320-9.e3.

32. Roblin X, Marotte H, Rinaudo M, Del Tedesco E, Moreau A, Phelip JM, et al. Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2014;12(1):80-4.e2.

33. Xu ZH, Lee H, Vu T, Hu C, Yan H, Baker D, et al. Population pharmacokinetics of golimumab in patients with ankylosing spondylitis: impact of body weight and immunogenicity. International journal of clinical pharmacology and therapeutics. 2010;48(9):596-607.

34. Xu Z, Vu T, Lee H, Hu C, Ling J, Yan H, et al. Population pharmacokinetics of golimumab, an anti-tumor necrosis factor-alpha human monoclonal antibody, in patients with psoriatic arthritis. Journal of clinical pharmacology. 2009;49(9):1056-70.

35. Hu C, Xu Z, Zhang Y, Rahman MU, Davis HM, Zhou H. Population approach for exposure-response modeling of golimumab in patients with rheumatoid arthritis. Journal of clinical pharmacology. 2011;51(5):639-48.

36. Thorlund K, Druyts E, Toor K, Mills EJ. Comparative efficacy of golimumab, infliximab, and adalimumab for moderately to severely active ulcerative colitis: a network meta-analysis accounting for differences in trial designs. Expert review of gastroenterology & hepatology. 2015;9(5):693-700.

37. 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;371(9613):660-7.

38. 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;386(10006):1825-34.

39. Osterman MT, Sandborn WJ, Colombel JF, Robinson AM, Lau W, Huang B, et al. Increased risk of malignancy with adalimumab combination therapy, compared with monotherapy, for Crohn's disease. Gastroenterology. 2014;146(4):941-9.

40. Colombel JF, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. The New England journal of medicine. 2010;362(15):1383-95.

41. 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;146(2):392-400.e3.

42. Lemann M, Mary JY, Duclos B, Veyrac M, Dupas JL, Delchier JC, et al. Infliximab plus azathioprine for steroid-dependent Crohn's disease patients: a randomized placebo-controlled trial. Gastroenterology. 2006;130(4):1054-61.

43. van Schaik T, Maljaars JP, Roopram RK, Verwey MH, Ipenburg N, Hardwick JC, et al. Influence of Combination Therapy with Immune Modulators on Anti-TNF Trough Levels and Antibodies in Patients with IBD. Inflammatory bowel diseases. 2014.

44. 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;146(3):681-8.e1.

45. Hanauer SB, Wagner CL, Bala M, Mayer L, Travers S, Diamond RH, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2004;2(7):542-53.

46. Steenholdt C, Al-khalaf M, Brynskov J, Bendtzen K, Thomsen OO, Ainsworth MA. Clinical implications of variations in anti-infliximab antibody levels in patients with inflammatory bowel disease. Inflammatory bowel diseases. 2012;18(12):2209-17.

47. Ungar B, Chowers Y, Yavzori M, Picard O, Fudim E, Har-Noy O, et al. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. Gut. 2014;63(8):1258-64.

48. Saito S, Shimizu U, Nan Z, Mandai N, Yokoyama J, Terajima K, et al. Economic impact of combination therapy with infliximab plus azathioprine for drug-refractory Crohn's disease: a cost-effectiveness analysis. Journal of Crohn's & colitis. 2013;7(2):167-74.

49. Beaugerie L, Brousse N, Bouvier AM, Colombel JF, Lemann M, Cosnes J, et al. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. Lancet. 2009;374(9701):1617-25.

50. Kandiel A, Fraser AG, Korelitz BI, Brensinger C, Lewis JD. Increased risk of lymphoma among inflammatory bowel disease patients treated with azathioprine and 6-mercaptopurine. Gut. 2005;54(8):1121-5.

51. Siegel CA, Marden SM, Persing SM, Larson RJ, Sands BE. Risk of lymphoma associated with combination anti-tumor necrosis factor and immunomodulator therapy for the treatment of Crohn's disease: a meta-analysis. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2009;7(8):874-81.

52. Sahasranaman S, Howard D, Roy S. Clinical pharmacology and pharmacogenetics of thiopurines. European journal of clinical pharmacology. 2008;64(8):753-67.

53. Dubinsky MC, Lamothe S, Yang HY, Targan SR, Sinnett D, Théorêt Y, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. Gastroenterology. 2000;118(4):705-13.

54. Lichtenstein GR, Diamond RH, Wagner CL, Fasanmade AA, Olson AD, Marano CW, et al. Clinical trial: benefits and risks of immunomodulators and maintenance infliximab for IBD-subgroup analyses across four randomized trials. Alimentary pharmacology & therapeutics. 2009;30(3):210-26.

55. Rahier JF. Management of IBD Patients with Current Immunosuppressive Therapy and Concurrent Infections. Digestive diseases (Basel, Switzerland). 2015;33 Suppl 1:50-6.

56. Ong DE, Kamm MA, Hartono JL, Lust M. Addition of thiopurines can recapture response in patients with Crohn's disease who have lost response to anti-tumor necrosis factor monotherapy. Journal of gastroenterology and hepatology. 2013;28(10):1595-9.

57. Ben-Horin S, Waterman M, Kopylov U, Yavzori M, Picard O, Fudim E, et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2013;11(4):444-7.

58. Roblin X, Marotte H, Leclerc M, Del Tedesco E, Phelip JM, Peyrin-Biroulet L, et al. Combination of C-reactive protein, infliximab trough levels, and stable but not transient antibodies to infliximab are associated with loss of response to infliximab in inflammatory bowel disease. Journal of Crohn's & colitis. 2015;9(7):525-31.

59. Dotan I, Ron Y, Yanai H, Becker S, Fishman S, Yahav L, et al. Patient factors that increase infliximab clearance and shorten half-life in inflammatory bowel disease: a population pharmacokinetic study. Inflammatory bowel diseases. 2014;20(12):2247-59.

60. Fasanmade AA, Adedokun OJ, Olson A, Strauss R, Davis HM. Serum albumin concentration: a predictive factor of infliximab pharmacokinetics and clinical response in patients with ulcerative colitis. International journal of clinical pharmacology and therapeutics. 2010;48(5):297-308.

61. Brandse JF, van den Brink GR, Wildenberg ME, van der Kleij D, Rispens T, Jansen JM, et al. Loss of Infliximab Into Feces Is Associated With Lack of Response to Therapy in Patients With Severe Ulcerative Colitis. Gastroenterology. 2015;149(2):350-5.e2.

62. Biancheri P, Brezski RJ, Di Sabatino A, Greenplate AR, Soring KL, Corazza GR, et al. Proteolytic cleavage and loss of function of biologic agents that neutralize tumor necrosis factor in the mucosa of patients with inflammatory bowel disease. Gastroenterology. 2015;149(6):1564-74.e3.

63. Gibson DJ, Heetun ZS, Redmond CE, Nanda KS, Keegan D, Byrne K, et al. An accelerated infliximab induction regimen reduces the need for early colectomy in patients with acute severe ulcerative colitis. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2015;13(2):330-5.e1.

64. Bressler B, Panaccione R, Fedorak RN, Seidman EG. Clinicians' guide to the use of fecal calprotectin to identify and monitor disease activity in inflammatory bowel disease. Can J Gastroenterol Hepatol. 2015;29(7):369-72.

65. Chang S, Malter L, Hudesman D. Disease monitoring in inflammatory bowel disease. World journal of gastroenterology : WJG. 2015;21(40):11246-59.

66. Mao R, Xiao YL, Gao X, Chen BL, He Y, Yang L, et al. Fecal calprotectin in predicting relapse of inflammatory bowel diseases: a meta-analysis of prospective studies. Inflammatory bowel diseases. 2012;18(10):1894-9.

67. Bartelds GM, Wijbrandts CA, Nurmohamed MT, Stapel S, Lems WF, Aarden L, et al. Anti-infliximab and anti-adalimumab antibodies in relation to response to adalimumab in infliximab switchers and anti-tumour necrosis factor naive patients: a cohort study. Annals of the rheumatic diseases. 2010;69(5):817-21.

68. Frederiksen MT, Ainsworth MA, Brynskov J, Thomsen OO, Bendtzen K, Steenholdt C. Antibodies Against Infliximab Are Associated with De Novo Development of Antibodies to Adalimumab and Therapeutic Failure in Infliximab-to-Adalimumab Switchers with IBD. Inflammatory bowel diseases. 2014.

69. Billiet T, Vande Casteele N, Van Stappen T, Princen F, Singh S, Gils A, et al. Immunogenicity to infliximab is associated with HLA-DRB1. Gut. 2015;64(8):1344-5.

70. Gisbert JP, Marín AC, McNicholl AG, Chaparro M. Systematic review with meta-analysis: the efficacy of a second anti-TNF in patients with inflammatory bowel disease whose previous anti-TNF treatment has failed. Alimentary pharmacology & therapeutics. 2015;41(7):613-23.

71. Summerton CB, Longlands MG, Wiener K, Shreeve DR. Faecal calprotectin: a marker of inflammation throughout the intestinal tract. European journal of gastroenterology & hepatology. 2002;14(8):841-5.

72. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflammatory bowel diseases. 2012;18(12):2218-24.

73. 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. Inflammatory bowel diseases. 2013;19(10):2111-7.

74. Travis S, Satsangi J, Lemann M. Predicting the need for colectomy in severe ulcerative colitis: a critical appraisal of clinical parameters and currently available biomarkers. Gut. 2011;60(1):3-9.

75. Dignass A, Lindsay JO, Sturm A, Windsor A, Colombel JF, Allez M, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. Journal of Crohn's & colitis. 2012;6(10):991-1030.

76. Van Assche G, Dignass A, Panes J, Beaugerie L, Karagiannis J, Allez M, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. Journal of Crohn's & colitis. 2010;4(1):7-27.

77. Yanai H, Lichtenstein L, Assa A, Mazor Y, Weiss B, Levine A, et al. Levels of Drug and Anti-drug Antibodies are Associated with Outcome of Interventions after Loss of Response to Infliximab or Adalimumab. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2014.

78. Afif W, Loftus EV, Jr., Faubion WA, Kane SV, Bruining DH, Hanson KA, et al. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. The American journal of gastroenterology. 2010;105(5):1133-9.

79. Roblin X, Rinaudo M, Del Tedesco E, Phelip JM, Genin C, Peyrin-Biroulet L, et al. Development of an algorithm incorporating pharmacokinetics of adalimumab in inflammatory bowel diseases. The American journal of gastroenterology. 2014;109(8):1250-6.

80. Katz L, Gisbert JP, Manoogian B, Lin K, Steenholdt C, Mantzaris GJ, et al. Doubling the infliximab dose versus halving the infusion intervals in Crohn's disease patients with loss of response. Inflammatory bowel diseases. 2012;18(11):2026-33.

81. Sandborn WJ, Feagan BG, Rutgeerts P, Hanauer S, Colombel JF, Sands BE, et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. The New England journal of medicine. 2013;369(8):711-21.

82. Feagan BG, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. The New England journal of medicine. 2013;369(8):699-710.

83. Kane S, Huo D, Aikens J, Hanauer S. Medication nonadherence and the outcomes of patients with quiescent ulcerative colitis. Am J Med. 2003;114(1):39-43.

84. Lopez A, Billioud V, Peyrin-Biroulet C, Peyrin-Biroulet L. Adherence to anti-TNF therapy in inflammatory bowel diseases: a systematic review. Inflammatory bowel diseases. 2013;19(7):1528-33.

85. Billioud V, Laharie D, Filippi J, Roblin X, Oussalah A, Chevaux JB, et al. Adherence to adalimumab therapy in Crohn's disease: a French multicenter experience. Inflammatory bowel diseases. 2011;17(1):152-9.

86. Kane SV, Chao J, Mulani PM. Adherence to infliximab maintenance therapy and health care utilization and costs by Crohn's disease patients. Advances in therapy. 2009;26(10):936-46.

87. Schnitzler F, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, et al. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. Inflammatory bowel diseases. 2009;15(9):1295-301.

88. Colombel JF, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. Gastroenterology. 2011;141(4):1194-201.

89. Steenholdt C, Brynskov J, Thomsen OO, Munck LK, Fallingborg J, Christensen LA, et al. Individualised therapy is more cost-effective than dose intensification in patients with Crohn's disease who lose response to anti-TNF treatment: a randomised, controlled trial. Gut. 2014;63(6):919-27.

90. Velayos FS, Kahn JG, Sandborn WJ, Feagan BG. A test-based strategy is more cost effective than empiric dose escalation for patients with Crohn's disease who lose responsiveness to infliximab. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2013;11(6):654-66.

91. Moreno N, Ripolles T, Paredes JM, Ortiz I, Martinez MJ, Lopez A, et al. Usefulness of abdominal ultrasonography in the analysis of endoscopic activity in patients with Crohn's disease: changes following treatment with immunomodulators and/or anti-TNF antibodies. Journal of Crohn's & colitis. 2014;8(9):1079-87.

92. Castiglione F, Testa A, Rea M, De Palma GD, Diaferia M, Musto D, et al. Transmural healing evaluated by bowel sonography in patients with Crohn's disease on maintenance treatment with biologics. Inflammatory bowel diseases. 2013;19(9):1928-34.

93. Koelbel G, Schmiedl U, Majer MC, Weber P, Jenss H, Kueper K, et al. Diagnosis of fistulae and sinus tracts in patients with Crohn disease: value of MR imaging. AJR Am J Roentgenol. 1989;152(5):999-1003.

94. Shoenut JP, Semelka RC, Magro CM, Silverman R, Yaffe CS, Micflikier AB. Comparison of magnetic resonance imaging and endoscopy in distinguishing the type and severity of inflammatory bowel disease. J Clin Gastroenterol. 1994;19(1):31-5.

95. Coorevits L, Baert FJ, Vanpoucke HJ. Faecal calprotectin: comparative study of the Quantum Blue rapid test and an established ELISA method. Clinical chemistry and laboratory medicine : CCLM / FESCC. 2013;51(4):825-31.

96. Molander P, Farkkila M, Salminen K, Kemppainen H, Blomster T, Koskela R, et al. Outcome after discontinuation of TNFalpha-blocking therapy in patients with inflammatory bowel disease in deep remission. Inflammatory bowel diseases. 2014;20(6):1021-8.

97. Louis E, Mary JY, Vernier-Massouille G, Grimaud JC, Bouhnik Y, Laharie D, et al. Maintenance of remission among patients with Crohn's disease on antimetabolite therapy after infliximab therapy is stopped. Gastroenterology. 2012;142(1):63-70.e5; quiz e31.

98. Steenholdt C, Molazahi A, Ainsworth MA, Brynskov J, Ostergaard Thomsen O, Seidelin JB. Outcome after discontinuation of infliximab in patients with inflammatory bowel disease in clinical remission: an observational Danish single center study. Scandinavian journal of gastroenterology. 2012;47(5):518-27.

99. Duron C, Goutte M, Pereira B, Bommelaer G, Buisson A. Factors influencing acute infusion reactions in inflammatory bowel disease patients treated with infliximab in the era of scheduled maintenance therapy. European journal of gastroenterology & hepatology. 2015;27(6):705-11.

100. Kugathasan S, Levy MB, Saeian K, Vasilopoulos S, Kim JP, Prajapati D, et al. Infliximab retreatment in adults and children with Crohn's disease: risk factors for the development of delayed severe systemic reaction. The American journal of gastroenterology. 2002;97(6):1408-14.

101. Han PD, Cohen RD. Managing immunogenic responses to infliximab: treatment implications for patients with Crohn's disease. Drugs. 2004;64(16):1767-77.

102. Lichtenstein L, Ron Y, Kivity S, Ben-Horin S, Israeli E, Fraser GM, et al. Infliximab-Related Infusion Reactions: Systematic Review. Journal of Crohn's & colitis. 2015;9(9):806-15.

103. Mosli MH, Zou G, Garg SK, Feagan SG, MacDonald JK, Chande N, et al. C-Reactive Protein, Fecal Calprotectin, and Stool Lactoferrin for Detection of Endoscopic Activity in Symptomatic Inflammatory Bowel Disease Patients: A Systematic Review and Meta-Analysis. The American journal of gastroenterology. 2015;110(6):802-19; quiz 20.

104. Elkjaer M, Shuhaibar M, Burisch J, Bailey Y, Scherfig H, Laugesen B, et al. E-health empowers patients with ulcerative colitis: a randomised controlled trial of the web-guided 'Constant-care' approach. Gut. 2010;59(12):1652-61.

105. Mould DR, Upton RN, Wojciechowski J. Dashboard systems: implementing pharmacometrics from bench to bedside. The AAPS journal. 2014;16(5):925-37.

# Chapter 7

## Combination immunosuppression in IBD

S. Bots, K. Gecse, M. Barclay, G.D'Haens

*Inflammatory Bowel Diseases 2018;24(3):539-545*

## Abstract

Whether to use biologic treatment for inflammatory bowel disease as monotherapy or in combination with immunosuppressives has been a matter of debate in the last 2 decades. Combination therapy was not superior in any of the registration trials for Crohn's disease and ulcerative colitis for TNF antagonists, vedolizumab, or ustekinumab. It needs to be mentioned, though, that none of these trials were powered to detect such differences, and that many patients entered the trial after having failed conventional immunosuppressives. Postmarketing studies revealed that patients on background immunosuppression have a lower risk of immunogenicity (often resulting in infusion/injection reactions) than patients on monotherapy. In the SONIC and UC-SUCCESS trials, superiority of the combination azathioprine-infliximab was demonstrated in Crohn's disease and ulcerative colitis, respectively. This trial design has not been used with any other biologic for IBD, so far. Meanwhile, it has also become clear that combination treatment with TNF antagonists is associated with increased toxicity, mainly infections, but also malignancy such as lymphoproliferative disease. This toxicity could perhaps be reduced by using lower doses of immunosuppressives, a strategy that has been shown to be equally potent in reducing immunogenicity. Additionally, combination treatment could be used for a limited period of time (12 months or even shorter) since most immunogenicity develops in the beginning of the biologic treatment. Patients who develop anti-drug-antibodies later on can often be rescued by reintroduction of thiopurines or methotrexate.

In summary, combination treatment is certainly beneficial with infliximab, at least in the first 12 months of treatment. With other TNF antagonists, vedolizumab, and ustekinumab, the available data do not offer clear guidance. In patients without increased risk of toxicity, and certainly in those with limited treatment options, it may be wise to offer combination treatment with all biologics for the time being and at least during the initiation phase.

## Introduction and historical background

Initial studies with the anti-TNF agent infliximab for Crohn's disease (CD) and ulcerative colitis (UC) did not demonstrate increased efficacy when this therapeutic antibody was combined with immunosuppressive agents, however in the Phase 3 study ACCENT-1 for CD, a trend towards greater efficacy of combination treatment was observed at week 30 ($P = 0.062$).[1, 2]

It also became rapidly clear that after discontinuation and reinitiation of infliximab (IFX) treatment, patients on background immunosuppressive treatment experienced fewer infusion reactions and loss of response, a phenomenon that was explained by lower anti-IFX antibody formation.[3] It took until 2008, when the results of the SONIC trial were published, before it was demonstrated that combination treatment of IFX with the immunosuppressive azathioprine (AZA) led to superior clinical and endoscopic results compared to either monotherapy in CD.[4] Combination treatment was characterized by higher IFX serum concentrations and a lower immunogenicity risk. This finding was replicated in the UC-SUCCESS trial.[5] Subsequently, combination treatment has been recommended for all patients in whom IFX is started.

The observations for other biologics, which are more humanized than the chimera IFX, are less convincing. In the Phase 3 trials with adalimumab (ADL), golimumab (GOLI), certolizumab pegol (CZP), vedolizumab (VEDO), and ustekinumab (UST), no evidence was found for an additional benefit from combined immunosuppression, although none of these trials were powered to answer this question specifically.[6-14] SONIC-like programs were never performed for biologics other than IFX. For most biologics some retrospective data or cohort studies suggest benefit of combination immunosuppression, but presently the results do not appear strong enough to recommend combined treatment for all patients receiving non IFX biologics.



## Mechanisms of action of combination therapy

Several mechanisms have been proposed to explain the increased effectiveness of combined immunosuppressives and anti-TNF therapy (mainly IFX) compared with anti-TNF therapy alone. The best documented feature of combination therapy in this regard is the reduction in risk of immunogenicity, (ie, anti-drug antibody formation) with both the thiopurines and methotrexate (MTX). Presence of anti-drug antibodies against IFX has been associated with a 4-fold increase in drug clearance, most likely due to enhanced clearance of drug/anti-drug antibody immune complexes.[15] Rapid drug clearance results in low or undetectable circulating drug concentrations, which are associated with lower success rates for induction of remission and with loss of response. The extent of reduction in antidrug antibodies appears similar for the thiopurines and MTX.[4, 5, 16, 17] The rate of antidrug antibody formation is lower with ADL than IFX and so the effect of suppression of anti-drug

antibodies with immunosuppressives may be less pronounced with ADL combination therapy.[18] Combination immunosuppressive therapy leads, however, to less immunogenicity and higher ADL serum concentrations.[19, 20] Of note, anti-drug antibody formation has been observed as early as 18 days after commencing IFX, leading to a greater chance of nonresponse, and so it seems advisable to commence immunosuppression as early as possible in combination with anti-TNF-alpha therapy.[15]

In addition to preventing immunogenicity, introduction of an immunosuppressant also has a high chance of reversing anti-drug antibody formation, with most cases reversing within 12 months using either a thiopurine or MTX.[21, 22] One study also has shown a median increase in IFX trough concentrations of 2.84 mg/L with addition of a thiopurine or MTX to IFX.[22]

It has been postulated that the addition of immunosuppression to anti-TNF-alpha therapy may have benefits beyond altered immunogenicity or pharmacokinetics, resulting in treatment synergy. There are limited data to support this. However, in vitro studies have shown that anti-TNF drugs induce regulatory macrophages that assist in wound and mucosal healing and that AZA, when combined with IFX, further increases the number and wound-healing properties of these macrophages, which then also display stronger immunosuppressive properties.[23-26] This would support the possibility of synergy at the pharmacological level.

## Efficacy of combined immunosuppression

The efficacy of IFX combined with immunosuppressive agents has been studied extensively. The SONIC trial showed that combination therapy resulted in higher rates of corticosteroid-free clinical remission and mucosal healing (absence of ulcers) after 26 weeks of treatment in CD patients (56.8% vs 44.4%; $P = 0.02$ and 43.9% vs 30.1%; $P = 0.06$, respectively).[4] A recent posthoc analysis of this trial showed significantly higher rates of anti-drug antibodies in the monotherapy patients (36% vs 8%). The benefit of combination therapy seemed mainly driven by the effect of AZA on the pharmacokinetics and immunogenicity of IFX in those on combination therapy.[27]

Likewise, the UC-SUCCESS trial showed increased corticosteroid-free remission in UC patients on combination therapy after 16 weeks of treatment (39.7% vs 22.1%, $P = 0.017$), although in this trial the mucosal healing rates (assessed by local investigators) were not higher with combination than with monotherapy (62.8% vs 54.6%, $P = 0.295$ in combination vs monotherapy).[5] The superiority of IFX combination therapy in UC patients also was shown in a systematic review and meta-analysis.[27]

IFX combined with parenteral MTX was studied in the COMMIT trial for CD. After 50 weeks of treatment starting with an induction regimen of prednisone, no improved clinical efficacy was observed in the combination group compared to monotherapy,

although patients receiving MTX had higher serum concentrations of IFX on average.[16] The corticosteroid induction treatment for up to 14 weeks may have affected the efficacy outcomes blurring the potential additional benefit of MTX. Also, unlike the SONIC trial, this trial had no endoscopic endpoint.

As stated above, the superiority of combination immunosuppression with ADL has been demonstrated less convincingly. The question has never been investigated in a prospective trial. The large Phase 3 trials with ADL for CD, CHARM, and ulcerative colitis, ULTRA, could not demonstrate an additional benefit of combined immunosuppression.[14, 28] A recent systematic review and meta-analysis included 24 CD studies and showed no difference for induction of clinical remission (OR 0.86; 95% CI: 0.70–1.06; $P$ = 0.19) and clinical response (OR 1.01; 95% CI: 0.62–1.65; $P$ = 0.96) and also no differences for maintenance of clinical remission (OR 0.97; 95% CI: 0.79–1.14; $P$ = 0.75) or response (OR 0.91; 95% CI: 0.54–1.54; $P$ = 0.74).[19]

A few isolated studies, nonetheless, reported clinical benefit of combination treatment with immunosuppressives. Matsumoto et al showed no difference in clinical efficacy in CD patients on combination therapy versus monotherapy in a 52-week prospective trial (remission rates 68% vs 72%). However, endoscopic improvement (the secondary outcome defined as a decrease of SES-CD of at least 8 points from the baseline, or SES-CD ≤ 4) was more frequently attained in patients after 26 weeks of combination treatment [84.2%, n = 5 vs 63.8%, n = 58 ($P$ = 0.019)].[20] Nevertheless, this endoscopic difference was not sustained after 52 weeks of treatment. Kariyawasam and colleagues conducted a retrospective study in 91 CD patients and found higher rates of induction and maintenance of clinical response on combination treatment (83% vs 61%; $P$ = 0.02 and 81% vs 60%; $P$ < 0.0001, respectively), but they did not assess endoscopic outcomes.[29] Reenaers showed lower risk of ADL failure in the first semester of combination treatment (5% vs 10%; $P$ = 0.04; OR 0.48) and fewer flares beyond 6 months of combination treatment in a retrospective dataset (14% vs 36%; $P$ = 0.02; OR 0.31).[30] Finally, Cosnes showed longer anti-TNF survival in patients on combination treatment with similar effect for ADL and IFX [adjusted HR 2.17 (95% CI: 1.71–2.70)].[31]

In conclusion, data regarding ADL combination therapy are conflicting and most studies that showed clinical benefit used a retrospective design. Therefore, adequate prospective studies that are properly powered are needed to clarify this issue.

The effect of combination therapy with the anti-TNF agents GOLI and CZP is unknown. The PURSUIT trials (GOLI in UC) and PRECISE trials (CZP in CD) did not report data on clinical efficacy of combination therapy.[6, 7, 11, 13] To our knowledge no other studies on combination therapy with these agents have been conducted.

The efficacy of VEDO combination therapy also remains uncertain. The Gemini I and II trials showed no clinical bene- fit of VEDO combined with immunosuppressive agents

in CD and UC, but the trials were not designed to answer this question.[9, 10] Following these Phase 3 trials, several postmarketing cohort studies equally did not show beneficial outcomes of VEDO combination therapy in either CD or UC when compared to monotherapy.[32-34] One multicenter cohort study showed different results indicating superior clinical efficacy after 54 weeks of VEDO treatment with an immunosuppressant in CD, with an odds ratio of 8.33 (95% CI 2.15–32.26).[35] This was a retrospective study, however, and only patients that responded to 14 weeks of induction treatment were included in this analysis. Therefore, it is difficult to draw firm conclusions based on this study.

Currently, there is no evidence supporting superiority of UST combination therapy. The UNITI trials did not show better outcomes for patients on background immunosuppressive therapy treated with UST, but these trials were evidently not powered to address this question.[8] Battat et al showed equivalent corticosteroid-free and endoscopic remission for UST monotherapy and combination therapy after 26 weeks of treatment in 62 CD patients.[36] However, the study was not designed, and was underpowered, to assess this outcome.

Long-term outcomes of combination therapy are relatively unknown. It is not clear what the effect of combination treatment is on outcomes such as surgery and disease behavior over many years. However, it is to be expected that adequate control of inflammation leads to better long-term outcomes. This is reflected by the fact that the number of hospitalizations and surgeries have diminished since the introduction of biologic agents.[37]

## Effect of immunosuppression on withdrawal of biologics

The timing of anti-TNF treatment withdrawal in IBD patients in remission has been a matter of discussion. According to the STORI trial, approximately 50% of patients with CD previously treated with >1 year of IFX in combination with an antimetabolite experienced a relapse within 18 months after discontinuation of IFX (with continued immunosuppression).[38] Risk factors for relapse included male sex, the absence of previous surgical resection, and elevated inflammatory biomarkers. Importantly, relapsing patients on continued immunosuppressives were successfully retreated with IFX after a median drug-holiday of 6.6 months. None of the patients experienced a significant infusion reaction, as followed-up to the third retreatment infusion. Although the follow-up is limited and pre-infusion corticosteroid prophylaxis was applied, median IFX trough levels were not significantly different between the baseline and third retreatment infusions. Additionally, no increase in the formation of anti-drug antibodies was detected in these patients with sustained antimetabolite treatment.

The IMID (immunosuppression withdrawal in Crohn's disease) study evaluated whether continued treatment with immunosuppressives beyond 6 months of combination

treatment offered benefit over scheduled IFX monotherapy in patients with CD in stable remission.[39] There was no significant difference in the proportion of patients requiring dose intensification of IFX or stopping IFX therapy among patients on IFX monotherapy or continued combined immunosuppression. However, discontinued immunosuppression was associated with lowering of median IFX concentrations, which correlated with elevated serum CRP levels and clinical scores.

Additionally, an open-label randomized trial recently showed that in IBD patients with durable remission on combination therapy, dose reduction of AZA to 1–1.25 mg/kg/day was as effective as treatment at full dose in terms of clinical relapse after 1 year.[40] Median IFX trough levels dropped significantly in the IFX monotherapy group compared to the combination treatment or reduced AZA dose groups.

## Effect of combined immunosuppression on immunogenicity and serum concentrations of biologic drug

Many studies have shown a significant reduction in IFX anti-drug antibody formation with combination therapy versus IFX monotherapy in patients on maintenance treatment.[1, 4, 5, 16, 41, 42] The degree of reduction in anti-drug-antibodies appears similar for the thiopurines and MTX, although no head-to-head studies have been performed specifically addressing this question.[4, 5, 16, 17] Reduction of immunogenicity results in higher IFX serum concentrations. On the other hand, the reduction in immunogenicity also could be explained by a boost in IFX serum concentrations caused by immunosuppressives. As an example, in the SONIC study, week 30 median trough IFX concentrations were 1.6 mg/L for IFX monotherapy vs. 3.5 mg/L for combination with AZA, and patients with higher trough concentrations had a higher chance of remission.[4] As mentioned above, a recent prospective trial conducted by Roblin et al. showed that lower doses of AZA were equally effective in maintaining adequate IFX concentrations and preventing antibody formation as full AZA doses.[40]

The rate of antidrug antibody formation is lower in patients treated with ADL when compared to IFX.[12, 43-45] Although ADL combination immunosuppressant therapy has generally not been shown to be more effective, it leads to less immunogenicity and higher ADL trough concentrations.[19, 20, 46] A systematic review showed that the presence of anti-drug antibodies was associated with a significant reduction in concentrations of IFX and ADL (−7.07, 95%CI 5.25-8.9).[46] It is unclear why ADL combination therapy is not as effective as IFX combination therapy. Other factors such as tissue concentrations may be of relevance here. Since ADL appears to be less immunogenic than IFX, reduction of antibody formation to ADL could be less important.

A post-hoc analysis of the PURSUIT trials showed a small increase in GOLI serum concentrations in patients receiving combination treatment with 50 mg GOLI every 4

weeks but not in patients receiving 100 mg every 4 weeks, but this did not influence clinical treatment efficacy.[47] The Precise I and II trials showed a small difference in the development of antibodies against CZP in mono versus combination therapy.[11, 13] The influence of immunogenicity on treatment efficacy and drug serum concentrations was not reported, but it is to be expected that lower antibody formation results in higher CZP serum concentrations. Concomitant immunosuppressive treatment also resulted in lower immunogenicity to VEDO in the GEMINI I and II trials.[9, 10] The immunogenicity of UST appears to be very low.[8, 36] The effect of combination therapy on the immunogenicity of UST has not been studied and remains unknown.

## Safety of combination therapy

There are 2 areas of particular interest when discussing the safety of combination treatment: the risk of infections and malignancy. Safety data for combination treatment is derived from pooled post-hoc analysis of registration trials, from dedicated trials on combination treatment, and from registries with long-term outcomes.

Data on the safety of combination treatment with biologic drugs derived from individual registration trials is limited by the relatively low number of events and short-term follow-up. However, a pooled analysis of sponsor-initiated trials on IFX in IBD, (mainly based on results from the ACCENT I, ACCENT II, SONIC, and ACT I and II studies) resulted in 1713 IFX-treated patients (and 406 placebo-treated patients) with or without concomitant immunosuppressives.[48] There was no increased incidence of infections, serious infections,

or malignancy with IFX monotherapy when compared to placebo. Immunosuppression in patients with UC was associated with a higher incidence of infections [120.07 (95% CI 110.66, 130.08)/100 patient-years vs 92.47 (84.54, 100.94)/100 patient-years]. The most common serious infections in IFX-treated patients were pneumonia, cellulitis, abdominal abscess, and perirectal abscess. Additionally, among placebo-treated patients with CD, those on immunosuppressives had a higher incidence of malignancy compared to no immunosuppressive treatment [1.84 (0.22, 6.66)/100 patient-years vs 0.00 (0.00, 0.00)/100 patient-years].[48]

Clinical registries play an important role in evaluating medication safety. They include larger numbers of patients with a long follow-up period compared to randomized trials and represent real-life practice. The Crohn's Therapy, Resource, Evaluation, and Assessment Tool (TREAT) Registry was a US-based prospective registry that was designed to examine the safety of CD medications, including IFX. The registry included 6273 patients with CD who were followed up for a median duration exceeding 6 years. In an exposure-based analysis, the use of immunosuppressives alone (OR 4.19; 95% CI 0.58–30.37; $P$ = 0.16) or in combination with IFX (OR 3.33; 95% CI 0.46– 24.06; $P$ = 0.23) was

associated with a numerically greater risk of malignancy than treatment with IFX alone (OR 1.96; 95% CI 0.23–17.02; *P* = 0.54), however, this was not statistically significant.[49]

In comparison, ENCORE was a European 5-year prospective safety registry, that included 2960 IFX-naive CD patients treated with IFX or conventional therapy.[50] The most common serious infections were abscess, pneumonia, peritonitis, and sepsis, but incidence rates were not different in the IFX and AZA/6-MP combination treatment group compared with the infliximab monotherapy group [25.4/1000 patient-years (95% CI 20.4, 31.4) vs. 39.1/1000 patient-years (20.2, 68.3)]. IFX was associated with an increased rate of benign haematological conditions. However, 45/46 of these incidents were reported in patients on combination therapy. Lymphoproliferative disorders and malignancy were reported in 49/1541 IFX-treated patients. The rates per 1000 patient-years exposure in patients with and without AZA/6-MP combination were 7.0 (95% CI 5.1, 9.3) and 14.5 (6.6, 27.5), respectively.

In a pooled analysis of 1594 patients with CD who participated in clinical trials of ADL (CLASSIC I and II, CHARM, GAIN, EXTEND, and ADHERE) giving a total of 3050 patient-years of exposure, 44% were receiving concomitant immunosuppressives (563 with thiopurines and 131 with MTX). There were 44 malignancies reported in 34 patients (2.1%), 12 events on ADL monotherapy and 32 events on combination treatment. In this analysis, incidence of nonmelanoma skin cancer (NMSC) or other cancers was not increased in patients receiving ADL monotherapy compared to the normal population. However, patients receiving combination therapy had a higher incidence of NMSC (standardized incidence ratio, 4.59; 95% CI 2.51–7.70) and other malignancies (standardized incidence ratio, 3.04; 95% CI 1.66–5.10). Patients receiving combination therapy had an increased risk for NMSC (relative risk, 3.46; 95% CI 1.08–11.06) and other malignancies (relative risk, 2.82; 95% CI 1.07–7.44), compared to patients receiving ADL monotherapy.[51]

The PYRAMID registry analysed long-term safety of ADL in patients with CD, which also included subgroup analysis on serious infections and malignancy in patients with ADL monotherapy compared to combination treatment.[52] Of 5061 patients enrolled, 2444 completed the 6-year follow-up, which resulted in a cumulative ADL exposure of 16,680.4 patient-years. There were 24.2% of patients who received concomitant immunosuppressives and an additional 11.6% of patients who received both corticosteroids and immunosuppressives at baseline. There was a significant difference in the incidence of treatment-emergent malignancies between the ADL monotherapy and ADL and thiopurine combination therapy groups (1.9 vs. 3.1%, *P* = 0.014). Nine of the 10 patients diagnosed with lymphoma received concomitant immunosuppressive treatment. Additionally, there was a significant difference in the incidence of treatment-emergent serious infections when comparing ADL monotherapy and combination therapy groups (9.6 vs. 12.7%, *P* = 0.007).

Data are limited with regard to the safety of combination treatment of immunosuppressives with VEDO and UST. In the GEMINI 1 and II studies, 19 (17%) and 16 (17%) patients treated with VEDO received concomitant immunosuppressive or glucocorticoids and immunosuppressives at baseline, respectively.[9, 10] A pooled analysis of 6 VEDO trials including 2830 patients with 4811 patient-years of VEDO exposure indicated that baseline immunosuppressive use was not associated with serious infections in either CD or UC.[53] In the maintenance trial of UST, 35%, 39%, and 33% of patients in the placebo, 90 mg UST every 12 weeks, and 90 mg UST every 8 weeks groups received stable doses of concomitant immunosuppressives, respectively, including overall 1281 patients.[36] During 1 year of treatment, 3 opportunistic infections occurred, including 1 case of Listeria meningitis (under UST and prednisolone treatment) and 2 cases of esophageal candidiasis (1 with UST treatment and 1with UST, MTX and prednisolone combination therapy). There were 8 NMSC events occurring in 5 patients, 2 of whom were receiving placebo and 3 receiving UST maintenance treatment. Of the 5 patients with NMSC 3 were currently using or had previously used immunosuppressives.

The SECURE registry was established to evaluate safety outcomes in patients with CD treated with CZP. However, no results are yet available from the interim analysis that compares CZP monotherapy with combination treatment.[54]

A systematic review and meta-analysis including 11,702 persons with immune-mediated diseases (RA, IBD, and psoriasis) and a prior diagnosis of cancer found that rates of cancer recurrence were similar among individuals who received no immunosuppression (37.5 per 1000 person-years), anti-TNF therapy (33.8 per 1000 person-years), immunosuppressive therapy (33.8 per 1000 person-years), or combination treatment (54.5 per 1000 person-years; $P > 0.1$ for all), although recurrence was numerically higher in the latter group.[55] A subgroup analysis of new and recurrent cancers separately, type of immunosuppressive therapy, or immune-mediated disease showed similar results, with no increase in risk. However, prolonged treatment with thiopurines appears to be associated with a small increase in risk of lymphoma.[56] Additionally, the ongoing I-CARE project aims to evaluate prospectively the presence and the extent of safety concerns (cancers, especially lymphoma, and serious infection risks) for anti-TNF monotherapy or combination treatment among IBD patients (NCT02377258).

In conclusion, risk of serious infections has been shown to be increased with combination immunosuppressive treatment in comparison to monotherapy.[48, 57] The rate of malignancy with thiopurine therapy has been reported to be 2- to 5-fold higher for both lymphoproliferative disorders and NMSC and has also been associated with increased risk of overall cancer compared with IBD patients not treated with thiopurines.[58-60] There is no current evidence to clearly indicate increased risk of malignancy with anti-TNF monotherapy or with combination MTX.[61] The increased risk of cancer with thiopurine and anti-TNF combination therapy is a safety risk in IBD. Based on current evidence, the

relative contribution of thiopurines to risk may be more important than that of anti-TNF drugs.[62] Combining immunosuppressives with VEDO or UST seems to be safe, although prospective data specifically addressing this topic are not currently available.

## Recommendations for clinical practice

Combination treatment is pivotal for successful monoclonal antibody treatment in IBD. Benefits and harms of combination treatment and recommendations for clinical practice are summarized in table 1. In patients starting on IFX, it should currently be recommended that the IFX be combined with thiopurines (and if not tolerated, with MTX) for at least 1 year. In combination treatment, it is probable that lower doses of immunosuppressives suffice compared to monotherapy doses of immunosuppressives. It remains to be confirmed if immunosuppressives can be completely abandoned in the presence of higher serum concentrations of monoclonal drugs. Safety and (potential) toxicity, mainly associated with thiopurines in combination with anti-TNF, has to be balanced against the clinical benefit with combination treatment. For instance, the small risk of lymphoma associated with prolonged thiopurine treatment could be considered when deciding to continue combination treatment beyond 1 year.

Immunosuppressives also play a role in the management of immunogenicity. Multiple studies have demonstrated that adding or switching immunosuppressives when anti-drug anti- bodies appear is often a successful intervention, with suppression of antidrug antibodies and increase of monoclonal drug concentrations, with recapture of clinical benefit.

Finally, immunosuppressives appear to have a beneficial effect when patients stop anti-TNF treatment. Patients who continue immunosuppressives have a significantly lower risk of relapse. It remains to be seen if the initiation of an immunosuppressive when the biologic drug is discontinued can maintain remission.

Combination treatment with VEDO and UST warrants further exploration. Although the immunogenicity of these agents is lower than that of IFX, initial combination with an immunosuppressive for a number of months may be beneficial.

**Table 1.** *Benefits and Harms of Combination Treatment in IBD and Recommendations for Practice.*

| | Benefits | Harms | Recommendations |
|---|---|---|---|
| IFX | -Lower immunogenicity<br>-Higher serum concentrations<br>-Increased efficacy<br>-Reverse antibody formation<br>-No increased infection risk | -Small increase in malignancy risk with thiopurines | -At least 1 year of treatment<br>-Lower IM doses may suffice |
| ADL | -Lower immunogenicity<br>-Higher serum concentrations<br>-Increased efficacy questionable<br>-Reverse antibody formation<br>-No increased infection risk | -Small increase in malignancy risk with thiopurines | |
| GOLI | -Slightly higher serum concentrations | -Unknown | -Questionable |
| CZP | -Slightly lower immunogenicity | -Unknown | -Questionable |
| VEDO | -Unknown | -Unknown | -Questionable |
| UST | -Unknown | -Unknown | -Questionable |

*IFX = infliximab; ADL = adalimumab; GOLI = golimumab; CZP = certolizumab pegol; VEDO = vedolizumab; UST = ustekinumab; IM = immunosuppressive*

## References


1. Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet. 2002;359(9317):1541-9.

2. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. The New England journal of medicine. 2005;353(23):2462-76.

3. Baert F, Noman M, Vermeire S, Van Assche G, G DH, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. The New England journal of medicine. 2003;348(7):601-8.

4. Colombel JF, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. The New England journal of medicine. 2010;362(15):1383-95.

5. 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;146(2):392-400.e3.

6. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146(1):96-109.e1.

7. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146(1):85-95; quiz e14-5.

8. Feagan BG, Sandborn WJ, Gasink C, Jacobstein D, Lang Y, Friedman JR, et al. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. The New England journal of medicine. 2016;375(20):1946-60.

9. Sandborn WJ, Feagan BG, Rutgeerts P, Hanauer S, Colombel JF, Sands BE, et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. The New England journal of medicine. 2013;369(8):711-21.

10. Feagan BG, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. The New England journal of medicine. 2013;369(8):699-710.

11. Schreiber S, Khaliq-Kareemi M, Lawrance IC, Thomsen OO, Hanauer SB, McColm J, et al. Maintenance therapy with certolizumab pegol for Crohn's disease. The New England journal of medicine. 2007;357(3):239-50.

12. Sandborn WJ, Hanauer SB, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh DG, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. Gut. 2007;56(9):1232-9.

13. Sandborn WJ, Feagan BG, Stoinov S, Honiball PJ, Rutgeerts P, Mason D, et al. Certolizumab pegol for the treatment of Crohn's disease. The New England journal of medicine. 2007;357(3):228-38.

14. Colombel JF, Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Panaccione R, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Gastroenterology. 2007;132(1):52-65.

15. Brandse JF, Mathot RA, van der Kleij D, Rispens T, Ashruf Y, Jansen JM, et al. Pharmacokinetic Features and Presence of Antidrug Antibodies Associate With Response to Infliximab Induction Therapy in Patients With Moderate to Severe Ulcerative Colitis. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2016;14(2):251-8.e1-2.

16. 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;146(3):681-8.e1.

17. Vermeire S, Noman M, Van Assche G, Baert F, D'Haens G, Rutgeerts P. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. Gut. 2007;56(9):1226-31.

18. van Schaik T, Maljaars JP, Roopram RK, Verwey MH, Ipenburg N, Hardwick JC, et al. Influence of Combination Therapy with Immune Modulators on Anti-TNF Trough Levels and Antibodies in Patients with IBD. Inflammatory bowel diseases. 2014.

19. Chalhoub JM, Rimmani HH, Gumaste VV, Sharara AI. Systematic Review and Meta-analysis: Adalimumab Monotherapy Versus Combination Therapy with Immunomodulators for Induction and Maintenance of Remission and Response in Patients with Crohn's Disease. Inflammatory bowel diseases. 2017;23(8):1316-27.

20. Matsumoto T, Motoya S, Watanabe K, Hisamatsu T, Nakase H, Yoshimura N, et al. Adalimumab Monotherapy and a Combination with Azathioprine for Crohn's Disease: A Prospective, Randomized Trial. Journal of Crohn's & colitis. 2016;10(11):1259-66.

21. Ben-Horin S, Waterman M, Kopylov U, Yavzori M, Picard O, Fudim E, et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2013;11(4):444-7.

22. Strik AS, van den Brink GR, Ponsioen C, Mathot R, Lowenberg M, D'Haens GR. Suppression of anti-drug antibodies to infliximab or adalimumab with the addition of an immunomodulator in patients with inflammatory bowel disease. Alimentary pharmacology & therapeutics. 2017;45(8):1128-34.

23. Bloemendaal FM, Levin AD, Wildenberg ME, Koelink PJ, McRae BL, Salfeld J, et al. Anti-Tumor Necrosis Factor with a Glyco-engineered Fc Region Has Increased Efficacy in Mice with Colitis. Gastroenterology. 2017.

24. Vos AC, Wildenberg ME, Arijs I, Duijvestein M, Verhaar AP, de Hertogh G, et al. Regulatory macrophages induced by infliximab are involved in healing in vivo and in vitro. Inflammatory bowel diseases. 2012;18(3):401-8.

25. Vos AC, Wildenberg ME, Duijvestein M, Verhaar AP, van den Brink GR, Hommes DW. Anti-tumor necrosis factor-alpha antibodies induce regulatory macrophages in an Fc region-dependent manner. Gastroenterology. 2011;140(1):221-30.

26. Wildenberg ME, Koelink PJ, Diederen K, Te Velde AA, Wolfkamp SC, Nuij VJ, et al. The ATG16L1 risk allele associated with Crohn's disease results in a Rac1-dependent defect in dendritic cell migration that is corrected by thiopurines. Mucosal immunology. 2017;10(2):352-60.

27. Christophorou D, Funakoshi N, Duny Y, Valats JC, Bismuth M, Pineton De Chambrun G, et al. Systematic review with meta-analysis: infliximab and immunosuppressant therapy vs. infliximab alone for active ulcerative colitis. Alimentary pharmacology & therapeutics. 2015;41(7):603-12.

28. Reinisch W, Sandborn WJ, Hommes DW, D'Haens G, Hanauer S, Schreiber S, et al. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. Gut. 2011;60(6):780-7.

29. Kariyawasam VC, Ward MG, Blaker PA, Patel KV, Goel R, Sanderson JD, et al. Thiopurines Dosed to a Therapeutic 6-Thioguanine Level in Combination with Adalimumab Are More Effective Than

Subtherapeutic Thiopurine-based Combination Therapy or Adalimumab Monotherapy During Induction and Maintenance in Patients with Long-standing Crohn's Disease. Inflammatory bowel diseases. 2017.

30. Reenaers C, Louis E, Belaiche J, Seidel L, Keshav S, Travis S. Does co-treatment with immunosuppressors improve outcome in patients with Crohn's disease treated with adalimumab? Alimentary pharmacology & therapeutics. 2012;36(11-12):1040-8.

31. Cosnes J, Sokol H, Bourrier A, Nion-Larmurier I, Wisniewski A, Landman C, et al. Adalimumab or infliximab as monotherapy, or in combination with an immunomodulator, in the treatment of Crohn's disease. Alimentary pharmacology & therapeutics. 2016;44(10):1102-13.

32. Eriksson C, Marsal J, Bergemalm D, Vigren L, Bjork J, Eberhardson M, et al. Long-term effectiveness of vedolizumab in inflammatory bowel disease: a national study based on the Swedish National Quality Registry for Inflammatory Bowel Disease (SWIBREG). Scandinavian journal of gastroenterology. 2017;52(6-7):722-9.

33. Dulai PS, Singh S, Jiang X, Peerani F, Narula N, Chaudrey K, et al. The Real-World Effectiveness and Safety of Vedolizumab for Moderate-Severe Crohn's Disease: Results From the US VICTORY Consortium. The American journal of gastroenterology. 2016;111(8):1147-55.

34. Stallmach A, Langbein C, Atreya R, Bruns T, Dignass A, Ende K, et al. Vedolizumab provides clinical benefit over 1 year in patients with active inflammatory bowel disease - a prospective multicenter observational study. Alimentary pharmacology & therapeutics. 2016;44(11-12):1199-212.

35. Allegretti JR, Barnes EL, Stevens B, Storm M, Ananthakrishnan A, Yajnik V, et al. Predictors of Clinical Response and Remission at 1 Year Among a Multicenter Cohort of Patients with Inflammatory Bowel Disease Treated with Vedolizumab. Digestive diseases and sciences. 2017;62(6):1590-6.

36. Battat R, Kopylov U, Bessissow T, Bitton A, Cohen A, Jain A, et al. Association Among Ustekinumab Trough Concentrations and Clinical, Biomarker, and Endoscopic Outcomes in Patients With Crohn's Disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2017.

37. van der Valk ME, Mangen MJ, Leenders M, Dijkstra G, van Bodegraven AA, Fidder HH, et al. Healthcare costs of inflammatory bowel disease have shifted from hospitalisation and surgery towards anti-TNFalpha therapy: results from the COIN study. Gut. 2014;63(1):72-9.

38. Louis E, Mary JY, Vernier-Massouille G, Grimaud JC, Bouhnik Y, Laharie D, et al. Maintenance of remission among patients with Crohn's disease on antimetabolite therapy after infliximab therapy is stopped. Gastroenterology. 2012;142(1):63-70.e5; quiz e31.

39. Van Assche G, Magdelaine-Beuzelin C, D'Haens G, Baert F, Noman M, Vermeire S, et al. Withdrawal of immunosuppression in Crohn's disease treated with scheduled infliximab maintenance: a randomized trial. Gastroenterology. 2008;134(7):1861-8.

40. Roblin X, Boschetti G, Williet N, Nancey S, Marotte H, Berger A, et al. Azathioprine dose reduction in inflammatory bowel disease patients on combination therapy: an open-label, prospective and randomised clinical trial. Alimentary pharmacology & therapeutics. 2017;46(2):142-9.

41. Hanauer SB, Wagner CL, Bala M, Mayer L, Travers S, Diamond RH, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2004;2(7):542-53.

42. Sands BE, Blank MA, Patel K, van Deventer SJ. Long-term treatment of rectovaginal fistulas in Crohn's disease: response to infliximab in the ACCENT II Study. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2004;2(10):912-20.

43. Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Colombel JF, Panaccione R, et al. Adalimumab induction therapy for Crohn disease previously treated with infliximab: a randomized trial. Annals of internal medicine. 2007;146(12):829-38.

44. Hanauer SB, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh D, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology. 2006;130(2):323-33; quiz 591.

45. Sandborn WJ, van Assche G, Reinisch W, Colombel JF, D'Haens G, Wolf DC, et al. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2012;142(2):257-65.e1-3.

46. Pecoraro V, De Santis E, Melegari A, Trenti T. The impact of immunogenicity of TNFalpha inhibitors in autoimmune inflammatory disease. A systematic review and meta-analysis. Autoimmunity reviews. 2017;16(6):564-75.

47. Adedokun OJ, Xu Z, Marano CW, Strauss R, Zhang H, Johanns J, et al. Pharmacokinetics and Exposure-response Relationship of Golimumab in Patients with Moderately-to-Severely Active Ulcerative Colitis: Results from Phase 2/3 PURSUIT Induction and Maintenance Studies. Journal of Crohn's & colitis. 2017;11(1):35-46.

48. Lichtenstein GR, Rutgeerts P, Sandborn WJ, Sands BE, Diamond RH, Blank M, et al. A pooled analysis of infections, malignancy, and mortality in infliximab- and immunomodulator-treated adult patients with inflammatory bowel disease. The American journal of gastroenterology. 2012;107(7):1051-63.

49. Lichtenstein GR, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Langholff W, et al. Drug therapies and the risk of malignancy in Crohn's disease: results from the TREAT Registry. The American journal of gastroenterology. 2014;109(2):212-23.

50. D'Haens G, Reinisch W, Colombel JF, Panes J, Ghosh S, Prantera C, et al. Five-year Safety Data From ENCORE, a European Observational Safety Registry for Adults With Crohn's Disease Treated With Infliximab [Remicade(R)] or Conventional Therapy. Journal of Crohn's & colitis. 2017;11(6):680-9.

51. Osterman MT, Sandborn WJ, Colombel JF, Robinson AM, Lau W, Huang B, et al. Increased risk of malignancy with adalimumab combination therapy, compared with monotherapy, for Crohn's disease. Gastroenterology. 2014;146(4):941-9.

52. D'Haens GRR, W.; Satsangi, J; Loftus, E.V.; Panaccione, R.; Alperovich, G.; Kalabic, J.; Bereswill, M.; Arikan, D.; Petersson, J.H.; Robinson, A.M. Long-Term Safety of Adalimumab in Patients with Crohn's Disease: Final Data from Pyramid Registry. Gastroenterology. 2017;152(5, Supplement 1):S137.

53. Colombel JF, Sands BE, Rutgeerts P, Sandborn W, Danese S, D'Haens G, et al. The safety of vedolizumab for ulcerative colitis and Crohn's disease. Gut. 2017;66(5):839-51.

54. Lee SW, D.; Feagan, B.; Lichtenstein, G.; Andrews, E.; Stidham, R.; Aberra, F.; Aguilar, H.; Kayhan, C.; Kosutic, G.; Sen, D.; Golembesky, A.; Hasan, I.; Spearman, M.; Loftus, E. P-104 SECURE: An Observational Study of Certolizumab Pegol in Crohn's Disease-Events of Interest Analysis with 5869 Patient-Years at Risk. Inflammatory bowel diseases. 2016.

55. Shelton E, Laharie D, Scott FI, Mamtani R, Lewis JD, Colombel JF, et al. Cancer Recurrence Following Immune-Suppressive Therapies in Patients With Immune-Mediated Diseases: A Systematic Review and Meta-analysis. Gastroenterology. 2016;151(1):97-109 e4.

56. 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. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2015;13(5):847-58.e4; quiz e48-50.

57. Toruner M, Loftus EV, Jr., Harmsen WS, Zinsmeister AR, Orenstein R, Sandborn WJ, et al. Risk factors for opportunistic infections in patients with inflammatory bowel disease. Gastroenterology. 2008;134(4):929-36.

58. Beaugerie L, Brousse N, Bouvier AM, Colombel JF, Lemann M, Cosnes J, et al. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. Lancet. 2009;374(9701):1617-25.

59. Peyrin-Biroulet L, Khosrotehrani K, Carrat F, Bouvier AM, Chevaux JB, Simon T, et al. Increased risk for nonmelanoma skin cancers in patients who receive thiopurines for inflammatory bowel disease. Gastroenterology. 2011;141(5):1621-28 e1-5.

60. Pasternak B, Svanstrom H, Schmiegelow K, Jess T, Hviid A. Use of azathioprine and the risk of cancer in inflammatory bowel disease. Am J Epidemiol. 2013;177(11):1296-305.

61. Nyboe Andersen N, Pasternak B, Basit S, Andersson M, Svanstrom H, Caspersen S, et al. Association between tumor necrosis factor-alpha antagonists and risk of cancer in patients with inflammatory bowel disease. JAMA. 2014;311(23):2406-13.

62. Annese V, Beaugerie L, Egan L, Biancone L, Bolling C, Brandts C, et al. European Evidence-based Consensus: Inflammatory Bowel Disease and Malignancies. Journal of Crohn's & colitis. 2015;9(11):945-65.

# Chapter 8

## Anti-Drug Antibody Formation Against Biologic Agents in Inflammatory Bowel Disease: A Systematic Review and Metaanalysis

S. Bots, C. Parker, J. Brandse, M. Löwenberg, B. Feagan, W. Sandborn, V. Jairath, G. D'Haens, N. Vande Casteele

*BioDrugs 2021;35(6):715-733*

## Abstract

### Background and aims

Immunogenicity with formation of anti-drug antibodies (ADA) to biologics is an important reason for treatment failure in inflammatory bowel disease (IBD). Our aim was to assess the rate of ADA, the effect of combination therapy with immunomodulators on ADA and the influence of ADA on efficacy and safety of biologics for IBD treatment.

### Methods

MEDLINE, Embase and Cochrane Central Register of Controlled Trials (CENTRAL) were searched from inception to April 2020 for trials of biologics that assessed immunogenicity. The overall certainty of evidence was evaluated using Grading of Recommendations, Assessment, Development and Evaluations (GRADE). The primary outcome was rate of ADA. Secondary outcomes included efficacy and safety outcomes among patients with detectable versus undetectable ADA. For dichotomous outcomes, pooled risk ratios (RR) and 95% confidence intervals (CI) were calculated.

### Results

Data from 68 studies were analysed and 33 studies (5850 patients) were included in the meta-analysis. Pooled ADA rates for biologic monotherapy were 28.0% for infliximab, 7.5% for adalimumab, 3.8% for golimumab, 10.9% for certolizumab, 6.2% for ustekinumab and 16.0% for natalizumab. Pooled ADA rates were 8.4% for vedolizumab and 5.0% for etrolizumab for combo- and monotherapy combined. In all biologics, ADA rates were underestimated by use of drug sensitive ADA assays and higher dose and/or frequency. ADA rate was significantly reduced in patients treated with combination therapy for infliximab (RR 0.52; 95% CI 0.44, 0.62), adalimumab (RR: 0.31; 95% CI 0.14, 0.69), golimumab (RR: 0.29; 95% CI 0.10, 0.83), certolizumab pegol (RR: 0.30; 95% CI 0.14, 0.67) and natalizumab (RR: 0.20; 95% CI 0.11, 0. 39). ADA to infliximab were associated with lower clinical response rates (RR: 0.75; 95% CI 0.61, 0.91) and higher rates of infusion reactions (RR: 2.36; 95% CI 1.85, 3.01).

### Conclusions

Differences in analytical methods to detect ADA hamper comparison of true ADA rates across biologics in IBD. Use of combination therapy with immunomodulators appeared to reduce ADA positivity for most biologics. For infliximab, ADA were associated with reduced drug efficacy and increased adverse events.

## Introduction

Although biologics were first introduced for the treatment of inflammatory bowel disease (IBD) in the late nineties guidelines regarding their optimal use are still evolving. Over the last several decades IBD-related healthcare costs have increased significantly due to increased utilization of tumor necrosis factor-alpha (TNF-$\alpha$) antagonists and other biologics.[1, 2] Accordingly, optimal use of biologics is necessary to enhance efficacy, reduce adverse effects and manage costs.

The TNF-$\alpha$ antagonists infliximab, adalimumab, golimumab and certolizumab pegol act by targeting and inhibiting TNF-$\alpha$, a pro-inflammatory cytokine that has a central role in mucosal inflammation in IBD.[3, 4] Ustekinumab binds to the common p40 subunit of the pro-inflammatory cytokines interleukin (IL)-12 and IL-23 which are also known to play a role in the pathophysiology of IBD.[5] Natalizumab binds to the $\alpha_4$ subunit of the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrin, thereby blocking the binding to respectively vascular cell adhesion molecule 1 (VCAM-1) and mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) and inhibiting the migration of mononuclear leukocytes to different tissues, predominantly the gut and central nervous system.[6] Vedolizumab recognizes a conformational epitope of the heterodimer $\alpha_4\beta_7$ which blocks binding to MAdCAM-1, thereby inhibiting the migration of gut-selective leukocytes.[7] Etrolizumab tartgets the $\beta_7$ unit of the heterodimeric integrins $\alpha_4\beta_7$ and $\alpha_E\beta_7$, thereby inhibiting migration of gut-selective leukocytes.[8]

A major concern when treating patients with biologics is the development of anti-drug antibodies (ADA), since ADA are associated with lower serum drug concentrations, loss of response, and adverse effects such as infusion and injection site reactions.[9-11] Several strategies for prevention of ADAs formation have been investigated. Combination therapy comprised of a biologic with an immunomodulator prevents ADA formation.[12, 13] Higher anti-TNF dosing is associated with less ADA detection.[14, 15] Pre-treatment with hydrocortisone has also been shown to prevent ADA formation and infusion reactions; however, data supporting this strategy are limited.[16] There is also some evidence that a decline in ADA may be achieved by adding or switching immunomodulators.[17, 18]

The incidence of immunogenicity varies considerably across studies and biologic agents. A critical factor related to this variability may be the sensitivity of the assay used to detect ADA.[19, 20] Qualitative terms are used to distinguish between drug 'tolerant' assays that are able to measure ADA in the presence of detectable drug concentrations, and drug 'sensitive' assays which are not. Drug tolerant assays are preferred for detecting ADA to assess the true ADA rate.

Strategies for optimal management of ADA formation are still evolving, and the exact influence of concomitant immunomodulator therapy on immunogenicity remains unclear. Therefore, the objectives of this systematic review were to determine the rate of ADA

formation in patients on monotherapy or combination therapy, and the impact of ADA formation on the pharmacokinetics, efficacy and safety of biologics in patients with IBD.

## Methods

This systematic review and meta-analysis was conducted using the methods described in the Cochrane Handbook of Systematic Reviews,[21] and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[22] The review protocol was registered in the Cochrane Database of Systematic Reviews.[23]

*Eligibility criteria*

Any trial of biologics that enrolled adult Crohn's disease (CD) or ulcerative colitis (UC) patients (16 years or older) and assessed immunogenicity was eligible for inclusion. Interventions of interest included adalimumab, certolizumab pegol, golimumab, infliximab, natalizumab, vedolizumab, ustekinumab and etrolizumab administered alone or in combination with another agent (i.e. corticosteroids [including pre-medication], thiopurines, or methotrexate).

*Comparisons and outcomes*

The primary outcomes of interest were ADA formation and ADA concentration among patients treated with monotherapy compared to combination therapy. Secondary outcomes included drug serum concentration and rates of response (clinical, endoscopic or biochemical), remission (clinical, endoscopic or biochemical) and adverse events (including acute, delayed or injection site reactions) in patients with detectable versus undetectable ADA. Response and remission rates were pooled for analysis irrespective of the definition employed in the original study.

*Search strategy*

MEDLINE, Embase, and the Cochrane Central Register of Controlled Trials (CENTRAL) were searched from inception to April 21, 2020 (Supplementary Appendix 1). No language or date restrictions were applied. The bibliographies of relevant articles and conference proceedings from Digestive Disease Week and United European Gastroenterology Week (2013 to 2020) were hand searched to identify additional studies.

*Screening and data extraction*

Two authors (SB and NVC) independently screened search results and extracted information on study design, participants, intervention, comparison, outcomes, and risk of bias using a standardized data collection form. Secondary outcome data were only collected if immunogenicity data were reported. Disagreements were resolved through discussion with a third author (JFB or CEP). For unclear or missing data, an attempt was made to contact the original study authors for clarification.

*Risk of bias assessment*

The Cochrane risk of bias tool was used to assess the methodological quality of randomized controlled trials (RCTs) [24]. The Newcastle-Ottawa Quality Assessment Scale (NOS) was used for quality assessment of the observational studies.[25]

*Heterogeneity*

Heterogeneity was assessed using the $Chi^2$ test ($p \leq 0.10$ was considered statistically significant) and the $I^2$ statistic.[26] $I^2$ values of 0%, <50%, ≥50% and ≥75% were interpreted as indicating no, low, moderate and high heterogeneity, respectively[26]

*Data synthesis and analysis*

Data from individual trials were pooled for meta-analysis if the intervention(s), population, and outcomes were sufficiently similar (determined by consensus). Data were not pooled for analysis if there was a high degree of heterogeneity ($I^2$ >75%).

For dichotomous outcomes, the risk ratio (RR) and corresponding 95% confidence interval (CI) were calculated. If outcome data were reported at multiple timepoints, the primary timepoint defined by the original study authors was used. A fixed-effect model was used to pool data, however, we planned to use a random-effects model in the case of significant, unexplained heterogeneity. Review Manager (RevMan 5.4; The Nordic Cochrane Centre for The Cochrane Collaboration, Copenhagen, Denmark) was used to perform data analysis according to the intention-to-treat principle.

*Sensitivity and subgroup analyses*

Sensitivity analyses were conducted to investigate potential sources of heterogeneity and assess the impact of removing low quality studies from the pooled analyses. Where possible, subgroup analyses were performed to assess the influence of the following factors on the overall RR estimate: study design (randomized versus observational; induction versus maintenance), patient population (CD versus UC versus IBD), combination therapy regimen (combination therapy versus monotherapy) and ADA assay type (drug sensitive versus drug tolerant).

*Quality of the evidence*

The Grading of Recommendations, Assessment, Development and Evaluations (GRADE) approach was used to assess the overall certainty of the evidence. Results from RCTs were initially considered high-quality, but potentially downgraded due to risk of bias; indirectness of evidence; unexplained heterogeneity; publication bias or sparse data.[27] Observational data were initially considered low quality. Outcomes with less than 35 events were reduced by two GRADE levels; outcomes with less than 300 events were reduced by one GRADE level.

## Results

*Search results*

The search identified 11881 records, from which 3368 duplicates were removed. Of the remaining 8513 records, 7994 were deemed ineligible based on title and abstract. Full-text review was required for 519 records, and 398 records were excluded. Most excluded studies did not report on ADA formation or had no control group. A total of 68 studies met the inclusion criteria (Figure 1). The included studies are described in Supplementary Table 1. ADA formation rates in each study are shown in Supplementary Table 2. Table 1 summarizes the main results and provides an overall assessment of the certainty of the evidence.

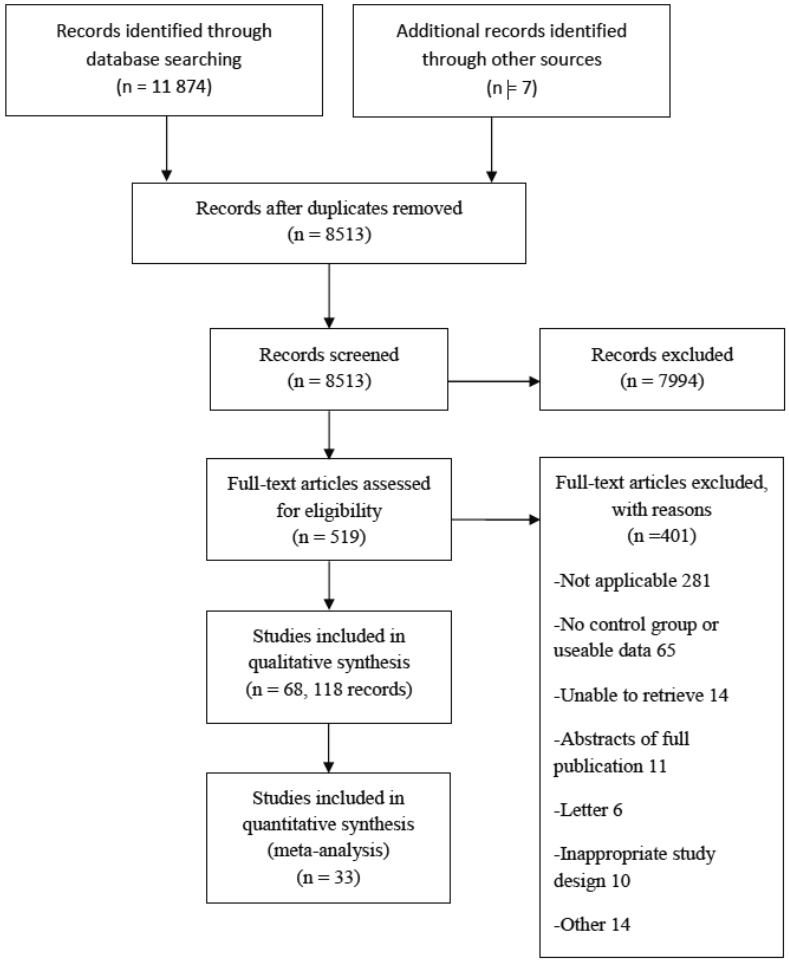



**Figure 1.** *Prisma flow diagram*

**Table 1.** *Summary of findings.*

| Outcome | Studies (N) | Participants (N) | Risk of Bias | Inconsistency | Indirectness | Imprecision | Reporting bias | Pooled RR (95% CI, p) | Quality |
|---|---|---|---|---|---|---|---|---|---|
| Comparison: Infliximab combination therapy vs. infliximab monotherapy | | | | | | | | | |
| ADA formation (all studies) | 13 | 1825 | High | Low | No indirectness | 405 events | None | 0.52 (0.44, 0.62; p<0.001) | ⊕⊕⊕⊕ High |
| ADA formation (randomized to monotherapy or combinationtherapy) | 3 | 413 | Low | No | No indirectness | 40 events | None | 0.13 (0.05, 0.33; p<0.001) | ⊕⊕⊕⊖ Moderate[1] |
| ADA formation (observational data) | 10 | 1412 | High | No | No indirectness | 365 events | None | 0.59 (0.49, 0.71; p<0.001) | ⊕⊕⊕⊕ High |
| ADA formation (induction treatment) | 3 | 352 | High | No | No indirectness | 197 events | None | 0.57 (0.47, 0.70; p<0.001) | ⊕⊕⊕⊖ Moderate[1] |
| ADA formation (maintenance treatment) | 10 | 1473 | High | Moderate | No indirectness | 208 events | None | 0.47 (0.35, 0.63; p<0.001) | ⊕⊕⊕⊖ Moderate[1] |
| ADA formation (CD patients) | 10 | 1471 | High | Low | No indirectness | 378 events | None | 0.52 (0.44, 0.62; p<0.001) | ⊕⊕⊕⊕ High |

**Table 1.** *(Continued).*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ADA formation (UC patients) | 1 | 68 | Low | Not applicable | Not applicable | 8 events | None | 0.12 (0.02, 0.92; p=0.04) | ⊕⊕⊖⊖ Low[2] |
| ADA formation (IBD patients) | 2 | 286 | High | No | No indirectness | 19 events | None | 0.83 (0.34, 2.03; p=0.68) | ⊕⊕⊖⊖ Low[2] |
| ADA formation (Thiopurines only) | 4 | 491 | High | Moderate | No indirectness | 108 events | None | 0.50 (0.37, 0.67; p<0.001) | ⊕⊕⊕⊖ Moderate[1] |
| ADA formation (MTX only) | 2 | 235 | High | Moderate | No indirectness | 81 events | None | 0.51 (0.36. 0.72; p<0.001) | ⊕⊕⊕⊖ Moderate[1] |
| ADA formation (Corticosteroids only) | 2 | 477 | Low | Low | No indirectness | 89 events | None | 0.80 (0.53, 1.22; p=0.30) | ⊕⊕⊕⊖ Moderate[1] |
| ADA formation (Drug sensitive assays) | 11 | 1561 | High | Low | No indirectness | 342 events | None | 0.49 (0.41, 0.60; p<0.001) | ⊕⊕⊕⊕ High |
| ADA formation (Drug tolerant assays) | 2 | 264 | High | High | No indirectness | 63 events | None | 0.67 (0.44, 1.04; p=0.07) | ⊕⊕⊖⊖ Low[3] |

**Table 1.** *(Continued).*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Comparison: Infliximab ADA positive vs ADA undetectable | | | | | | | | | |
| Clinical response | 7 | 1127 | High | No | No indirectness | 573 events | None | 0.75 (0.61, 0.91; p=0.004) | ⊕⊕⊕⊕ High |
| Infusion reactions | 5 | 1242 | Low | Moderate | No indirectness | 230 events | None | 2.36 (1.85, 3.01; p<0.001) | ⊕⊕⊕⊖ Moderate[1] |
| Comparison: Adalimumab combination therapy vs. adalimumab monotherapy | | | | | | | | | |
| ADA formation (all data) | 5 | 698 | Low | No | No indirectness | 38 events | None | 0.34 (0.16, 0.75; p=0.007) | ⊕⊕⊕⊖ Moderate[1] |
| ADA formation (maintenance treatment) | 4 | 539 | Low | No | No indirectness | 38 events | None | 0.31 (0.14, 0.69; p=0.004) | ⊕⊕⊕⊖ Moderate[1] |
| Comparison: Golimumab combination therapy vs. golimumab monotherapy | | | | | | | | | |
| ADA formation | 1 | 1103 | Low | Not applicable | Not applicable | 32 events | None | 0.29 (0.10, 0.83; p=0.02) | ⊕⊕⊕⊖ Moderate |

**Table 1.** *(Continued).*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Comparison: Golimumab ADA positive vs ADA undetectable | | | | | | | | | |
| Clinical response (week 6) | 1 | 720 | Low | Not applicable | Not applicable | 374 events | None | 1.26 (0.91, 1.75; p=0.17) | ⊕⊕⊕⊕ High |
| Clinical response (week 54) | 1 | 263 | Low | Not applicable | Not applicable | 146 events | None | 0.51 (0.16, 1.65; p=0.26) | ⊕⊕⊕⊖ Moderate[1] |
| Clinical remission (week 6) | 1 | 720 | Low | Not applicable | Not applicable | 135 events | None | 1.35 (0.62, 2.92; p=0.45) | ⊕⊕⊕⊖ Moderate[1] |
| Clinical remission (week 54) | 1 | 263 | Low | Not applicable | Not applicable | 77 events | None | 0.48 (0.08, 2.98; p=0.43) | ⊕⊕⊕⊖ Moderate[1] |
| Mucosal healing (week 6) | 1 | 720 | Low | Not applicable | Not applicable | 316 events | None | 1.26 (0.84, 1.89; p=0.26) | ⊕⊕⊕⊕ High |
| Mucosal healing (week 54) | 1 | 263 | Low | Not applicable | Not applicable | 127 events | None | 0.59 (0.18, 1.90; p=0.37) | ⊕⊕⊕⊖ Moderate[1] |

**Table 1.** *(Continued).*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Comparison: Certolizumab pegol combination therapy vs. certolizumab pegol monotherapy | | | | | | | | | |
| ADA formation (all data) | 2 | 1139 | Low | Low | No indirectness | 43 events | None | 0.30 (0.14, 0.67; p=0.003) | ⊕⊕⊕⊖ Moderate[1] |
| Comparison: Ustekinumab combination therapy vs. ustekinumab monotherapy | | | | | | | | | |
| ADA formation (all data) | 2 | 917 | Low | Low | No indirectness | 50 events | None | 0.59 (0.30, 1.18; p<0.13) | ⊕⊕⊕⊖ Moderate[1] |
| Comparison: Natalizumab combination therapy vs. natalizumab monotherapy | | | | | | | | | |
| ADA formation (all data) | 3 | 763 | Low | No | No indirectness | 70 events | None | 0.17 (0.08, 0.38; p<0.001) | ⊕⊕⊕⊖ Moderate[1] |

[1] *Downgraded 1 level due to sparse data.* [2] *Downgraded 2 levels due to sparse data.* [3] *Downgraded 2 levels due to sparse data & high heterogeneity*

*ADA: anti-drug antibodies; IBD: inflammatory bowel disease; CD: Crohn's disease; UC: ulcerative colitis; MTX: methotrexate.*

*Risk of bias assessment*

The risk of bias assessments are reported in Supplementary Tables 3 and 4. The RCTs scored low or unclear risk of bias for most domains. One study was rated as high risk of bias with respect to attrition bias, and seven studies were rated as high risk of bias with respect to blinding. For the observational studies, one study was deemed to be at high risk of bias (NOS *score 6), and 13 studies were deemed to be at low risk of bias (*range 7-9).

## Infliximab

The search identified 26 eligible studies for infliximab, 5 of which were RCTs and 21 were observational. Thirteen studies allowed us to evaluate combination therapy to monotherapy. Eleven studies allowed us to evaluate ADA-positive to ADA-negative or ADA-undetectable patients. Seventeen studies included patients with CD, six studies included patients with UC, and two studies included IBD patients (Supplementary Table 1).

*ADA formation*

Thirteen studies allowed us to evaluate ADA formation rates among patients treated with infliximab monotherapy versus combination therapy with an immunomodulator (supplementary table 1). Follow-up ranged between 12 weeks and 36 months (median). ADA development occurred in 15.8% (136/863) of patients on combination therapy compared to 28.0% (269/962) of patients on monotherapy. The pooled RR was 0.52 (95% CI 0.44, 0.62, 13 studies, p<0.001; GRADE high; Figure 2.1.1) with low heterogeneity ($I^2$= 40%, p=0.07).

The pooled RR for RCT studies was 0.13 (95% CI 0.05, 0.33, 3 studies, p<0.001, GRADE moderate; Figure 2.1.2) with no heterogeneity detected ($I^2$= 0%, p=0.49). The pooled RR for observational studies was 0.59 (95% CI 0.49, 0.71, 10 studies, p<0.001, GRADE high; Figure 2.1.3). The RR estimates continued to demonstrate a statistically significant effect in favour of combination therapy when data were pooled based on study design (induction versus maintenance [Figures 2.1.4 and 2.1.5] and CD versus UC [Figures 2.1.6 and 2.1.7], with between-study heterogeneity remaining low. The RR estimate did not show a statistically significant effect in the mixed population (Figure 2.1.8).

ADA formation resulting from infliximab combination therapy with azathioprine was evaluated in four studies. The pooled RR was 0.50 (95% CI 0.37, 0.67, p<0.001, GRADE moderate; Figure 2.1.9). The between study heterogeneity was moderate ($I^2$=74%). When removing the study by Vermeire et al. (a study on episodic infliximab treatment) from the analysis, the pooled RR was 0.15 (95% CI 0.05, 0.41; p<0.001, GRADE moderate; Supplementary Figure 1).

Two studies compared the presence of ADA formation among patients treated with infliximab monotherapy relative to infliximab in combination with methotrexate (Figure 2.1.10) with a

pooled RR of 0.51 (95% CI 0.36, 0.72, p<0.001; GRADE moderate). The between-study heterogeneity was moderate ($I^2$=61%).

Two studies explored the effect of combined infliximab and corticosteroid therapy on ADA formation, without the use of concomitant immunomodulators (Figure 2.1.11). Sixteen percent (32/200) of patients on combination therapy developed ADAs, compared to 21% (57/277) of patients on monotherapy. This effect was not statistically significant (RR 0.80, 95% CI 0.53, 1.22, p=0.30; GRADE moderate), and the between-study heterogeneity was low ($I^2$ = 33%).

The pooled RR for studies that used a drug sensitive assay was 0.49 (95% CI 0.41, 0.60, 11 studies, p<0.001; GRADE high), and the between study heterogeneity was low ($I^2$ = 31%) (Figure 2.1.12). For two studies that used a drug tolerant assay, ADA development occurred in 19% (24/128) of patients receiving combination therapy versus 29% (39/136) of patients receiving monotherapy. The pooled RR was 0.51 (95% CI 0.13-2.01; p=0.34 GRADE low) and the between-study heterogeneity was high ($I^2$ = 78%) (Figure 2.2). When removing Oh et al. (a prospective observational study) from the analysis, the RR was 0.23 (95% CI 0.07, 0.77; p=0.02, GRADE low; Supplementary Figure 2).

*ADA concentration*

Four infliximab studies reported ADA concentrations. We did not combine quantitative data for analysis due to the heterogeneous units of measurement used to define ADA concentrations and heterogeneity in statistical reporting.

For the Baert et al. study, the median ADA concentration was higher in patients receiving monotherapy relative to combination therapy.[9] The median concentration of ADAs to infliximab was 13.8 μg/ml (95% CI 7.9-16.2) among patients with luminal disease receiving monotherapy therapy, compared to 1.3 μg/ml (95% CI 0.6-3.2) among patients with luminal disease receiving combination therapy. In patients with fistulizing disease receiving monotherapy, the median concentration of ADAs to IFX was 21.4 μg/ml (95% CI 13.2-24.5) compared to 1.5 μg/ml (95% CI 0.4-8.8) among patients with fistulizing disease receiving combination therapy.

In a study by Farrell et al., ADA concentrations were lower in patients pretreated with hydrocortisone compared to placebo (median 2.9 versus 11.1 μg/ml at week 8 and 1.6 versus 3.4 μg/ml at week 16).[16]

Among 96 patients who developed ADA to infliximab, Vermeire et al. reported 27 (28%), 30 (31%) and 39 (41%) patients with ADA concentrations of below 8, above 8 or above 20 μg/ml respectively.[28]

Using a drug tolerant assay, Oh et al. showed a lower median ADA concentration of 8.064 AU/mL (IQR 6.929-9.908) in patients in remission compared to 11.209 AU/mL (IQR 8.008-118.835) in patients with active disease.[29]

*Serum drug concentrations*

Five infliximab studies compared serum drug concentrations among ADA-positive and ADA-negative patients. We did not combine data for analysis due the heterogeneous cut-off values used to define ADA-positive and ADA-negative patients. A post-hoc analysis of the ACT-1 and ACT-2 trials showed that patients with ADA formation had a higher likelihood of low drug serum concentrations ($p<0.001$).[30, 31] Seow et al. showed that of 66/108 patients with undetectable drum serum concentrations, 44 (66.7%) were ADA-positive and 22 (33.3%) were ADA negative.[32] Vermeire et al. showed lower median concentrations of 7.55 μg/mL (IQR2.65-13.73) after 1 infusion of infliximab in patients who later became ADA-positive, compared with median drug serum concentrations of 11.15 μg/mL [IQR 5.98-18.98] in patients who remained ADA-negative.[28] Oh et al. reported median trough levels of 0.141 μg/mL (IQR 0.002-0.869) in ADA-positive patients versus median trough levels of 1.415 μg/mL (IQR 0.570-2.495) in ADA-negative patients, using a drug tolerant assay.[29] Van Stappen et al. reported an inverse correlation between ADA concentration, as measured with a drug 'tolerant' assay, and infliximab trough concentrations in a posthoc analysis of the TAXIT trial.[33] Patients in the highest quadrant of ADA concentrations had lower trough concentrations when compared to the two lowest quadrants and ADA-negative patients ($p<0.001$). Median trough concentrations were lower in ADA-positive patients when using a drug tolerant and a drug sensitive assay (0.0, IQR 0.0-0.0 μg/ml) compared with ADA-negative patients (1.8, IQR 1.4-2.4 μg/ml) ($p<0.001$) and patients who were ADA-positive with a drug tolerant assay only (1.7, IQR 0.7-2.3, $p<0.001$). There was no difference in trough concentration between ADA-negative patients and patients who were ADA-positive with a drug tolerant assay only ($p=1.0$). Indicating that low concentration ADA that are undetectable at trough using a drug 'sensitive' assay may be pharmacologically less relevant.

*Clinical outcomes*

A lower overall clinical response to infliximab rate was observed in ADA-positive compared with ADA-undetectable patients (RR 0.75, 95% CI 0.61, 0.91, $p=0.004$, 7 studies, GRADE high; Figure 3). The heterogeneity was low for this comparison ($I^2= 0\%$, $p=0.51$).

*Endoscopic outcomes*

Two infliximab studies reported on endoscopic outcomes in relation to ADA status. Data were not pooled due to high heterogeneity. Seow et al. showed no difference in endoscopic improvement between ADA-positive and ADA-undetectable patients (25% vs 35%; $p=0.61$). However, detectable infliximab serum drug concentrations were associated with endoscopic improvement (76% vs 28%; $P<0.001$).[32] Reguiero et al. observed a higher endoscopic recurrence rate after ileocecal resection in ADA positive (64.7%, 11/17) vs ADA negative (46.7%, 7/15) or ADA inconclusive (30.1%, 22/73) patients.[34]

*Biochemical outcomes*

No infliximab studies reported on the relationship between biochemical disease activity and ADA formation.

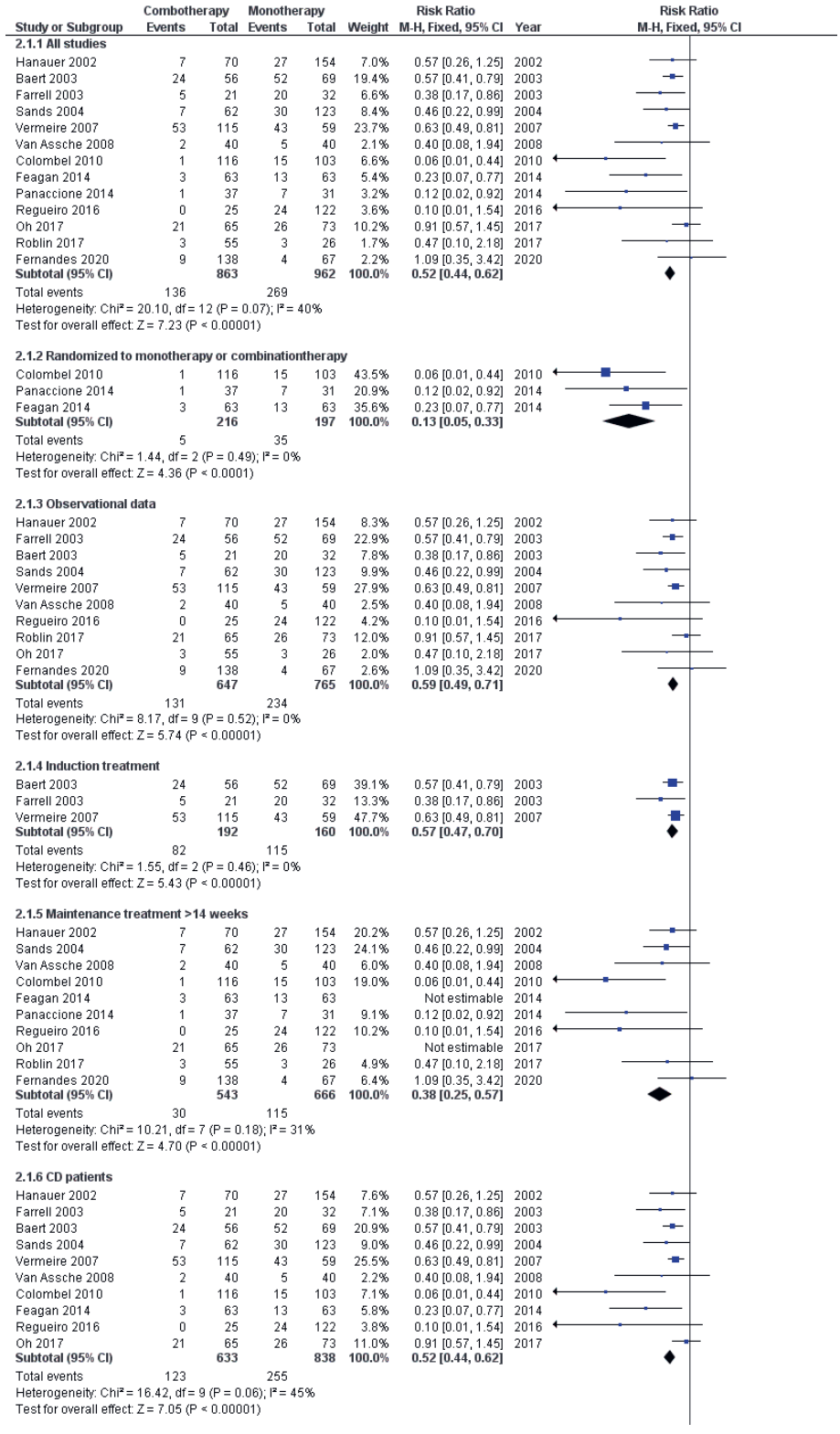
*Adverse events*

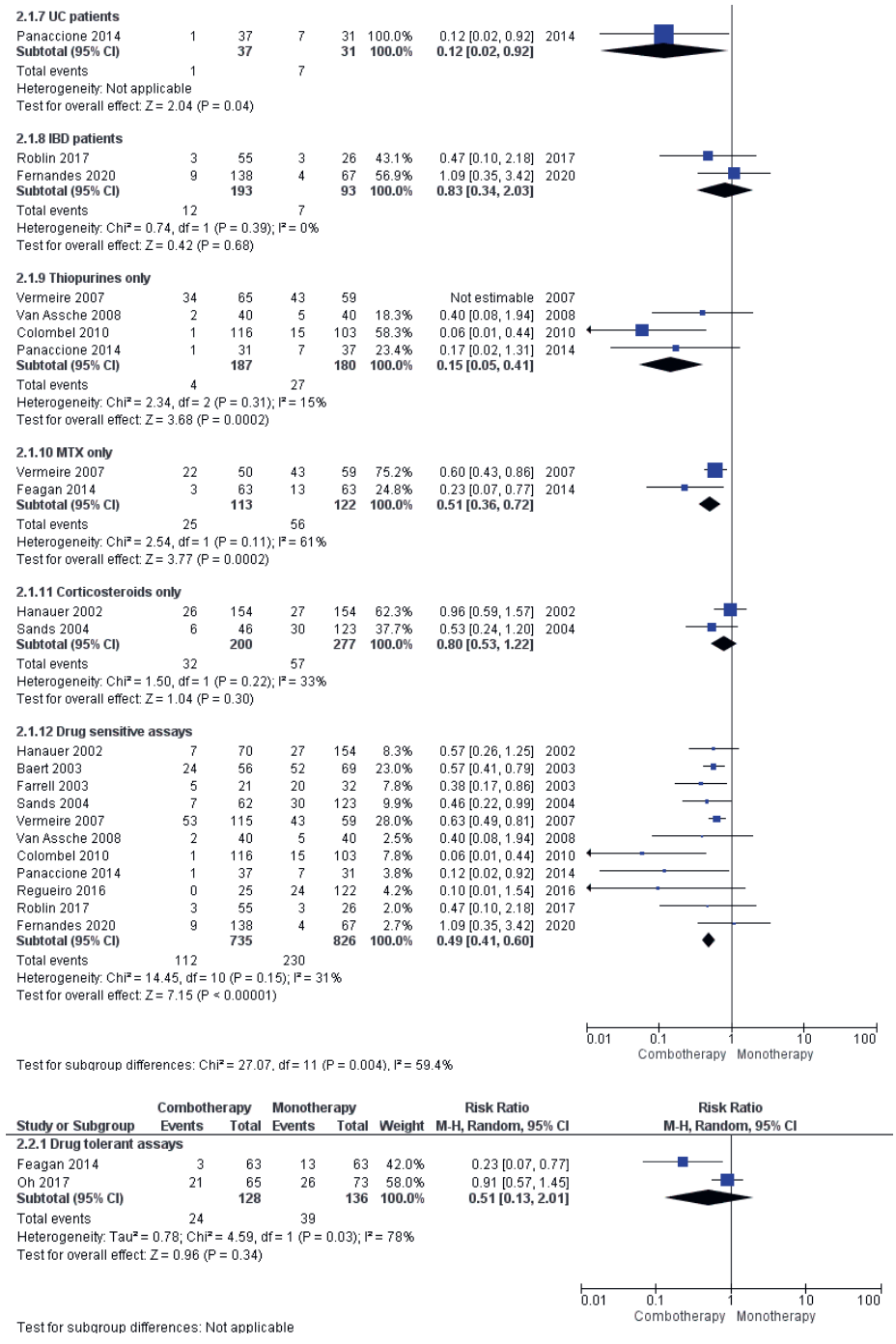
Infliximab infusion reactions occurred in 65/175 (37.1%) of ADA-positive patients versus 165/1067 (15.5%) ADA-undetectable patients (RR 2.36, 95% CI 1.85, 3.01, p<0.001, 5 studies, GRADE moderate; Figure 4). Moderate heterogeneity was detected ($I^2$= 59%, p=0.04).

One study reported significantly higher median ADA titers in patients with infusion reactions compared to those without (20.1 vs 3.2 μg/ml) [9]. Concentrations of 8 μg/ml or higher predicted a higher risk of infusion reactions (RR 2.40; 95% CI 1.65, 3.66; P<0.001). A single study by Ye et al. reported that 2/2 patients that had an infusion reaction at week 30 were ADA positive. In total, 19/220 infusion reactions were reported but ADA formation was not reported in these cases.[35]

*Antibody assays*

In four infliximab studies, a drug-tolerant assay was used for the assessment of ADA formation. In these studies, the reported ADA formation rates were 47/138 (34.1%), 16/126 (12.7%), 21/122 (17.2%) and 13/42 (31%), respectively.


Combotherapy
Monotherapy
Risk Ratio
Risk Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI Year
M-H, Fixed, 95% CI
2.1.1 All studies
Hanauer 2002 7 70 27 154 7.0% 0.57 [0.26, 1.25] 2002
Baert 2003 24 56 52 69 19.4% 0.57 [0.41, 0.79] 2003
Farrell 2003 5 21 20 32 6.6% 0.38 [0.17, 0.86] 2003
Sands 2004 7 62 30 123 8.4% 0.46 [0.22, 0.99] 2004
Vermeire 2007 53 115 43 59 23.7% 0.63 [0.49, 0.81] 2007
Van Assche 2008 2 40 5 40 2.1% 0.40 [0.08, 1.94] 2008
Colombel 2010 1 116 15 103 6.6% 0.06 [0.01, 0.44] 2010
Feagan 2014 3 63 13 63 5.4% 0.23 [0.07, 0.77] 2014
Panaccione 2014 1 37 7 31 3.2% 0.12 [0.02, 0.92] 2014
Regueiro 2016 0 25 24 122 3.6% 0.10 [0.01, 1.54] 2016
Oh 2017 21 65 26 73 10.2% 0.91 [0.57, 1.45] 2017
Roblin 2017 3 55 3 26 1.7% 0.47 [0.10, 2.18] 2017
Fernandes 2020 9 138 4 67 2.2% 1.09 [0.35, 3.42] 2020
Subtotal (95% CI) 863 962 100.0% 0.52 [0.44, 0.62]
Total events 136 269
Heterogeneity: Chi² = 20.10, df = 12 (P = 0.07); I² = 40%
Test for overall effect: Z = 7.23 (P < 0.00001)
2.1.2 Randomized to monotherapy or combinationtherapy
Colombel 2010 1 116 15 103 43.5% 0.06 [0.01, 0.44] 2010
Panaccione 2014 1 37 7 31 20.9% 0.12 [0.02, 0.92] 2014
Feagan 2014 3 63 13 63 35.6% 0.23 [0.07, 0.77] 2014
Subtotal (95% CI) 216 197 100.0% 0.13 [0.05, 0.33]
Total events 5 35
Heterogeneity: Chi² = 1.44, df = 2 (P = 0.49); I² = 0%
Test for overall effect: Z = 4.36 (P < 0.0001)
2.1.3 Observational data
Hanauer 2002 7 70 27 154 8.3% 0.57 [0.26, 1.25] 2002
Farrell 2003 24 56 52 69 22.9% 0.57 [0.41, 0.79] 2003
Baert 2003 5 21 20 32 7.8% 0.38 [0.17, 0.86] 2003
Sands 2004 7 62 30 123 9.9% 0.46 [0.22, 0.99] 2004
Vermeire 2007 53 115 43 59 27.9% 0.63 [0.49, 0.81] 2007
Van Assche 2008 2 40 5 40 2.5% 0.40 [0.08, 1.94] 2008
Regueiro 2016 0 25 24 122 4.2% 0.10 [0.01, 1.54] 2016
Roblin 2017 21 65 26 73 12.0% 0.91 [0.57, 1.45] 2017
Oh 2017 3 55 3 26 2.0% 0.47 [0.10, 2.18] 2017
Fernandes 2020 9 138 4 67 2.6% 1.09 [0.35, 3.42] 2020
Subtotal (95% CI) 647 765 100.0% 0.59 [0.49, 0.71]
Total events 131 234
Heterogeneity: Chi² = 8.17, df = 9 (P = 0.52); I² = 0%
Test for overall effect: Z = 5.74 (P < 0.00001)
2.1.4 Induction treatment
Baert 2003 24 56 52 69 39.1% 0.57 [0.41, 0.79] 2003
Farrell 2003 5 21 20 32 13.3% 0.38 [0.17, 0.86] 2003
Vermeire 2007 53 115 43 59 47.7% 0.63 [0.49, 0.81] 2007
Subtotal (95% CI) 192 160 100.0% 0.57 [0.47, 0.70]
Total events 82 115
Heterogeneity: Chi² = 1.55, df = 2 (P = 0.46); I² = 0%
Test for overall effect: Z = 5.43 (P < 0.00001)
2.1.5 Maintenance treatment >14 weeks
Hanauer 2002 7 70 27 154 20.2% 0.57 [0.26, 1.25] 2002
Sands 2004 7 62 30 123 24.1% 0.46 [0.22, 0.99] 2004
Van Assche 2008 2 40 5 40 6.0% 0.40 [0.08, 1.94] 2008
Colombel 2010 1 116 15 103 19.0% 0.06 [0.01, 0.44] 2010
Feagan 2014 3 63 13 63 Not estimable 2014
Panaccione 2014 1 37 7 31 9.1% 0.12 [0.02, 0.92] 2014
Regueiro 2016 0 25 24 122 10.2% 0.10 [0.01, 1.54] 2016
Oh 2017 21 65 26 73 Not estimable 2017
Roblin 2017 3 55 3 26 4.9% 0.47 [0.10, 2.18] 2017
Fernandes 2020 9 138 4 67 6.4% 1.09 [0.35, 3.42] 2020
Subtotal (95% CI) 543 666 100.0% 0.38 [0.25, 0.57]
Total events 30 115
Heterogeneity: Chi² = 10.21, df = 7 (P = 0.18); I² = 31%
Test for overall effect: Z = 4.70 (P < 0.00001)
2.1.6 CD patients
Hanauer 2002 7 70 27 154 7.6% 0.57 [0.26, 1.25] 2002
Farrell 2003 5 21 20 32 7.1% 0.38 [0.17, 0.86] 2003
Baert 2003 24 56 52 69 20.9% 0.57 [0.41, 0.79] 2003
Sands 2004 7 62 30 123 9.0% 0.46 [0.22, 0.99] 2004
Vermeire 2007 53 115 43 59 25.5% 0.63 [0.49, 0.81] 2007
Van Assche 2008 2 40 5 40 2.2% 0.40 [0.08, 1.94] 2008
Colombel 2010 1 116 15 103 7.1% 0.06 [0.01, 0.44] 2010
Feagan 2014 3 63 13 63 5.8% 0.23 [0.07, 0.77] 2014
Regueiro 2016 0 25 24 122 3.8% 0.10 [0.01, 1.54] 2016
Oh 2017 21 65 26 73 11.0% 0.91 [0.57, 1.45] 2017
Subtotal (95% CI) 633 838 100.0% 0.52 [0.44, 0.62]
Total events 123 255
Heterogeneity: Chi² = 16.42, df = 9 (P = 0.06); I² = 45%
Test for overall effect: Z = 7.05 (P < 0.00001)




*IBD: inflammatory bowel disease; CD: Crohn's disease; UC: ulcerative colitis; MTX: methotrexate.*

**Figure 2.** *ADA formation in infliximab combo- versus monotherapy.*

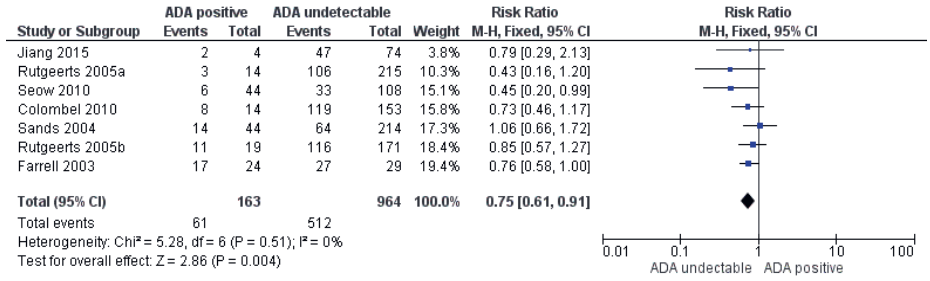



**Figure 3.** *Clinical response to infliximab in patients with positive versus undetectable ADA.*

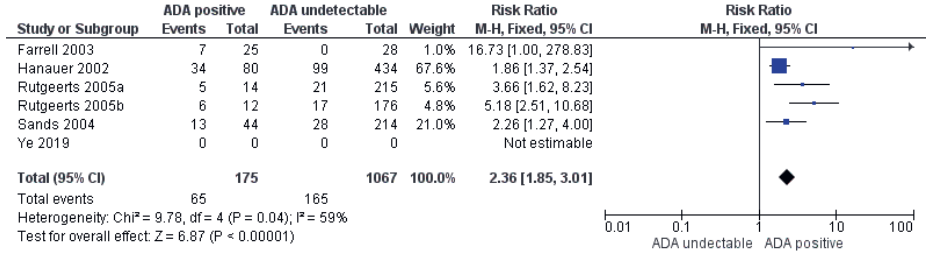



**Figure 4.** *Infusions reactions in infliximab treated patients with positive versus undetectable ADA.*

## Adalimumab

The search identified 10 RCTs of adalimumab treatment that met the eligibility criteria. Seven studies reported data on combination therapy. One study randomized patients to receive adalimumab in combination with immunomodulators or as monotherapy. No studies compared ADA-positive to ADA-negative patients. The majority of studies included patients with CD and two studies included patients with UC (Supplementary Table 1).

*ADA formation*

ADA formation occurred in 2.2% (6/273) of patients receiving adalimumab combination therapy compared to 7.5% (32/425) of patients treated with monotherapy. The pooled RR was 0.31 (95% CI 0.14, 0.69, 5 studies, p=0.004, GRADE moderate; Figure 5.1.1). No heterogeneity was detected ($I^2$ = 0%, P=0.82). The RR estimate continued to demonstrate a statistically significant protective effect in favour of combination therapy when data were pooled based on study design (maintenance treatment; Figure 5.1.2). Data on the influence of methotrexate, thiopurines or corticosteroids were unavailable.

*Other outcomes*

Data on the impact of ADA formation on clinical, biochemical and endoscopic outcomes or serum drug concentrations and adverse events were not available.

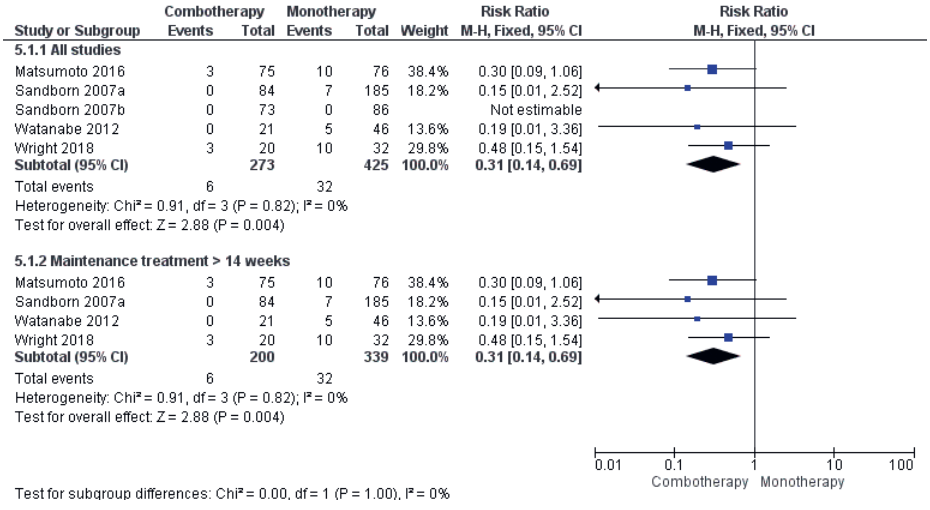



**Figure 5.** *ADA formation in adalimumab combo- versus monotherapy.*

## Infliximab and adalimumab

One study by Roblin et al. showed increased incidence of ADA development and undetectable drug concentrations in the monotherapy group after switching TNF antagonist therapy (i.e. from infliximab to adalimumab or from adalimumab to infliximab) because of clinical failure and immunogenicity.[36] Starting the subsequent biologic with combination therapy significantly reduced ADA development and the incidence of undetectable drug concentrations (adalimumab and azathioprine: Hazard ratio [HR] 0.12; 95% CI 0.03, 0.40; p<0.001; infliximab and azathioprine: HR 0.16 95%; CI 0.06, 0.37; p<0.001). This effect was consistent only for infliximab and not for adalimumab when ADA were measured using a drug tolerant assay (adalimumab and azathioprine: HR 0.61; 95% CI 0.30, 1.24; p=0.17; and infliximab and azathioprine: HR 0.18; 95% CI 0.08, 0.41).

## Golimumab

The search yielded three RCTs of golimumab treatment that met the eligibility criteria. None of the studies randomized patients to monotherapy or combination therapy. No studies compared ADA-positive to ADA-negative patients. All three studies analyzed UC patients (Supplementary Table 1).

*ADA formation*

Sandborn et al. reported data on the protective effect of combination therapy on ADA formation.[37, 38] ADA formation occurred in 1.1% (4/362) of patients on combination therapy compared to 3.8% (28/741) of patients on monotherapy. The calculated RR was 0.29 (95% CI 0.10, 0.83; p=0.02; GRADE moderate) (Supplementary Figure 3). A post-hoc analysis of PURSUIT-SC, PURSUIT-M and PURSUIT-IV showed much higher ADA detection when using a drug tolerant assay (21.8% versus 2.8%). ADA rates were higher in patients in the PURSUIT-M study who were randomized to placebo after induction treatment (31.6% versus 20.2%). ADA rates were lower in patients receiving immunomodulators (11.8% versus 26.9%; p<0.001) and ADA rates were lower in patients receiving golimumab monotherapy doses of 100mg versus 50mg (22.4% versus 37.1%; p-value unavailable). Additionally, ADA-positive patients with a drug-tolerant assay had lower ADA titers as opposed to ADA-positive patients with a drug-sensitive assay. Trough serum golimumab concentrations were lower in ADA-positive patients 0.51 vs 0.85 μg/mL [50 mg q4w]; 0.85 vs 1.60 μg/mL [100 mg q4w]).

*Other outcomes*

A post-hoc analysis of Sandborn et al. reported clinical and endoscopic outcomes in relation to ADA status at weeks 6 and 54 and no significant associations were found.[39] However, in the post-hoc analysis by Adedokun et al., clinical response rates were lower at week 54 in ADA-positive versus ADA-negative patients when using a drug-tolerant assay (38.1% versus 52.8%; p=0.047). ADA-formation did not have an impact on injection site reactions. Other data on the effect of ADA formation on biochemical outcomes or adverse events were not available.

## Certolizumab pegol

The search yielded six RCTs that evaluated treatment with certolizumab pegol that met the eligibility criteria. None of the studies randomized patients to monotherapy or combination therapy. Two studies compared ADA-positive to ADA-negative patients. All studies analyzed CD patients (Supplementary Table 1).

*ADA formation*

Three certolizumab pegol studies provided data on the effect of combination therapy on ADA formation, of which two could be compared. ADA formation occurred in 3.3% (7/213) of patients on combination therapy compared to 10.9% (36/331) of patients on monotherapy (RR 0.30, 95% CI 0.14, 0.67; 2 studies; p=0.003, GRADE moderate; Figure 6). Heterogeneity was low ($I^2$ = 0%, p=0.43). In all studies, patients received scheduled maintenance treatment for more than 14 weeks. In the PRECiSE 3 study, an extension of PRECiSE 1 and PRECiSE 2, ADA formation occurred in 14.6% (31/213) of patients on combination therapy compared to 27% (103/382) of patients who received monotherapy (RR 0.54; 95% CI 0.37, 0.78; p<0.001). Data on the effect of methotrexate, thiopurines or corticosteroids on ADA formation were unavailable.

In the PRECiSE 2 trial it was shown that immunogenicity rates were higher in patients receiving induction treatment who were then assigned to placebo when compared to patients receiving maintenance treatment (18% versus 8%). This was mainly reflected by patients receiving monotherapy (24% ADA positive).

*Serum drug concentrations*

An integrated analysis of 5-year follow-up of the PRECiSE trial showed a lower range of mean drug serum concentrations in ADA-positive versus ADA-negative patients (0.88-15.25 vs 8.33-29.89 μg/ml) and no influence of ADA-formation on adverse events.[40]

*Clinical outcomes*

Sandborn et al. reported that of the 17 patients with positive tests for ADA against certolizumab pegol, 12 (71%) had a response through week 26, compared with 62% (121/196) of patients with negative antibody tests.[41] In a 7-year analysis of the PRECiSE 3 trial, Sandborn et al. found no difference in clinical disease activity between persistently ADA-positive and ADA-undetectable patients.[42]

*Biochemical outcomes*

Sandborn et al. demonstrated that median CRP and fecal calprotectin concentrations were higher ($p<0.05$ at some visits) and plasma CZP concentrations were significantly lower ($p<0.0001$ at all visits) in patients with persistent ADAs when compared to the ADA negative group.[42]

*Other outcomes*

Data on the influence of ADA formation on endoscopic outcomes and antibody assays were not available.

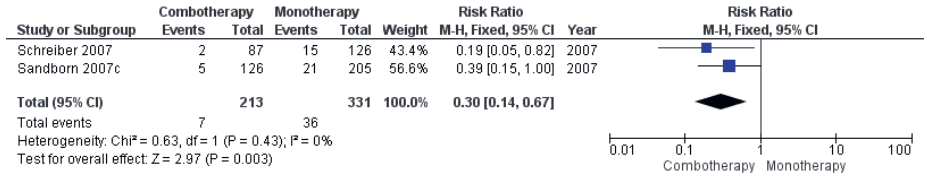



**Figure 6.** *ADA formation in certolizumab pegol combo- versus monotherapy.*

## Vedolizumab

The search yielded nine RCTs that met eligibility criteria. No eligible observational studies were identified. None of the studies randomized patients to vedolizumab with or without an immunomodulator. None of the studies compared ADA-positive to ADA-negative patients. Four studies analyzed CD patients and five studies analyzed UC patients (Supplementary Table 1).

*ADA formation*

ADA formation occurred in 8.4% of patients treated with vedolizumab. Numerical data on ADA formation in patients treated with monotherapy or concomitant immunomodulator therapy were unavailable. Feagan et al. reported that concomitant immunomodulator therapy was associated with decreased immunogenicity.[43] No other data were available regarding the influence of combination therapy on antibody formation to vedolizumab.

*Infusion reactions*

Three studies reported on infusion reactions in patients treated with vedolizumab. The number of infusion reactions was very low, however most patients with an infusion reaction were ADA-positive. Wyant et al. reported no relationship between immunogenicity and safety in an long-term safety analysis of the GEMINI studies[44]

*Other outcomes*

Feagan et al. studied ADA formation against MLN02 (a predecessor compound of vedolizumab which had a modified amino acid sequence and was expressed in a different system) at dosages of 0.5 mg/kg and 2.0 mg/kg, and showed that ADAs were more frequently detected at lower dosages.[45] Additionally, it was reported that patients with clinically significant ADA titers had lower remission rates. Sandborn et al. showed that ADA to subcutaneous or intravenous vedolizumab resulted in lower drug exposure and reduced efficacy, although the number of ADA-positive patients was very low.[46] It was also observed that patients receiving vedolizumab induction treatment who were then randomized to placebo had higher ADA rates (30%). Other relevant data on the influence of ADA formation

on clinical, biochemical and endoscopic outcomes or serum drug concentrations and adverse events were not available .

## Ustekinumab

The search yielded four RCTs that met the eligibility criteria. No eligible observational studies were identified. None of the studies randomized patients to ustekinumab in combination with immunomodulators or ustekinumab monotherapy. One study compared ADA-positive to ADA-negative patients. All studies analyzed CD patients (Supplementary Table 1).

*ADA formation*

ADA development occurred in 3.7% (10/273) of patients on combination therapy compared to 6.2% (40/644) of patients treated with monotherapy (RR 0.59; 95% CI 0.30, 1.12; 2 studies; p=0.13; GRADE moderate; Supplementary Figure ). Low heterogeneity was detected ($I^2$= 30%, p=0.23).

*Other outcomes*

In a post-hoc analysis of UNIFI, Adedokun et al. reported lower serum ustekinumab concentrations in ADA-positive versus ADA-negative patients at week 24 (0.31 μg/ml; IQR 0.11-2.14, vs 2.76 μg/ml; IQR 1.87-4.18). They also showed that there was no relationship between ADA status and clinical efficacy. Furthermore they reported no difference in endoscopic response between ADA positive and negative patients and no relationship between ADA positivity and injection site reactions or anaphylactic reactions.[47, 48] Other data on the influence of antibody formation on clinical, biochemical and endoscopic outcomes or serum drug concentrations and adverse events were not available.

## Natalizumab

The search yielded six RCTs and one prospective study that met the eligibility criteria. None of the studies randomized patients to natalizumab in combination with immunomodulators or natalizumab monotherapy. None of the studies compared ADA positive to ADA negative patients. All studies analyzed CD patients (Supplementary Table 1).

*ADA formation*

ADA development occurred in 2.6% (10/389) of patients treated with combination therapy compared to 16.0% (60/374) of patients assigned to monotherapy (RR 0.20; 95% CI 0.11, 0.39; 2 studies, p<0.001; GRADE moderate; Supplementary Figure 5). No heterogeneity was detected ($I^2$ = 0%, P = 0.48). Only one study assessed maintenance treatment (>14 weeks), thus no subgroup analysis could be performed. Data on the effect of methotrexate, thiopurines or corticosteroids on ADA formation were unavailable.

*Other outcomes*

Data on the influence of ADA formation on clinical, biochemical and endoscopic outcomes or serum drug concentrations and adverse events were not available. None of the studies used drug tolerant assays.

## Etrolizumab

The search yielded two RCTs that met the eligibility criteria. No eligible observational studies were identified. None of the studies randomized patients to etrolizumab in combination with immunomodulators or etrolizumab monotherapy. None of the studies compared ADA-positive to ADA-negative patients. Both studies analyzed UC patients (Supplementary Table 1).

*ADA formation*

ADAs were measured in 119 patients. Reported ADA formation rates were 4.9% (4/81) (Vermeire et al.) and 5.3% (2/38) (Rutgeerts et al.).[8, 49] ADAs were detected with a drug tolerant assay in both studies. None of the studies reported on the effect of combination therapy on antibody formation. Other data on the influence of ADA formation on clinical, biochemical and endoscopic outcomes or serum drug concentrations and adverse events were not available.

## Discussion

The results of this systematic review and meta-analysis showed that ADA formation to biologic agents was reduced in patients treated with combination therapy compared to patients treated with monotherapy. Combination therapy has been the preferred strategy for patients starting infliximab treatment, however our findings are consistent with the notion that starting combination therapy with immunomodulators may also be an effective strategy for reducing ADA formation in patients treated with adalimumab, golimumab, certolizumab pegol, and natalizumab. Although comparative evidence is lacking, it is likely that combination therapy reduces ADA formation for other approved biologics agents (i.e., ustekinumab, etrolizumab and vedolizumab). For instance, Feagan et al. noted lower immunogenicity in patients treated with vedolizumab in combination with an immunomodulator, yet quantitative data are lacking.[43] ADA formation with ustekinumab was lower in patients treated with combination therapy compared to monotherapy (4% vs 6%), however this difference was not statistically significant, which may be a consequence of the low number of patients developing ADA.

ADA formation has a negative impact on clinical, biochemical, and endoscopic outcomes. For example, ADA formation to infliximab has usually been associated with lower clinical response rates and Vande Casteele et al. demonstrated higher concentrations of CRP in ADA positive patients.[50] Nonetheless, Seow et al. found that ADA positivity had no influence on endoscopic outcomes in patients treated with infliximab.[32] Sandborn et al. found higher median CRP and fecal calprotectin concentrations in patients persistently positive for ADA to certolizumab pegol, however this was not reflected in clinical outcomes.[42] Based on the available evidence, no robust conclusions can be drawn regarding the effect of ADA formation on biochemical and endoscopic treatment outcomes. Importantly, none of these studies were powered to assess the influence of ADA formation on these outcomes. Additionally, it is relevant to note that ADA positive patients may have sufficient serum drug concentrations during the majority of the treatment interval, especially for drugs that are more frequently administered (e.g. adalimumab). Lastly, patients who are in remission and develop ADAs are unlikely to immediately lose response due to pharmacodynamic carryover.

Several studies reported on ADA formation and infusion reactions to infliximab or vedolizumab.[9, 16, 31, 45, 51-53] ADA formation was associated with an increased risk for infusion reactions with infliximab (RR 2.36; 95% CI 1.85, 2.81), however the data regarding vedolizumab were insufficient for meta-analysis.

Rates of ADA formation differed substantially amongst biologics, and across different studies of the same agent. The highest reported incidence of ADA formation in patients receiving scheduled infliximab, adalimumab or golimumab monotherapy was 40.7% (44/108), 16.7% (1/6) and 37.1%, respectively. For scheduled certolizumab pegol and vedolizumab treatment, the highest reported incidence of ADA formation was 24.2% (30/124) and 10.8% (4/37),

respectively. Lower rates were reported in patients receiving scheduled natalizumab, ustekinumab and etrolizumab monotherapy (4.1% [5/123], 11.1% [2/18], 4.6% [23/505] and 4.9% [4/81], respectively), despite the use of drug tolerant assays for the newer molecules (ustekinumab and etrolizumab). Higher proportions of ADAs were seen in the older studies where patients were treated episodically, or only received induction treatment with one to three infusions (i.e. phase 2 studies). For instance, the highest reported incidence of ADA formation for episodic infliximab was 60.8% (76/125).[9] Additionally, ADA rates were higher for episodic vedolizumab, certolizumab pegol and golimumab treatment.[14, 45, 54-56] For the other biologics, the impact of episodic treatment is unknown. Another reason for differences in immunogenicity could be differences in structure of the agent (i.e. infliximab is a chimeric monoclonal antibody). Although the described differences in ADA rates should be interpreted cautiously because of differences between assays it is likely that immunogenicity is of less concern with newer biologics especially given that these agents have been assessed, in the most part, using highly sensitive drug-tolerant assays.

There were several aspects that we could not assess in this review. Only a few studies used drug tolerant assays and a meta-analysis of studies comparing different assays could therefore not be made. However, immunogenicity rates for infliximab, vedolizumab and golimumab are higher when measuring with a drug tolerant assay.[14, 33, 55, 57] Furthermore, we were unable to assess the impact of drug dose on immunogenicity due to lack of sufficient data. Thus it is important to note that higher drug concentrations in patients treated with higher doses may have masked the detection of ADA. Despite those limitations, there are data suggesting that higher doses of infliximab and golimumab result in less immunogenicity.[14, 15] It has been shown that ADA formation results in low serum drug concentrations due to accelerated clearance, which is the main reason for treatment failure due to ADA formation, together with drug neutralization.[58, 59] Some of the included studies showed lower serum drug concentrations in ADA-positive patients, but we could not perform a meta-analysis due to scarce data and data heterogeneity. We could also not account for the impact of route of administration, which may also be a factor influencing immunogenicity. For instance, there is evidence that subcutaneous infliximab is less immunogenic than intravenous infliximab.[60] Furthermore, we did not assess the difference between neutralizing versus non-neutralizing and transient vs. sustained antibodies, since most studies did not distinguish between the two. Transient ADAs are probably clinically less relevant and sustained ADAs are more likely to result in treatment failure.[61, 62] Additionally, we did not evaluate the risk of developing ADAs when rechallenging with the same biologic agent or when switching to a second biologic. For instance, it has been shown that the risk of ADA formation is higher when switching to a second TNF antagonist.[63, 64] It has also been shown that re-treatment with the same TNF antagonist after stopping maintenance treatment ('drug holiday') is associated with a higher risk of adverse events such as infusion reactions and ADA development, which is also seen with episodic treatment.[9, 65, 66] We did also not assess risk of immunogenicity when switching from originator to biosimilar biologicals since this was not within the scope of this review.

Nevertheless, is has been shown in previous studies that switching to a biosimilar is safe, effective and not associated with increased immunogenicity.[67, 68] Lastly, there is also some evidence that genetic factors play a role in immunogenicity.[69] Thus, these results should be interpreted with caution and in the context of important aforementioned data gaps. The incidence of ADA for different biologics is likely higher than reported, since most studies used drug sensitive assays. Additionally, there are several important factors impacting ADA formation that could not be properly assessed in this review. However, this does not negate the fact that ADA formation is associated with poorer treatment outcomes and that combination therapy with an immunomodulator results in less immunogenicity, which is mainly of importance for agents with considerable immunogenic potential. Given the fact that immunogenicity is much lower for the newest agents (e.g. anti-integrins and ustekinumab), the benefit of combination therapy for prevention of ADA formation is probably trivial in these agents.

Reduced ADA formation in patients treated with infliximab in combination with an immunomodulator has also been shown in several uncontrolled or retrospective studies.[10, 11, 70, 71] Another systematic review, studying the immunogenicity of TNF antagonists in autoimmune inflammatory diseases found similar results.[72] However, most of the studies in this review were not conducted in patients with IBD. ADA formation was also associated with inferior treatment outcomes. Nevertheless, in other cohort studies it has been shown that immunogenicity increases drug clearance, resulting in lower serum drug concentrations that are, in turn, associated with poorer treatment outcomes for various biologic agents.[10, 11]

Despite the available evidence, several knowledge gaps remain. For instance, not much is known on how ADA titers are associated with serum drug concentrations and what ADA titers result in sub-therapeutic serum drug concentrations (i.e. patients could have sub-therapeutic drug concentrations due to ADA formation which is not detected by drug sensitive assays). To evaluate this question, studies with drug tolerant assays should be conducted. Drug sensitive assays only show ADAs when serum drug concentrations are low or undetectable and thus an inverse correlation between both continuous measures is a self-fulfilling prophecy. Detecting ADAs earlier on, before serum drug concentrations become sub-therapeutic, could be of clinical value and should be evaluated in future studies. In this review, we identified some studies that used a drug tolerant assay. However, the studies did not evaluate this issue. A retrospective study showed that a cut-off in ADA lower than 282 ng/mL was valuable for decision making for interval shortening and dose doubling in infliximab.[73] Controlled prospective studies for different biologics are needed to further assess this phenomenon. These questions also highlight the importance of harmonizing ADA detection methods. Usage of uniform assays and standards would result in more comparable data for meta-analysis and would ultimately lead to improved clinical utility. Additionally, small retrospective cohort studies have suggested that adding an immunomodulator in

monotherapy patients who develop ADAs may reverse ADA formation.[17, 18] However, no prospective controlled studies have been conducted.

With respect to combination therapy, the optimal doses of immunomodulators that suffice for prevention of ADA formation are unknown. To our knowledge only one study has addressed this issue.[74] Roblin et al. showed that lower doses of azathioprine worked equally well for ADA prevention with infliximab. More studies on this topic should be conducted in the future. However, it has been shown that lower doses of methotrexate suffice for prevention of immunogenicity in rheumatic diseases.[75]

This study has several strengths. To our knowledge this is the first comprehensive systematic review and meta-analysis studying immunogenicity and comparing monotherapy with combination therapy in IBD patients. An extensive literature search was conducted and all available biologics for treatment of IBD were evaluated. In doing so, we highlighted several knowledge gaps that need to be addressed in the future. This review also has some limitations. Firstly, the search was conducted in April 2020. New data may have become available in the meantime, especially regarding newer agents. Given the scale of this project it was not possible to finish the manuscript within one year after the search. Therefore, this review should be updated in the future when more data regarding the newer agents has become available. Furthermore, ADA formation was not the primary outcome in any of the studies. As a result, many studies reported on ADA formation in a smaller subset of patients with available blood samples and not in the entire cohort which could potentially lead to over or underestimation of ADA rates. Additionally, factors such as the amount of blood samples taken, the time-points for measuring, assay types, and treatment duration were not always comparable across studies. Moreover, in the older studies with infliximab, patients were treated episodically, resulting in higher immunogenicity rates which is not applicable to the scheduled treatment regimens used today. Such heterogeneity has likely influenced the results of the meta-analysis. Nevertheless, we believe that most evidence was sufficient to draw reliable conclusions regarding ADA formation and combination therapy with immunomodulators.

In conclusion, our analyses showed that combination therapy reduced ADA formation for most biologics for which data were available. The protective effect of combination therapy on ADA formation may be greater for those biologics with higher immunogenic potential and thus the risks associated with combination therapy may not outweigh the potential benefits for newer, less immunogenic agents. Combination therapy for a subsequent biologic agent should be considered when switching from a biologic agent in a sensitized patient because of persistent loss of response and presence of ADA. Future studies should focus on harmonizing ADA assays, determining clinically relevant concentration cut-offs for ADA and optimal dosing of immunomodulators to prevent ADA formation.

## References


1. van der Valk ME, Mangen MJ, Leenders M, Dijkstra G, van Bodegraven AA, Fidder HH, et al. Healthcare costs of inflammatory bowel disease have shifted from hospitalisation and surgery towards anti-TNFalpha therapy: results from the COIN study. Gut. 2014;63(1):72-9.

2. Bots SJA, Hoekman DR, Benninga MA, Ponsioen CY, D'Haens GR, Lowenberg M. Patterns of anti-TNF use and associated treatment outcomes in inflammatory bowel disease patients: results from an analysis of Dutch health insurance claims data. The Netherlands journal of medicine. 2017;75(10):432-42.

3. Plevy SE, Landers CJ, Prehn J, Carramanzana NM, Deem RL, Shealy D, et al. A role for TNF-alpha and mucosal T helper-1 cytokines in the pathogenesis of Crohn's disease. Journal of immunology (Baltimore, Md : 1950). 1997;159(12):6276-82.

4. Masuda H, Iwai S, Tanaka T, Hayakawa S. Expression of IL-8, TNF-alpha and IFN-gamma m-RNA in ulcerative colitis, particularly in patients with inactive phase. Journal of clinical & laboratory immunology. 1995;46(3):111-23.

5. Benson JM, Peritt D, Scallon BJ, Heavner GA, Shealy DJ, Giles-Komar JM, et al. Discovery and mechanism of ustekinumab: a human monoclonal antibody targeting interleukin-12 and interleukin-23 for treatment of immune-mediated disorders. mAbs. 2011;3(6):535-45.

6. Hamann A, Andrew DP, Jablonski-Westrich D, Holzmann B, Butcher EC. Role of alpha 4-integrins in lymphocyte homing to mucosal tissues in vivo. Journal of immunology (Baltimore, Md : 1950). 1994;152(7):3282-93.

7. Soler D, Chapman T, Yang LL, Wyant T, Egan R, Fedyk ER. The binding specificity and selective antagonism of vedolizumab, an anti-alpha4beta7 integrin therapeutic antibody in development for inflammatory bowel diseases. The Journal of pharmacology and experimental therapeutics. 2009;330(3):864-75.

8. Rutgeerts PJ, Fedorak RN, Hommes DW, Sturm A, Baumgart DC, Bressler B, et al. A randomised phase I study of etrolizumab (rhuMAb beta7) in moderate to severe ulcerative colitis. Gut. 2013;62(8):1122-30.

9. Baert F, Noman M, Vermeire S, Van Assche G, G DH, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med. 2003;348(7):601-8.

10. Karmiris K, Paintaud G, Noman M, Magdelaine-Beuzelin C, Ferrante M, Degenne D, et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. Gastroenterology. 2009;137(5):1628-40.

11. O'Meara S, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in patients with inflammatory bowel disease: a systematic review and meta-analysis. Inflammatory bowel diseases. 2014;20(1):1-6.

12. Colombel JF, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. The New England journal of medicine. 2010;362(15):1383-95.

13. 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;146(2):392-400.e3.

14. Adedokun OJ, Gunn GR, Leu JH, Gargano C, Xu Z, Sandborn WJ, et al. Immunogenicity of Golimumab and its Clinical Relevance in Patients With Ulcerative Colitis. Inflammatory bowel diseases. 2019;25(9):1532-40.

15. Hanauer SB, Wagner CL, Bala M, Mayer L, Travers S, Diamond RH, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. Clinical Gastroenterology & Hepatology. 2004;2:542-53.

16. Farrell RJ, Alsahli M, Jeen YT, Falchuk KR, Peppercorn MA, Michetti P. Intravenous hydrocortisone premedication reduces antibodies to infliximab in Crohn's disease: a randomized controlled trial. Gastroenterology. 2003;124(4):917-24.

17. Ben-Horin S, Waterman M, Kopylov U, Yavzori M, Picard O, Fudim E, et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2013;11(4):444-7.

18. Strik AS, van den Brink GR, Ponsioen C, Mathot R, Lowenberg M, D'Haens GR. Suppression of anti-drug antibodies to infliximab or adalimumab with the addition of an immunomodulator in patients with inflammatory bowel disease. Alimentary pharmacology & therapeutics. 2017;45(8):1128-34.

19. Hart MH, de Vrieze H, Wouters D, Wolbink GJ, Killestein J, de Groot ER, et al. Differential effect of drug interference in immunogenicity assays. Journal of immunological methods. 2011;372(1-2):196-203.

20. Steenholdt C, Ainsworth MA, Tovey M, Klausen TW, Thomsen OO, Brynskov J, et al. Comparison of techniques for monitoring infliximab and antibodies against infliximab in Crohn's disease. Therapeutic Drug Monitoring. 2013;35(4):530-8.

21. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020). Cochrane, 2020. Available from www.training.cochrane.org/handbook.

22. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Journal of clinical epidemiology. 2009;62(10):1006-12.

23. Bots S, Vande Casteele N, Brandse JF, Lowenberg M, Feagan BG, Sandborn WJ, et al. Antibody development against biologic agents used for the treatment of inflammatory bowel disease and antibody prevention with immunosuppressives. Cochrane Database of Systematic Reviews. 2016(5).

24. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.

25. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M. The Newcastle-Ottawa Scale (NOS) for assessing the quality of non randomised studies in meta-analyses 2017 [Available from: www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

26. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557-60.

27. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336(7650):924-6.

28. Vermeire S, Noman M, Van Assche G, Baert F, D'Haens G, Rutgeerts P. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. Gut. 2007;56(9):1226-31.

29. Oh EH, Ko DH, Seo H, Chang K, Kim GU, Song EM, et al. Clinical correlations of infliximab trough levels and antibodies to infliximab in South Korean patients with Crohn's disease. World journal of gastroenterology : WJG. 2017;23(8):1489-96.

30. Adedokun OJ, Sandborn WJ, Feagan BG, Rutgeerts P, Xu Z, Marano CW, et al. Association between serum concentration of infliximab and efficacy in adult patients with ulcerative colitis. Gastroenterology. 2014;147(6):1296-307 e5.

31. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. The New England journal of medicine. 2005;353(23):2462-76.

32. Seow CH, Newman A, Irwin SP, Steinhart AH, Silverberg MS, Greenberg GR. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut. 2010;59(1):49-54.

33. Van Stappen T, Vande Casteele N, Van Assche G, Ferrante M, Vermeire S, Gils A. Clinical relevance of detecting anti-infliximab antibodies with a drug-tolerant assay: post hoc analysis of the TAXIT trial. Gut. 2018;67(5):818-26.

34. Regueiro M, Feagan BG, Zou B, Johanns J, Blank MA, Chevrier M, et al. Infliximab Reduces Endoscopic, but Not Clinical, Recurrence of Crohn's Disease After Ileocolonic Resection. Gastroenterology. 2016;150(7):1568-78.

35. Ye BD, Pesegova M, Alexeeva O, Osipenko M, Lahat A, Dorofeyev A, et al. Efficacy and safety of biosimilar CT-P13 compared with originator infliximab in patients with active Crohn's disease: an international, randomised, double-blind, phase 3 non-inferiority study. Lancet. 2019;393(10182):1699-707.

36. Roblin X, Williet N, Boschetti G, Phelip JM, Del Tedesco E, Berger AE, et al. Addition of azathioprine to the switch of anti-TNF in patients with IBD in clinical relapse with undetectable anti-TNF trough levels and antidrug antibodies: a prospective randomised trial. Gut. 2020;69(7):1206-12.

37. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146(1):96-109.e1.

38. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146(1):85-95; quiz e14-5.

39. Adedokun OJ, Xu Z, Marano CW, Strauss R, Zhang H, Johanns J, et al. Pharmacokinetics and Exposure-response Relationship of Golimumab in Patients with Moderately-to-Severely Active Ulcerative Colitis: Results from Phase 2/3 PURSUIT Induction and Maintenance Studies. Journal of Crohn's & colitis. 2017;11(1):35-46.

40. Sandborn W, Dubinsky M, Kosutic G, Parker G, Spearman M, Hasan I, et al. Incidence of anti-drug antibodies in crohn's disease patients during 5 years of certolizumab pegol therapy. Inflammatory bowel diseases. 2016;22:S41.

41. Sandborn WJ, Feagan BG, Stoinov S, Honiball PJ, Rutgeerts P, Mason D, et al. Certolizumab pegol for the treatment of Crohn's disease. The New England journal of medicine. 2007;357(3):228-38.

42. Sandborn WJ, Wolf DC, Kosutic G, Parker G, Schreiber S, Lee SD, et al. Effects of Transient and Persistent Anti-drug Antibodies to Certolizumab Pegol: Longitudinal Data from a 7-Year Study in Crohn's Disease. Inflammatory bowel diseases. 2017;23(7):1047-56.

43. Feagan BG, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. The New England journal of medicine. 2013;369(8):699-710.

44. Wyant T, Yang L, Lirio R, Rosario M. Long-term immunogenicity of vedolizumab in ulcerative colitis and Crohn's disease (GEMINI Programme). Journal of Crohn's and Colitis. 2019;13 (Supplement 1):S331.

45. Feagan BG, Greenberg GR, Wild G, Fedorak RN, Pare P, McDonald JW, et al. Treatment of active Crohn's disease with MLN0002, a humanized antibody to the alpha4beta7 integrin. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2008;6(12):1370-7.

46. Sandborn WJ, Baert F, Danese S, Krznarić Ž, Kobayashi T, Yao X, et al. Efficacy and Safety of Vedolizumab Subcutaneous Formulation in a Randomized Trial of Patients With Ulcerative Colitis. Gastroenterology. 2020;158(3):562-72.e12.

47. Adedokun OJ, Xu Z, Marano C, O'Brien C, Szapary P, Zhang H, et al. Ustekinumab Pharmacokinetics and Exposure Response in a Phase 3 Randomized Trial of Patients With Ulcerative Colitis: Ustekinumab PK and exposure-response in UC. Clinical Gastroenterology & Hepatology. 2019;06:06.

48. Sands BE, Sandborn WJ, Panaccione R, O'Brien CD, Zhang H, Johanns J, et al. Ustekinumab as induction and maintenance therapy for ulcerative colitis. New England Journal of Medicine. 2019;381(13):1201-14.

49. Vermeire S, O'Byrne S, Keir M, Williams M, Lu TT, Mansfield JC, et al. Etrolizumab as induction therapy for ulcerative colitis: a randomised, controlled, phase 2 trial. Lancet. 2014;384(9940):309-18.

50. Vande Casteele N, Ferrante M, Van Assche G, Ballet V, Compernolle G, Van Steen K, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology. 2015;148(7):1320-9 e3.

51. Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet. 2002;359(9317):1541-9.

52. Parikh A, Leach T, Wyant T, Scholz C, Sankoh S, Mould DR, et al. Vedolizumab for the treatment of active ulcerative colitis: a randomized controlled phase 2 dose-ranging study. Inflammatory bowel diseases. 2012;18(8):1470-9.

53. Sands BE, Anderson FH, Bernstein CN, Chey WY, Feagan BG, Fedorak RN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. The New England journal of medicine. 2004;350(9):876-85.

54. Feagan BG, Greenberg GR, Wild G, Fedorak RN, Pare P, McDonald JW, et al. Treatment of ulcerative colitis with a humanized antibody to the alpha4beta7 integrin. The New England journal of medicine. 2005;352(24):2499-507.

55. Sandborn WJ, Baert F, Danese S, Krznaric Z, Kobayashi T, Yao X, et al. Efficacy and Safety of Vedolizumab Subcutaneous Formulation in a Randomized Trial of Patients With Ulcerative Colitis. Gastroenterology. 2020;158(3):562-72.e12.

56. Schreiber S, Khaliq-Kareemi M, Lawrance IC, Thomsen OO, Hanauer SB, McColm J, et al. Maintenance therapy with certolizumab pegol for Crohn's disease. The New England journal of medicine. 2007;357(3):239-50.

57. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OO, Ainsworth MA. Clinical implications of measuring drug and anti-drug antibodies by different assays when optimizing infliximab treatment failure in Crohn's disease: post hoc analysis of a randomized controlled trial. The American journal of gastroenterology. 2014;109(7):1055-64.

58. Brandse JF, Mould D, Smeekes O, Ashruf Y, Kuin S, Strik A, et al. A Real-life Population Pharmacokinetic Study Reveals Factors Associated with Clearance and Immunogenicity of Infliximab in Inflammatory Bowel Disease. Inflammatory bowel diseases. 2017;23(4):650-60.

59. Papamichael K, Chachu KA, Vajravelu RK, Vaughn BP, Ni J, Osterman MT, et al. Improved Long-term Outcomes of Patients With Inflammatory Bowel Disease Receiving Proactive Compared With Reactive

Monitoring of Serum Concentrations of Infliximab. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2017;15(10):1580-8.e3.

60. Schreiber S. LJ, Dudkowiak R., Lahat A. , Gawdis-, Wojnarska B. PA, Horynski M. , Farkas K. , Kierkus J. , Kowalski M. ,, Ben-Horin S. YBD, Lee S.J. , Kim S.H. , Kim M.R. , Kim H.N. ,, W. R. Noninferiority of novel subcutaneous infliximab (ct-p13) to intravenous infliximab (ct-p13) in patients with active crohn's disease and ulcerative colitis: week 30 results from a multicentre, randomised controlled pivotal trial. Unit Eur Gastroenterol J.7(10):1412.

61. Ungar B, Chowers Y, Yavzori M, Picard O, Fudim E, Har-Noy O, et al. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. Gut. 2014;63(8):1258-64.

62. Roblin X, Marotte H, Leclerc M, Del Tedesco E, Phelip JM, Peyrin-Biroulet L, et al. Combination of C-reactive protein, infliximab trough levels, and stable but not transient antibodies to infliximab are associated with loss of response to infliximab in inflammatory bowel disease. Journal of Crohn's & colitis. 2015;9(7):525-31.

63. Bartelds GM, Wijbrandts CA, Nurmohamed MT, Stapel S, Lems WF, Aarden L, et al. Anti-infliximab and anti-adalimumab antibodies in relation to response to adalimumab in infliximab switchers and anti-tumour necrosis factor naive patients: a cohort study. Annals of the rheumatic diseases. 2010;69(5):817-21.

64. Roblin X, Vérot C, Paul S, Duru G, Williet N, Boschetti G, et al. Is the Pharmacokinetic Profile of a First Anti-TNF Predictive of the Clinical Outcome and Pharmacokinetics of a Second Anti-TNF? Inflammatory bowel diseases. 2018;24(9):2078-85.

65. Casanova MJ, Chaparro M, Garcia-Sanchez V, Nantes O, Leo E, Rojas-Feria M, et al. Evolution After Anti-TNF Discontinuation in Patients With Inflammatory Bowel Disease: A Multicenter Long-Term Follow-Up Study. The American journal of gastroenterology. 2017;112(1):120-31.

66. Torres J, Boyapati RK, Kennedy NA, Louis E, Colombel JF, Satsangi J. Systematic Review of Effects of Withdrawal of Immunomodulators or Biologic Agents From Patients With Inflammatory Bowel Disease. Gastroenterology. 2015;149(7):1716-30.

67. Albshesh A, Ben-Horin S. CT-P13: a review on a biosimilar to infliximab in the treatment of inflammatory bowel disease. Expert opinion on biological therapy. 2019;19(10):971-8.

68. García-Beloso N, Altabás-González I, Samartín-Ucha M, Gayoso-Rey M, De Castro-Parga ML, Salgado-Barreira Á, et al. Switching between reference adalimumab and biosimilars in chronic immune-mediated inflammatory diseases: A systematic literature review. British journal of clinical pharmacology. 2021.

69. Sazonovs A, Kennedy NA, Moutsianas L, Heap GA, Rice DL, Reppell M, et al. HLA-DQA1*05 Carriage Associated With Development of Anti-Drug Antibodies to Infliximab and Adalimumab in Patients With Crohn's Disease. Gastroenterology. 2020;158(1):189-99.

70. Steenholdt C, Svenson M, Bendtzen K, Thomsen OO, Brynskov J, Ainsworth MA. Severe infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with inflammatory bowel disease. Alimentary pharmacology & therapeutics. 2011;34(1):51-8.

71. West R, Woude C, Hansen B, Felt-Bersma R, Tilburg A, Drapers J, et al. Clinical and endosonographic effect of ciprofloxacin on the treatment of perianal fistulae in Crohn's disease with infliximab: a double-blind placebo-controlled study. Alimentary pharmacology & therapeutics [Internet]. 2004; 20(11-12):[1329-36 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/054/CN-00514054/frame.html.

72. Pecoraro V, De Santis E, Melegari A, Trenti T. The impact of immunogenicity of TNFalpha inhibitors in autoimmune inflammatory disease. A systematic review and meta-analysis. Autoimmunity reviews. 2017;16(6):564-75.

73. Dreesen E, Van Stappen T, Ballet V, Peeters M, Compernolle G, Tops S, et al. Anti-infliximab antibody concentrations can guide treatment intensification in patients with Crohn's disease who lose clinical response. Alimentary pharmacology & therapeutics. 2018;47(3):346-55.

74. Roblin X, Boschetti G, Williet N, Nancey S, Marotte H, Berger A, et al. Azathioprine dose reduction in inflammatory bowel disease patients on combination therapy: an open-label, prospective and randomised clinical trial. Alimentary pharmacology & therapeutics. 2017;46(2):142-9.

75. Schaeverbeke T, Truchetet ME, Kostine M, Barnetche T, Bannwarth B, Richez C. Immunogenicity of biologic agents in rheumatoid arthritis patients: lessons for clinical practice. Rheumatology (Oxford, England). 2016;55(2):210-20.

## Supplementary material

**Supplementary Appendix 1.** *Search strategies.*

*MEDLINE (OVID; 1946-present)*

1. random$.tw. 2. factorial$.tw. 3. (crossover$ or cross over$ or cross-over$).tw. 4. placebo$.tw. 5. single blind.mp. 6. double blind.mp. 7. triple blind.mp. 8. (singl$ adj blind$).tw. 9. (double$ adj blind$).tw. 10. (tripl$ adj blind$).tw. 11. assign$.tw. 12. allocat$.tw. 13. crossover procedure/ 14. double blind procedure/ 15. single blind procedure/ 16. triple blind procedure/ 17. randomized controlled trial/ 18. or/1-17 19. (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.) 20. 18 not 19 21. exp Crohn disease/ or crohn*.mp. 22. (colitis and ulcerat*).mp. 23. ulcerative colitis.mp. or exp ulcerative colitis/ 24. inflammatory bowel disease*.mp. 25. IBD.mp. 26. 21 or 22 or 23 or 24 or 25 27. 20 and 26 28. exp monoclonal antibody/ 29. anti-tum*.mp. or exp anti tumor necrosis factor/ 30. exp tumor necrosis factor antibody/ or exp tumor necrosis factor alpha antibody/ or anti-TNF.mp. or anti TNF.mp. 31. anti-alpha.mp. 32. infliximab.mp. or exp infliximab/ or cA2.mp. 33. ustekinumab.mp. or CNTO 1275.mp. or exp ustekinumab/ 34. exp certolizumab pegol/ or certolizumab*.mp. or CDP870.mp. 35. natalizumab.mp. or exp natalizumab/ or alpha-4.mp. or alpha4.mp. 36. vedolizumab.mp. or exp vedolizumab/ or alpha4beta7.mp or alpha-4beta-7.mp or MLN02.mp or MLN-02.mp. 37. adalimumab.mp. or exp adalimumab/ 38. exp golimumab/ or golimumab.mp. or CNTO*148.mp. 39. exp mucosal addressin cell adhesion molecule 1/ or anti-madcam.mp. 40.etrolizumab.mp.or rhuMAb Beta7.mp. or exp etrolizumab/ 41. 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 42. 27 and 41

*EMBASE (Ovid; 1947-present)*

1. random$.tw. 2. factorial$.tw. 3. (crossover$ or cross over$ or cross-over$).tw. 4. placebo$.tw. 5. single blind.mp. 6. double blind.mp. 7. triple blind.mp. 8. (singl$ adj blind$).tw. 9. (double$ adj blind$).tw. 10. (tripl$ adj blind$).tw. 11. assign$.tw. 12. allocat$.tw. 13. crossover procedure/ 14. double blind procedure/ 15. single blind procedure/ 16. triple blind procedure/ 17. randomized controlled trial/ 18. or/1-17 19. (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.) 20. 18 not 19 21. exp Crohn disease/ or crohn*.mp. 22. (colitis and ulcerat*).mp. 23. ulcerative colitis.mp. or exp ulcerative colitis/ 24. inflammatory bowel disease*.mp. 25. IBD.mp. 26. 21 or 22 or 23 or 24 or 25 27. 20 and 26 28. exp monoclonal antibody/ 29. anti-tum*.mp. or exp anti tumor necrosis factor/ 30. exp tumor necrosis factor antibody/ or exp tumor necrosis factor alpha antibody/ or anti-TNF.mp. or anti TNF.mp. 31. anti-alpha.mp. 32. infliximab.mp. or exp infliximab/ or cA2.mp. 33. ustekinumab.mp. or CNTO 1275.mp. or exp ustekinumab/ 34. exp certolizumab pegol/ or certolizumab*.mp. or CDP870.mp. 35. natalizumab.mp. or exp natalizumab/ or alpha-4.mp. or alpha4.mp. 36. vedolizumab.mp. or exp vedolizumab/ or alpha4beta7.mp or alpha-4beta-7.mp or MLN02.mp or MLN-02.mp. 37. adalimumab.mp. or exp adalimumab/ 38. exp golimumab/ or golimumab.mp. or CNTO*148.mp. 39. exp mucosal addressin cell adhesion molecule 1/ or anti-madcam.mp. 40.etrolizumab.mp.or rhuMAb Beta7.mp. or exp etrolizumab/ 41. 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 42. 27 and 41

*COCHRANE LIBRARY*

1. Crohn 2. Colitis 3. Inflammatory bowel disease 4. IBD 5. monoclonal antibody 6. anti-tum* or anti tumor necrosis factor or tumor necrosis factor antibody or tumor necrosis factor alpha antibody or anti-TNF or anti TNF or anti-alpha 7. infliximab or cA2 8. ustekinumab or CNTO 1275 9. certolizumab or CDP870 10. natalizumab or alpha-4or alpha4 11. vedolizumab or alpha4beta7 or alpha-4beta-7 or MLN02 or MLN-02. 12. Adalimumab 13. Golimumab or CNTO148 14. mucosal addressin cell adhesion molecule 1 or anti-madcam 15. Etrolizumab 16. #1 or #2 or #3 or #4 17. #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 18. #16 and #17

*Cochrane IBD/FBD Group Register*

anti-tum* or anti tumor necrosis factor or tumor necrosis factor antibody or tumor necrosis factor alpha antibody or anti-TNF or anti TNF or anti-alpha or infliximab or cA2 or ustekinumab or CNTO 1275 natalizumab or alpha-4or alpha4 or vedolizumab or alpha4beta7 or alpha-4beta-7 or MLN02 or MLN-02 or adalimumab or golimumab or CNTO148 or mucosal addressin cell adhesion molecule 1 or anti-madcam or etrolizumab

**Supplementary Table 1.** *Characteristics of included studies.*

**INFLIXIMAB**

| | Study & records | N | Sample size | Population | Study type | Data type | Intervention/ Population | Control | Outcomes of interest | Assay |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Baert 2003[1] | 125 | 125 | CD | Observational | Observational | Infliximab + IM | Infliximab | -ADA formation<br>-ADA concentration<br>-Adverse events | Drug sensitive |
| 2 | Colombel 2010[2]<br>*(SONIC)*<br>*Records:*<br>*-Colombel 2017*[3] | 508 | 219 | CD | RCT | Randomized | Infliximab + azathioprine | Azathioprine + placebo<br>Infliximab + placebo | -ADA formation<br>-Clinical outcomes | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | | |
| 3 | D'Haens 2018[4] | 122 | 122 | CD | RCT | Observational | Dose intensification algorithm | Dose intensification based on symptoms | -ADA formation | Drug tolerant |
| 4 | Farrell 2003[5] | 53 | 53 | CD | Observational | Observational | Infliximab ADA positive | Infliximab ADA negative | -ADA formation<br>-Clinical outcomes | Drug sensitive |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 | Farrel 2003[5] | 80 | 68 | | RCT | Randomized | Infliximab + hydrocortisone premedication | Infliximab | -ADA formation<br>-ADA concentration<br>-Adverse events | |
| 6 | Feagan 2014[6] | 126 | 126 | CD | RCT | Randomized | Infliximab + methotrexate | Infliximab + placebo | -ADA formation<br>-Clinical outcomes | Drug tolerant |
| 7 | Fernandes 2020[7] | 205 | | | Observational | Observational | Treatment escalation based on TDM | Retrospective cohort without TDM | -ADA formation | Drug sensitive |
| | | | | | | | Infliximab + IM | Infliximab | -ADA formation | |
| 8 | Hanauer 2002[8] *(ACCENT-1‡)*<br>*Records:*<br>*-Rutgeerts 2004[9]*<br>*-Hanauer 2004[10]* | 573 | 442 | CD | RCT | Observational | Infliximab + IM | Infliximab | -ADA formation<br>-Adverse events | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | | |
| 9 | Jiang 2015[11] | 123 | 78 | UC | RCT | Observational | ADA positive | ADA negative or inconclusive | -Clinical outcomes | Drug sensitive |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | Oh 2017[12] | 138 | 138 | CD | Observational | Observational | IFX ADA positive | IFX ADA negative | -Drug concentration | Drug tolerant |
| | | | | | | | IFX + IM | IM | -ADA formation | |
| | | | | | | | Clinical response | No response | -ADA concentration | |
| 11 | Panaccione 2014[13] | 239 | 68 | UC | RCT | Randomized | Infliximab + azathioprine | Infliximab + placebo | -ADA formation | Drug sensitive |
| | | | | | | | | Azathioprine + placebo | | |
| 12 | Present 1999[14] | 94 | 92 | CD | RCT | Observational | Infliximab | placebo | -ADA formation | Drug sensitive |
| 13 | Regueiro 2016[15] | 297 | 147 | CD* | RCT | Observational | Infliximab + IM | Infliximab | -ADA formation | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | -Endoscopic outcomes | |
| 14 | Roblin 2017[16] | 81 | 81 | IBD | Open label trial | Observational | Infliximab + continued azathioprine | Infliximab + placebo | -ADA formation | Drug sensitive |
| | | | | | | | Infliximab + lowered azathioprine | | | |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 15 | Rutgeerts 1999[17] | 73 | 47 | CD | RCT | Observational | Infliximab | Placebo | -ADA formation | Drug sensitive |
| 16.1 | Rutgeerts 2005a[18] *(ACT-1) Records: -Reinisch 2012[19] -Adedokun 2014[20] -Vande Casteele 2019[21]* | 364 | 229 | UC | RCT | Observational | ADA positive | ADA negative or inconclusive | -Clinical outcomes -Drug concentration -Adverse events | Drug sensitive |
| 16.2 | Rutgeerts 2005b[18] *(ACT-2) Records: -Reinisch 2012[19] -Adedokun 2014[20] -Vande Casteele 2019[21]* | 364 | 188 | UC | RCT | Observational | ADA positive | ADA negative or inconclusive | -Clinical outcomes -Drug concentration -Adverse events | Drug sensitive |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 17 | Sands 2004[22] | 306 | 258 | CD | RCT | Observational | Infliximab + IM<br>Infliximab + CS<br>Infliximab + IM + CS | Infliximab | -ADA formation<br>-Adverse events | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | | |
| 18 | Seow 2010[23] | 115 | 108 | UC | Observational | Observational | ADA positive | ADA negative | -Clinical outcomes<br>-Drug concentration | Drug sensitive |
| 19 | Steenholdt 2014[24]<br>*Records:*<br>*-Steenholdt 2015[25]*<br>*-Edlund 2017[26]* | 69 | 69 | CD | RCT | Observational | Infliximab intensification based on ADAs | Infliximab intensification based on algorithm | -ADA formation | Drug tolerant |
| 20 | Targan 1997[27] | 108 | 101 | CD | RCT | Observational | Infliximab | Placebo | -ADA formation | Drug sensitive |
| 21 | Van Assche 2008[28] | 80 | 80 | CD | RCT | Randomized | Infliximab + azathioprine | Infliximab + placebo (withdrawal of azathioprine) | -ADA formation | Drug sensitive |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 22 | Vande Casteele 2015[29]<br>*TAXIT Records:*<br>*-van Stappen 2017*[30]<br>*-van Stappen 2018*[31] | 263 | 275<br><br><br><br>76 | IBD | RCT | Observational | Infliximab intensification based on ADAs | Infliximab intensification based on symptoms | -ADA formation | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | -Drug concentration | |
| | | | | | | | Drug sensitive assay | Drug tolerant assay | -ADA formation<br>-Clinical relevance | Drug sensitive and drug tolerant |
| 23 | Vermeire 2007[32] | 174 | 174 | CD | Observational | Observational | Infliximab + methotrexate<br><br>Infliximab + azathioprine | Infliximab + placebo | -ADA formation<br>-ADA concentration | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | -Drug concentration | |
| 24 | Ye 2019[33] | 220 | | CD | RCT | Observational | Biosimilar IFX | Originator IFX | -ADA formation | Drug sensitive |
| 25 | Yokoyama 2017[34] | 21 | 21 | UC | Observational | Observational | LOR | No LOR | -ADA formation | Unknown |

**Supplementary Table 1.** *(Continued).*

| | ADALIMUMAB | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Study** | **N** | **Sample size** | **Population** | **Study type** | **Data type** | **Intervention/ Population** | **Control** | **Outcomes of interest** | **Assay** |
| 26 | Bodini 2014[35] *Records: -Savarino 2013[36]* | 6 | 6 | CD* | RCT | Observational† | Adalimumab | Azathioprine Mesalamine | -ADA formation | Drug tolerant |
| 27 | Hanauer 2006[37] *(CLASSIC-I)* | 299 | 225 | CD | RCT | Observational | Adalimumab | Placebo | -ADA formation | Drug sensitive |
| 28 | Matusumoto 2016[38] *Records: -Motoya 2017[39] -Nakase 2017[40]* | 176 | 151 | CD | RCT | Randomized | Adalimumab + azathioprine | Adalimumab + placebo | -ADA formation | Drug sensitive |
| 29 | Sandborn 2007a[41] *(CLASSIC II)* | 276 | 269 | CD | RCT | Observational | Adalimumab + IM | Adalimumab | -ADA formation | Drug sensitive |
| 30 | Sandborn 2007b[42] *(CLASSIC I)* | 325 | 158 | CD | RCT | Observational | Adalimumab + IM | Adalimumab | -ADA formation | Drug sensitive |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 31 | Sandborn 2012a[43] ULTRA II *Records: -Awni 2013[44] -Sandborn 2013[45]* | 518 | 245 | UC | RCT | Observational | Adalimumab | Placebo | -ADA formation | Drug sensitive |
| 32 | Suzuki 2014[46] *Records: -Suzuki 2017[47]* | 274 | 240 | UC | RCT | Observational | Adalimumab | Placebo | -ADA formation | Drug sensitive |
| 33 | Watanabe 2012[48] *Records: -Watanabe 2014[49]* | 90 | 67 | CD | RCT | Observational | Adalimumab + IM | Adalimumab | -ADA formation | Drug sensitive |
| 34 | Wright 2018[50] | 52 | 52 | CD | RCT subanalysis | Observational | Adalimumab + IM | Adalimumab | -ADA formation | Drug tolerant |
| | | | | | | | ADA positive | ADA negative | -Adalimumab concentration | |
| 35 | Wu 2016[51] | 30 | 30 | CD | RCT | Observational | Adalimumab | Placebo | -ADA formation | Drug sensitive |

**Supplementary Table 1.** *(Continued).*

| | INFLIXIMAB and ADALIMUMAB | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Study** | **N** | **Sample size** | **Population** | **Study type** | **Data type** | **Intervention/ Population** | **Control** | **Outcomes of interest** | **Assay** |
| 36 | Roblin 2020[52] | 90 | 90 | IBD | RCT | Observational | Anti-TNF + IM | Anti-TNF | -ADA formation<br>-ADA disappearance | Drug tolerant & drug sensitive |
| | **GOLIMUMAB** | | | | | | | | | |
| | **Study** | **N** | **Sample size** | **Population** | **Study type** | **Data type** | **Intervention/ Population** | **Control** | **Outcomes of interest** | **Assay** |
| 37 | Hibi 2017[53] *(PURSUIT-J)* | 123 | 123 | UC | RCT | Observational | Golimumab | Placebo | -ADA formation | Drug sensitive |
| 38 | Rutgeerts 2015[54] *(PURSUIT-IV)* | 291 | 206 | UC | RCT | Observational | Golimumab i.v. | Placebo | -ADA formation | Drug sensitive |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 39 | Sandborn 2014[55, 56]<br>*Records:*<br>*-Adedokun 2013 (2x)[57, 58]*<br>*-Adedokun 2017[59]*<br>*-Sandborn 2017[60]*<br>*-Reinisch 2018[61]*<br>-Adedokun 2019[62] | 1528 | 1103 | UC | RCT | Observational | Golimumab + IM | Golimumab | -ADA formation | Drug sensitive & Drug tolerant |
| | **CERTOLIZUMAB PEGOL** | | | | | | | | | |
| | **Study** | **N** | **Sample size** | **Population** | **Study type** | **Data type** | **Intervention/ Population** | **Control** | **Outcomes of interest** | **Assay** |
| 40 | Sandborn 2007c[63]<br>PRECISE-1<br>*Records:*<br>*-Sandborn 2015[64]* | 662 | 331 | CD | RCT | Observational | Certolizumab pegol + IM | Certolizumab pegol | -ADA formation | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | -Drug concentration | |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 41 | Sandborn 2010b[65] *(PRECiSE 4)* *Records:* *-Sandborn 2015[64]* *-Sandborn 2016[66]* | 124 | 124 | CD | RCT | Observational | Certolizumab pegol | Placebo | -ADA formation | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | -Drug concentration | |
| 42 | Sandborn 2011[67] | 438 | 223 | CD | RCT | Observational | Certolizumab pegol | Placebo | -ADA formation | Drug sensitive |
| 43 | Sandborn 2017[68] *(PRECiSE-3)* *Records:* *-Lichtenstein 2010[69]* *-Sandborn 2015[64]* | 595 | 595 | CD | RCT | Observational | Certolizumab pegol + IM | Certolizumab pegol | -ADA formation | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | -Biochemical outcomes -Drug concentration | |
| 44 | Schreiber 2005[70] | 292 | 73 | CD | RCT | Observational | Certolizumab pegol | Placebo | -ADA formation | Drug sensitive |
| 45 | Schreiber 2007[71] PRECISE-2 *Records:* *-Sandborn 2015[64]* | 668 | 668 | CD | RCT | Observational | Certolizumab pegol + IM | Certolizumab pegol | -ADA formation -Drug concentration | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | -Clinical outcomes | |

**Supplementary Table 1.** *(Continued).*

| VEDOLIZUMAB | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Study** | **N** | **Sample size** | **Population** | **Study type** | **Data type** | **Intervention/ Population** | **Control** | **Outcomes of interest** | **Assay** |
| 46 | Feagan 2005[72] | 181 | 30 | UC | RCT | Observational | MLN02 (0.5 mg)<br>MLN02 (2.0 mg) | Placebo | -ADA formation | Drug sensitive |
| 47 | Feagan 2008[73] | 185 | 128 | CD | RCT | Observational | MLN02 (0.5 mg)<br>MLN02 (2.0 mg) | Placebo | -ADA formation | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | -Adverse events<br>-Clinical outcomes | |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 48 | Feagan 2013[74] (GEMINI 1) *Records: -Rosario 2015[75] -Feagan 2015[76] -Feagan 2017[77] -Loftus 2017[78] -Wyant 2019[79]* | 895 | 620 | UC | RCT | Observational | Vedolizumab | Placebo | -ADA formation | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | -Adverse events | |
| 49 | Motoya 2019[80] | 292 | 167 | UC | RCT | Observational | Vedolizumab | Placebo | -ADA formation | Unknown |
| 50 | Parikh 2012[81] *Records: -Parikh 2013 (extension)[82]* | 46<br>72 | 37<br>72 | UC | RCT | Observational | Vedolizumab | Placebo | -ADA formation | Drug sensitive |
| | | | | | | | ADA positive | ADA negative or inconclusive | -Adverse events | |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 51 | Sandborn 2013[83]<br><br>*Records:*<br>*-Feagan 2015[76]*<br>*-Vermeire 2017[84]*<br>*-Wyant 2019[79]* | 1115 | 814 | CD | RCT | Observational | Vedolizumab | Placebo | -ADA formation | Drug sensitive |
| 52 | Sandborn 2020[85] (VISIBLE)<br>*Records:*<br>*-Sandborn 2019[86]* | 383 | 106 s.c.<br>54 i.v. | UC | RCT | Observational | Vedolizumab i.v. + s.c. maintenance | Placebo | -ADA formation | Drug tolerant |
| 53 | Sands 2014[87] | 416 | 209 | CD | RCT | Observational | Vedolizumab | Placebo | -ADA formation | Drug sensitive |
| 54 | Watanabe 2020[88] | 157 | 63 | CD | RCT | Observational | Vedolizumab | Placebo | -ADA formation | Unknown |
| | | | | | **NATALIZUMAB** | | | | | |
| | **Study** | **N** | **Sample size** | **Population** | **Study type** | **Data type** | **Intervention/ Population** | **Control** | **Outcomes of interest** | **Assay** |
| 55 | Ghosh 2003[89] | 248 | 185 | CD | RCT | Observational | Natalizumab | Placebo | -ADA formation | Drug sensitive |
| 56 | Gordon 2001[90] | 30 | 18 | CD | RCT | Observational | Natalizumab | Placebo | -ADA formation | Drug sensitive |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 57 | Gordon 2002[91] | 10 | 10 | UC | Open-label | Observational | Natalizumab | N/A | -ADA formation | Drug sensitive |
| 58 | Sandborn 2005a[92] *(ENACT-1)* | 905 | 723 | CD | RCT | Observational | Natalizumab + IM | Natalizumab | -ADA formation | Drug sensitive |
| 59 | Sandborn 2005b[92] *(ENACT-2)* | 339 | 214 | CD | RCT | Observational | Natalizumab + IM | Natalizumab | -ADA formation | Drug sensitive |
| 60 | Targan 2007[93] | 509 | 241 | CD | RCT | Observational | Natalizumab + IM | Natalizumab | -ADA formation | Drug sensitive |
| 61 | Sands 2007[94] | 79 | 52 | CD | RCT | Observational | Natalizumab | Placebo | -ADA formation | Drug sensitive |
| | | | | | **USTEKINUMAB** | | | | | |
| | **Study** | **N** | **Sample size** | **Population** | **Study type** | **Data type** | **Intervention/ Population** | **Control** | **Outcomes of interest** | **Assay** |
| 62 | Sands 2019[95] (UNIFI) *Records:* -Adedokun 2019 (2x)[96, 97] | 960 | 680 | UC | Post-Hoc RCT | Observational | Ustekinumab | Placebo | -ADA formation | Drug tolerant |

**Supplementary Table 1.** *(Continued).*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 63 | Feagan 2016[98] *(UNITI-1, UNITI-2, IM-UNITI)*<br>*Records:*<br>*-Hibi 2017[99]*<br>*-Sandborn 2016[100]*<br>*-Sandborn 2018[101]*<br>*-Sandborn 2019 (IM-UNITI)[102]*<br>*-Ghosh 2019 (IM-UNITI LTE)[103]*<br>*-Hanauer 2020 (IM-UNITI LTE)[104]* | 741<br>628<br>397<br><br>718 | 1154<br><br>237 | CD | RCT | Observational | Ustekinumab | Placebo | -ADA formation | Drug tolerant |
| 64 | Sandborn 2008[105] (UCDS) | 104 | 99 | CD | RCT | Observational | Ustekinumab | Placebo | -ADA formation | Drug sensitive |
| 65 | Sandborn 2012b[106] (CERTIFI) | 395 | 427 | CD | RCT | Observational | Ustekinumab | Placebo | -ADA formation | Drug sensitive |

**Supplementary Table 1.** *(Continued).*

| | ETROLIZUMAB | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Study** | **N** | **Sample size** | **Population** | **Study type** | **Data type** | **Intervention/ Population** | **Control** | **Outcomes of interest** | **Assay** |
| 66 | Rutgeerts 2013[107] | 48 | 38 | UC | RCT | Observational | Etrolizumab | Placebo | -ADA formation | Drug tolerant |
| 67 | Vermeire 2014[108] | 124 | 81 | UC | RCT | Observational | Etrolizumab | Placebo | -ADA formation | Drug tolerant |

*CD, Crohn's disease; IM, immunosuppressive; ADA, anti-drug antibody; RCT, randomized controlled trial; TDM, therapeutic drug monitoring; UC, ulcerative colitis*

**Supplementary Table 2.** *ADA formation rates and measurement time-points.*

| Infliximab | | | | | |
|---|---|---|---|---|---|
| **Study** | **Nr. Positive** | **Sample-size** | **Proportion** | **Measurement time-points** | **Time follow-up** |
| Baert 2003[1] | 76 | 125 | 60.8% | Unknown (variable) | 36 months (median) |
| Colombel 2010[2] *SONIC* | 16 | 219 | 7.3% | weeks 0, 30, 46 | 30 weeks |
| D'Haens 2018[4] | 21 | 122 | 17.2% | Weeks 0,2 ,4 ,6 , 12, 14, every 4 weeks | 54 weeks |
| Farrell 2003[5] | 19 | 53 | 35.8% | weeks 0, 24 | 20 weeks (median) |
| Farrell 2003[5] | 27 | 68 | 39.7% | weeks 0, 8, 16 weeks | 16 weeks |
| Feagan 2014[6] | 16 | 126 | 12.7% | weeks 1, 3, 7, 14, 22, 30, 38, 46, 50 | 50 weeks |
| Fernandes 2020[7] | 13 | 205 | 6.3% | Week 14 and every 2 infusions | 104 weeks |
| Hanauer 2002[8] *ACCENT-1*‡ | 64 | 442 | 14.5% | Weeks 0, 14, 22, 54 | 54 weeks |
| Jiang 2015[11] | 4 | 78 | 5.1% | Weeks 0, 30 | 30 weeks |
| Oh 2017[12] | 47 | 138 | 34.1% | Every infusion | 47 months (median) |
| Panaccione 2014[13] | 8 | 68 | 11.8% | Weeks 0, 16 | 16 weeks |
| Present 1999[14] | 3 | 92 | 3.3% | Weeks 0, 2, 6 | 12 weeks |
| Regueiro 2016[15] | 24 | 147 | 16.3% | Weeks 0, 72 | 104 weeks |
| Roblin 2017[16] | 6 | 81 | 7.4% | Every infusion | 56 weeks |
| Rutgeerts 1999 | 7 | 47 | 14.9% | Unknown | 48 weeks |
| Rutgeerts 2005a[18] *ACT-1* | 14 | 229 | 6.1% | Weeks 0, 30, 54 | 54 weeks |
| Rutgeerts 2005b[18] *ACT-2* | 12 | 188 | 6.4% | Weeks 0, 30 | 30 weeks |
| Sands 2004[22] | 44 | 258 | 17.1% | Weeks 0, 14, 30, 54 | 54 weeks |
| Seow 2010[23] | 44 | 108 | 40.7% | 10.7 months (median)<br>23 patients at week 1, 2, 4, 6, 14, 25<br>25 patients randomly for second measurement median 20 weeks after first measurement. | 54 weeks |
| Steenholdt 2015[25] | 13 | 42 | 31.0% | At time of IFX failure | 12 weeks |

**Supplementary Table 2.** *(Continued).*

| | | | | | |
|---|---|---|---|---|---|
| Targan 1997[27] | 6 | 101* | 5.9% | Week 12 | 12 weeks |
| Van Assche 2008[28] | 7 | 80 | 8.8% | Every 16 weeks | 104 weeks |
| Vande Casteele 2015[29] *TAXIT* | 18 | 275 | 2.9% | Screening measurement | Screening |
| Vande Casteele 2015[29] *TAXIT* | 3 | 226 | 1.3% | Every infusion | 52 weeks |
| Vermeire 2007[32] | 96 | 174 | 55.1% | Before and 4 weeks after each infusion (unscheduled) | 42 weeks (median) |
| Ye 2019[33] | 40 | 220 | 18.2% | Weeks 0, 14, 30, 54 | 54 weeks |
| Yokoyama 2017[34] | 1 | 21 | 4.8% | Unknown | 54 weeks |
| **Adalimumab** | | | | | |
| Bodini 2014[35] | 1 | 6 | 16.7% | Every 8 weeks | 104 weeks |
| Hanauer 2006[37] *CLASSIC-1* | 1 | 225 | 0.4% | Weeks 0, 1, 2, 4 | 4 weeks |
| Matusumoto 2016[38] | 13 | 151 | 8.6% | Week 26 | 52 weeks |
| Sandborn 2007a[41] *CLASSIC II* | 7 | 269* | 2.6% | Week 0, 2, 4, 8, 12, 20, 24, 32, 40, 48, 56 | 56 weeks |
| Sandborn 2007b[42] *CLASSIC I* | 0 | 159 | 0% | Week 0, 1, 2, 4 | 4 weeks |
| Sandborn 2012a[43] | 7 | 245 | 2.9% | Week 0, 8, 32, 52, early termination | 52 weeks |
| Suzuki 2014[46] | 12 | 240 | 5.0% | Week 2, 4, 8, 32, 52 | 52 weeks |
| Watanabe 2012[48] | 5 | 67 | 7.5% | Week 0, 4, 8, 12, 16, 20, 24, 36, 52 | 52 weeks |
| Wright 2018[50] | 15 | 52 | 28.8% | Month 6, 12 and 18 | 18 months |
| Wu 2016[51] | 0 | 30 | 0% | Week 0, 4 and 8 | 10 weeks |

**Supplementary Table 2.** *(Continued).*

| | | | | | |
|---|---|---|---|---|---|
| **Infliximab and adalimumab** | | | | | |
| Roblin 2020[52] | 90 | unknown | unknown | Month 6, 12, 18, 24 | 24 months |
| **Vedolizumab** | | | | | |
| Feagan 2005[72] | 13 | 30 | 43.3% | Weeks 4, 8 | 8 weeks |
| Feagan 2008[73] | 29 | 128 | 22.7% | Days 1, 43, 155, 267, 379, 491 | 8 weeks |
| Feagan 2013[74] | 23 | 620 | 3.7% | Every 12 weeks | 52 weeks |
| Parikh 2012[81] | 4 | 37 | 10.8% | Days 1, 43, 155, 267, 379, 491 | 78 weeks |
| Parikh 2013 (extension) | 3 | 72 | 4.2% | | |
| Sandborn 2013[83] | 33 | 814 | 4.2% | Unknown | 52 weeks |
| Sands 2014[87] | 3 | 209 | 1.4% | Week 0, 6, 10 | 22 weeks |
| Motoya 2019[80] | 5 | 167 | 3.0% | Week 0, 10, 30, 60 | 60 weeks |
| Sandborn 2020[85] | 6 | 106 | 5.7% | Week 0, 6, 8, 14, 22, 30, 38, 46, 52 | 52 weeks |
| Watanabe 2020[88] | 1 | 60 | 1.7% | Week 0, 10, 30, 60, 76 | 60 weeks |
| **Natalizumab** | | | | | |
| Ghosh 2003[89] | 13 | 185 | 7.0% | Week 6 | 6 weeks |
| Gordon 2001[90] | 2 | 18 | 11.1% | Week 1, 2, 4, 8, 12 | 12 weeks |
| Gordon 2002[91] | 1 | 10 | 10.0% | Week 2 | 2 weeks |
| Sandborn 2005a[92] *ENACT-1* | 53 | 723 | 7.3% | Week 10 | 10 weeks |
| Sandborn 2005b[92] *ENACT-2* | 36 | 214 | 16.8% | Week 36 | 36 weeks |
| Targan 2007[93] | 23 | 241 | 9.5% | Week 8 | 12 weeks |
| Sands 2007[94] | 2 | 52 | 3.8% | Week 10 | 10 weeks |
| **Ustekinumab** | | | | | |
| Feagan 2016[98] *UNITI-1* *UNITI-2* *IM-UNITI* | 44 | 1154 | 3.8% | Week 0, 6, 12, 24, 36, 44 | 52 weeks |

**Supplementary Table 2.** *(Continued).*

| | | | | | |
|---|---|---|---|---|---|
| Sands 2019[95] (UNIFI) | 23 | 505 | 4.6% | Week 0, 4, 8, 12, 16, 24, 36, 44 | 52 weeks |
| Sandborn 2008[105] (UCDS) | 0 | 99 | 0% | Week 0, 16, 28, 54 | 28 weeks |
| Sandborn 2012b[106] (CERTIFI) | 3 | 427 | 0.7% | Week 0, 22, 36 | 36 weeks |
| **Certolizumab Pegol** | | | | | |
| Sandborn 2007c[63] PRECISE-1 | 26 | 331 | 7.9% | Week 0, 2, 4, 6, 8, 12, 16, 20, 24, 26 | 26 weeks |
| Sandborn 2010b[65] *PRECiSE 4* | 30 | 124 | 24.2% | Week 0, 2, 4, 6, 8, 12, 12, 16, 18, 20, 22, ,24, 26 | 26 weeks |
| Sandborn 2011[67] | 7 | 223 | 3.1% | Week 2, 4, 6 | 6 weeks |
| Sandborn 2017[68] *PRECISE-3* | 134 | 595 | 22.5% | Week 0, every 4 weeks to week 106, week 130, 158, 182, 234, 258, 318 | 80 weeks |
| Schreiber 2005[70] | 9 | 73 | 12.3% | Unknown | 12 weeks |
| Schreiber 2007[71] PRECISE-2 | 58 | 668 | 8.7% | Week 0, 2, 4, 6, 8, 12, 16, 20, 24, 26 | 26 weeks |
| **Golimumab** | | | | | |
| Hibi 2017[53] *PURSUIT-J* | 5 | 123 | 4.1% | Week 0, 6, 28, 30, 52, 54, 68 | 54 weeks |
| Rutgeerts 2015[54] *PURSUIT-IV* | 0 | 206 | 0% | Week 0, 6, 30, 54 week | 54 weeks |
| Sandborn 2014[55, 56] | 32 | 1103 | 2.9% | Week 0, 6, 30, 54 | 54 weeks |
| **Etrolizumab** | | | | | |
| Rutgeerts 2013[107] | 2 | 38 | 5.3% | Day 1, 15, 29, 57, 99, 127 in single ascending dose stage<br>Day 1, 29, 57, 64, 85, 113, 141, 169, 197 in maintenance dose stage | 197 days |

**Supplementary Table 2.** *(Continued).*

| | | | | | |
|---|---|---|---|---|---|
| Vermeire 2014[108] | 4 | 81 | 4.9% | Day 1, 5, 15, 29, 43, 57, 61, 71, 85, 113, 141 | 10 weeks |

*Sample-size including placebo

**Supplementary Table 3.** *Quality assessment of the randomized controlled studies.*

| Study | Random sequence allocation | Allocation concealment | Blinding of participants and personnel | Blinding of outcome assessment | Incomplete outcome data | Selective Reporting | Other sources of bias |
|---|---|---|---|---|---|---|---|
| **Infliximab** | | | | | | | |
| Colombel 2010[2] *SONIC* | Low risk | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk |
| D'Haens 2018[4] | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| Farrell 2003[5] (study 2) | Low risk | Unclear risk | Low risk | Low risk | Unclear risk | Low risk | Low risk |
| Feagan 2014[6] | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| Hanauer 2002[8] *ACCENT-1* | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Jiang 2015[11] | Low risk | Unclear risk | Low risk | Unclear risk | High risk | Low risk | Low risk |
| Panaccione 2014[13] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Present 1999[14] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Regueiro 2016[15] | Low risk | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk |
| Roblin 2017[16] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Rutgeerts 1999[17] | Unclear risk | Unclear risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Rutgeerts 2005a[18] *ACT-1* | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| Rutgeerts 2005b[18] *ACT-2* | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| Sands 2004[22] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Steenholdt 2014[25] | Low risk | Low risk | Low risk | Unclear risk | Unclear risk | Low risk | Low risk |
| Targan 1997[27] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |

**Supplementary Table 3.** *(Continued).*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Van Assche 2008[28] | Unclear risk | Low risk | High risk | Unclear risk | Low risk | Low risk | Low risk |
| Vande Casteele 2015[29] | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| Ye 2019[33] | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| **Adalimumab** | | | | | | | |
| Bodini 2014[35] | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| Hanauer 2006[37] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Matsumoto 2016[38] | Low risk | Low risk | High risk | High risk | Low risk | Low risk | Low risk |
| Sandborn 2007a[41] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2007b[42] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Suzuki 2014[46] | Unclear risk | Unclear risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2012a[43] | Low risk | Unclear risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Watanabe 2012[48] | Low risk | Unclear risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Wright 2018[50] | Low risk | Unclear risk | High risk | Low risk | Low risk | Low risk | Low risk |
| Wu 2016[51] | Low risk | Unclear risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| **Infliximab and adalimumab** | | | | | | | |
| Roblin 2020[52] | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| **Golimumab** | | | | | | | |
| Hibi 2017[53] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Rutgeerts 2015[54] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2014[55, 56] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| **Certolizumab Pegol** | | | | | | | |
| Sandborn 2007c[63] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2010a[109] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2010b[65] | Low risk | Low risk | High risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2011[67] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2017[68] | Low risk | Low risk | High risk | Low risk | Low risk | Low risk | Low risk |

**Supplementary Table 3.** *(Continued).*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Schreiber 2005[70] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Schreiber 2007[71] | Low risk | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk |
| **Vedolizumab** | | | | | | | |
| Feagan 2005[72] | Low risk | Unclear risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Feagan 2008[73] | Unclear risk | Unclear risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Feagan 2013[74] | Low risk | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk |
| Motoya 2019[80] | Low risk | Low risk | High risk | Unclear risk | Low risk | Low risk | Low risk |
| Parikh 2012[81] | Low risk | Unclear risk | High risk | High risk | Unclear risk | Low risk | Low risk |
| Sandborn 2013[83] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sands 2014[87] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2020[85] | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| Watanabe 2020[88] | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| **Natalizumab** | | | | | | | |
| Ghosh 2003[89] | Low risk | Unclear risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Gordon 2001[90] | Unclear risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2005a[92] | Unclear risk | Unclear risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2005b[92] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Targan 2007[93] | Unclear risk | Unclear risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sands 2007[94] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| **Ustekinumab** | | | | | | | |
| Feagan 2016[98] UNITI-1 | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| UNITI-2 | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| IM-UNITI | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| Sandborn 2008[105] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |
| Sandborn 2012b[106] | Low risk | Low risk | Low risk | Unclear risk | Low risk | Low risk | Low risk |

**Supplementary Table 3.** *(Continued).*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Sands 2019[95] | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |
| **Etrolizumab** | | | | | | | |
| Rutgeerts 2013[107] | Low risk | Unclear risk | Low risk | Low risk | Unclear risk | Low risk | Low risk |
| Vermeire 2014[108] | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk |

**Supplementary Table 4.** *Quality assessment of the observational studies*.*

| Study | Selection (Max 4) | Comparability (Max 2) | Outcome (Max 3) | Total (Max 9) |
|---|---|---|---|---|
| **Infliximab** | | | | |
| Baert 2003[1] | **** | ** | *** | 9 |
| Farrell 2003[5] (study 1) | **** | ** | *** | 9 |
| Fernandes 2020[7] | | | | |
| Oh 2017[12] | **** | ** | **[1] | 8 |
| Seow 2010[23] | **** | ** | *** | 9 |
| Vermeire 2007[32] | **** | ** | *** | 9 |
| Yokoyama 2017[34] | **** | - | **[1] | 6 |
| **Adalimumab** | | | | |
| Regueiro 2016[15] | ***[3] | ** | **[1] | 8 |
| Roblin 2017[16] | **** | ** | **[1] | 8 |
| Rutgeerts 2005a[18] | **** | ** | **[1] | 8 |
| Rutgeerts 2005b[18] | **** | ** | **[1] | 8 |
| Sands 2004[22] | ***[2] | ** | **[1] | 7 |
| **Certolizumab pegol** | | | | |
| Gordon 2002[91] | **** | ** | **[1] | 8 |
| Sandborn 2016[110] | **** | ** | **[1] | 8 |

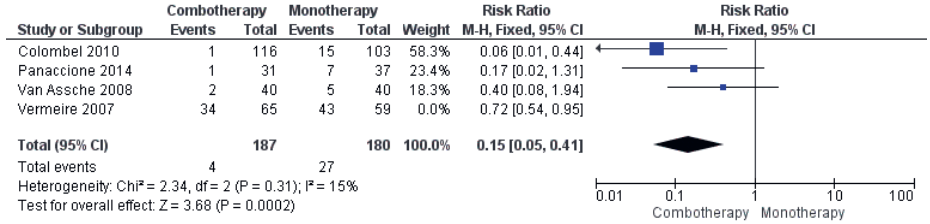
**The Newcastle-Ottawa Quality Assessment Scale for case-control studies was used for observational data. Comparisons of monotherapy versus combination therapy were considered observational if the patients were not randomized to monotherapy or combination therapy and the study reported event(s) of interest in these two groups.*

[1]*Unclear whether the assessment was independent and blinded*

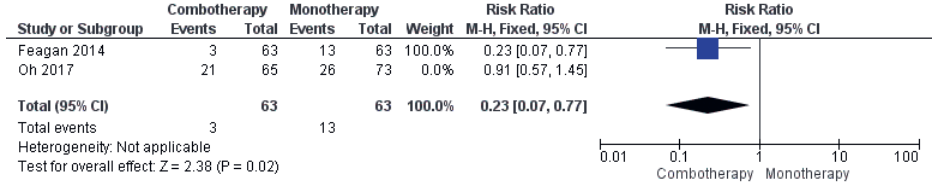
[2]*Only included patients with fistulizing Crohn's disease*

[3]*Only included patients with CD who had undergone ileocolonic resection with ileocolonic anastomosis*

[4]*Single-country study*

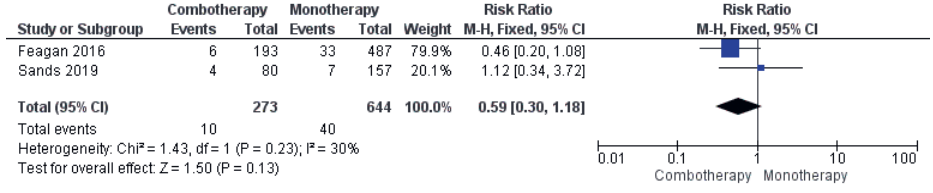



**Supplementary Figure 1.** *ADA formation in infliximab combotherapy with thiopurine versus monotherapy (sensitivity analysis).*

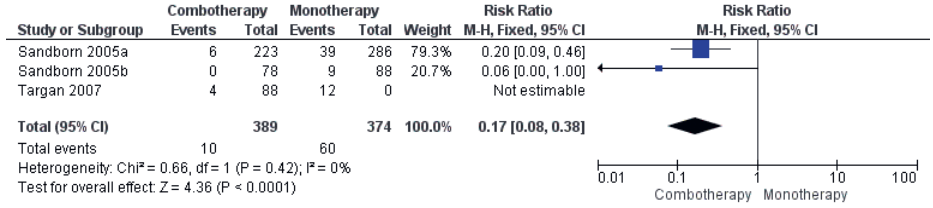



**Supplementary Figure 2.** *ADA formation in infliximab combotherapy versus monotherapy in drug tolerant assays (sensitivity analysis).*


**Supplementary figure 3.** *ADA formation in golimumab combo- versus monotherapy.*




**Supplementary figure 4.** *ADA formation in ustekinumab combotherapy versus monotherapy.*




**Supplementary Figure 5.** *ADA formation in natalizumab combotherapy versus monotherapy.*

## Supplementary references


1. Baert F, Noman M, Vermeire S, Van Assche G, G DH, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med. 2003;348(7):601-8.

2. 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;362(15):1383-95.

3. Colombel JF, Adedokun OJ, Gasink C, Tang K, Cornillie F, D'Haens G, et al. Higher levels of infliximab may alleviate the need of azathioprine comedication in the treatment of patients with Crohn's disease: a SONIC post hoc analysis. Journal of crohn's and colitis Conference: 12th congress of the european crohn's and colitis organisation, ECCO 2017 Spain. 2017;11(Supplement 1):S135 - S6.

4. 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;154(5):1343 - 51.e1.

5. Farrell RJ, Alsahli M, Jeen YT, Falchuk KR, Peppercorn MA, Michetti P. Intravenous hydrocortisone premedication reduces antibodies to infliximab in Crohn's disease: a randomized controlled trial. Gastroenterology. 2003;124(4):917-24.

6. 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;146(3):681-8 e1.

7. Fernandes SR, Bernardo S, Simoes C, Goncalves AR, Valente A, Baldaia C, et al. Proactive Infliximab Drug Monitoring Is Superior to Conventional Management in Inflammatory Bowel Disease. Inflammatory Bowel Diseases. 2020;26(2):263-70.

8. Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet. 2002;359(9317):1541-9.

9. Rutgeerts P, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Comparison of scheduled and episodic treatment strategies of infliximab in Crohn's disease. Gastroenterology. 2004;126(2):402-13.

10. Hanauer SB, Wagner CL, Bala M, Mayer L, Travers S, Diamond RH, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. Clinical Gastroenterology & Hepatology. 2004;2:542-53.

11. Jiang XL, Cui HF, Gao J, Fan H. Low-dose Infliximab for Induction and Maintenance Treatment in Chinese Patients With Moderate to Severe Active Ulcerative Colitis. Journal of clinical gastroenterology. 2015;49(7):582-8.

12. Oh EH, Ko D-H, Seo H, Chang K, Kim G-U, Song EM, et al. Clinical correlations of infliximab trough levels and antibodies to infliximab in South Korean patients with Crohn's disease. World journal of gastroenterology. 2017;23(8):1489-96.

13. 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;146(2):392-400.e3.

14. Present DH, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. New England Journal of Medicine. 1999;340(18):1398-405.

15. Regueiro M, Feagan BG, Zou B, Johanns J, Blank MA, Chevrier M, et al. Infliximab Reduces Endoscopic, but Not Clinical, Recurrence of Crohn's Disease After Ileocolonic Resection. Gastroenterology. 2016;150(7):1568-78.

16. Roblin X, Boschetti G, Williet N, Nancey S, Marotte H, Berger A, et al. Azathioprine dose reduction in inflammatory bowel disease patients on combination therapy: an open-label, prospective and randomised clinical trial. Alimentary pharmacology & therapeutics. 2017;46(2):142-9.

17. Rutgeerts P, D'Haens G, Targan S, Vasiliauskas E, Hanauer SB, Present DH, et al. Efficacy and safety of retreatment with anti-tumor necrosis factor antibody (infliximab) to maintain remission in Crohn's disease. Gastroenterology. 1999;117(4):761-9.

18. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2005;353(23):2462-76.

19. Reinisch W, Sandborn WJ, Rutgeerts P, Feagan BG, Rachmilewitz D, Hanauer SB, et al. Long-term infliximab maintenance therapy for ulcerative colitis: the ACT-1 and -2 extension studies. Inflamm Bowel Dis. 2012;18(2):201-11.

20. Adedokun OJ, Sandborn WJ, Feagan BG, Rutgeerts P, Xu Z, Marano CW, et al. Association between serum concentration of infliximab and efficacy in adult patients with ulcerative colitis. Gastroenterology. 2014;147(6):1296-307.e5.

21. Vande Casteele N, Jeyarajah J, Jairath V, Feagan BG, Sandborn WJ. Infliximab Exposure-Response Relationship and Thresholds Associated With Endoscopic Healing in Patients With Ulcerative Colitis. Clinical Gastroenterology & Hepatology. 2019;17(9):1814-21.e1.

22. Sands BE, Anderson FH, Bernstein CN, Chey WY, Feagan BG, Fedorak RN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med. 2004;350(9):876-85.

23. Seow CH, Newman A, Irwin SP, Steinhart AH, Silverberg MS, Greenberg GR. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut. 2010;59(1):49-54.

24. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OO, Ainsworth MA. Clinical implications of measuring drug and anti-drug antibodies by different assays when optimizing infliximab treatment failure in Crohn's disease: post hoc analysis of a randomized controlled trial. The American journal of gastroenterology. 2014;109(7):1055-64.

25. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OO, Munck LK, Christensen LA, et al. Changes in serum trough levels of infliximab during treatment intensification but not in anti-infliximab antibody detection are associated with clinical outcomes after therapeutic failure in Crohn's disease. Journal of Crohn's & colitis. 2015;9(3):238-45.

26. Edlund H, Steenholdt C, Ainsworth MA, Goebgen E, Brynskov J, Thomsen OO, et al. Magnitude of Increased Infliximab Clearance Imposed by Anti-infliximab Antibodies in Crohn's Disease Is Determined by Their Concentration. AAPS Journal. 2017;19(1):223-33.

27. Targan SR, Hanauer SB, van Deventer SJ, Mayer L, Present DH, Braakman T, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. N Engl J Med. 1997;337(15):1029-35.

28. Van Assche G, Magdelaine-Beuzelin C, D'Haens G, Baert F, Noman M, Vermeire S, et al. Withdrawal of immunosuppression in Crohn's disease treated with scheduled infliximab maintenance: a randomized trial. Gastroenterology. 2008;134(7):1861-8.

29. Vande Casteele N, Ferrante M, Van Assche G, Ballet V, Compernolle G, Van Steen K, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology. 2015;148(7):1320-9 e3.

30. Van Stappen T, Vande Casteele N, Van Assche G, Ferrante M, Vermeire S, Gils A. Clinical relevance of detecting anti-infliximab antibodies with a drug-tolerant assay: post-hoc analysis of the taxit trial. Journal of Crohn's & colitis. 2017;11:S44 - S5.

31. Van Stappen T, Vande Casteele N, Van Assche G, Ferrante M, Vermeire S, Gils A. Clinical relevance of detecting anti-infliximab antibodies with a drug-tolerant assay: post hoc analysis of the TAXIT trial. Gut. 2018;67(5):818-26.

32. Vermeire S, Noman M, Van Assche G, Baert F, D'Haens G, Rutgeerts P. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. Gut. 2007;56(9):1226-31.

33. Ye BD, Pesegova M, Alexeeva O, Osipenko M, Lahat A, Dorofeyev A, et al. Efficacy and safety of biosimilar CT-P13 compared with originator infliximab in patients with active Crohn's disease: an international, randomised, double-blind, phase 3 non-inferiority study. Lancet. 2019;393(10182):1699-707.

34. Yokoyama K, Kawagishi K, Kobayashi K, Koizumi W. Current status of the effectiveness of infliximab in patients with ulcerative colitis. Journal of crohn's and colitis Conference: 12th congress of the european crohn's and colitis organisation, ECCO 2017 Spain. 2017;11(Supplement 1):S370 - S1.

35. Bodini G, Savarino V, Peyrin-Biroulet L, de Cassan C, Dulbecco P, Baldissarro I, et al. Low serum trough levels are associated with post-surgical recurrence in Crohn's disease patients undergoing prophylaxis with adalimumab. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2014;46(11):1043-6.

36. Savarino E, Bodini G, Dulbecco P, Assandri L, Bruzzone L, Mazza F, et al. Adalimumab is more effective than azathioprine and mesalamine at preventing postoperative recurrence of Crohn's disease: a randomized controlled trial. The American journal of gastroenterology. 2013;108(11):1731-42.

37. Hanauer SB, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh D, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology. 2006;130(2):323-33; quiz 591.

38. Matsumoto T, Motoya S, Watanabe K, Hisamatsu T, Nakase H, Yoshimura N, et al. Adalimumab Monotherapy and a Combination with Azathioprine for Crohn's Disease: A Prospective, Randomized Trial. Journal of Crohn's & colitis. 2016;10(11):1259-66.

39. Motoya S, Nakase H, Matsumoto T, Watanabe K, Hisamatsu T, Yoshimura N, et al. Association between pharmacokinetics of adalimumab and disease outcome in Japanese patients with biologics naive Crohn's disease: A subanalysis of DIAMOND trial. Journal of Crohn's and Colitis. 2017;11 (Supplement 1):S345-S6.

40. Nakase H, Motoya S, Matsumoto T, Watanabe K, Hisamatsu T, Yoshimura N, et al. Association between pharmacokinetics of adalimumab and disease outcome in Japanese patients with biologics naïve Crohn's disease: a subanalysis of diamond study. Gastroenterology. 2017;152(5):S746 - .

41. Sandborn WJ, Hanauer SB, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh DG, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. Gut. 2007;56(9):1232-9.

42. Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Colombel JF, Panaccione R, et al. Adalimumab induction therapy for Crohn disease previously treated with infliximab: a randomized trial. Annals of internal medicine. 2007;146(12):829-38.

43. Sandborn WJ, van Assche G, Reinisch W, Colombel JF, D'Haens G, Wolf DC, et al. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2012;142(2):257-65.e1-3.

44. Awni W, Eckert D, Sharma S, Mostafa N, Noertersheuser P, Pradhan R, et al. P412 Pharmacokinetics of adalimumab in adult patients with moderately to severely active ulcerative colitis. Journal of Crohn's and Colitis. 2013;7(Supplement_1):S175-S.

45. Sandborn WJ, Colombel JF, D'Haens G, Van Assche G, Wolf D, Kron M, et al. One-year maintenance outcomes among patients with moderately-to-severely active ulcerative colitis who responded to induction therapy with adalimumab: subgroup analyses from ULTRA 2. Alimentary pharmacology & therapeutics. 2013;37(2):204-13.

46. Suzuki Y, Motoya S, Hanai H, Matsumoto T, Hibi T, Robinson AM, et al. Efficacy and safety of adalimumab in Japanese patients with moderately to severely active ulcerative colitis. Journal of gastroenterology. 2014;49(2):283-94.

47. Suzuki Y, Motoya S, Hanai H, Hibi T, Nakamura S, Lazar A, et al. Four-year maintenance treatment with adalimumab in Japanese patients with moderately to severely active ulcerative colitis.[Erratum appears in J Gastroenterol. 2017 Apr 12;:; PMID: 28405767]. Journal of gastroenterology. 2017;20:20.

48. Watanabe M, Hibi T, Lomax KG, Paulson SK, Chao J, Alam MS, et al. Adalimumab for the induction and maintenance of clinical remission in Japanese patients with Crohn's disease. Journal of Crohn's & colitis. 2012;6(2):160-73.

49. Watanabe M, Hibi T, Mostafa NM, Chao J, Arora V, Camez A, et al. Long-term safety and efficacy of adalimumab in Japanese patients with moderate to severe Crohn's disease. Journal of Crohn's & colitis. 2014;8(11):1407-16.

50. Wright EK, Kamm MA, De Cruz P, Hamilton AL, Selvaraj F, Princen F, et al. Anti-TNF therapeutic drug monitoring in postoperative Crohn's disease. Journal of Crohn's and Colitis. 2018;12(6):653-61.

51. Wu KC, Ran ZH, Gao X, Chen M, Zhong J, Sheng JQ, et al. Adalimumab induction and maintenance therapy achieve clinical remission and response in Chinese patients with Crohn's disease. Intestinal research. 2016;14(2):152-63.

52. Roblin X, Williet N, Boschetti G, Phelip JM, Del Tedesco E, Berger AE, et al. Addition of azathioprine to the switch of anti-TNF in patients with IBD in clinical relapse with undetectable anti-TNF trough levels and antidrug antibodies: a prospective randomised trial. Gut. 2020;24:24.

53. Hibi T, Imai Y, Senoo A, Ohta K, Ukyo Y. Efficacy and safety of golimumab 52-week maintenance therapy in Japanese patients with moderate to severely active ulcerative colitis: a phase 3, double-blind, randomized, placebo-controlled study-(PURSUIT-J study). Journal of gastroenterology. 2017;52(10):1101-11.

54. Rutgeerts P, Feagan BG, Marano CW, Padgett L, Strauss R, Johanns J, et al. Randomised clinical trial: a placebo-controlled study of intravenous golimumab induction therapy for ulcerative colitis. Alimentary pharmacology & therapeutics. 2015;42(5):504-14.

55. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146(1):85-95; quiz e14-5.

56. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146(1):96-109.e1.

57. Adedokun O, Xu Z, Marano C, Strauss R, Zhang H, Johanns J, et al. Effects of immunomodulators on the pharmacokinetics and efficacy of golimumab in patients with moderately to severely active ulcerative colitis: Results from phase 2/3 pursuit-SC induction and maintenance studies. American Journal of Gastroenterology. 2013;108:S517.

58. Adedokun OJ, Xu Z, Marano CW, Strauss R, Zhang H, Johanns J, et al. Pharmacokinetics and exposure-response relationship of golimumab in patients with moderately to severely active ulcerative colitis: Results from phase2/3 induction and maintenance studies. Gastroenterology. 2013;1):S228-S9.

59. Adedokun OJ, Xu Z, Marano CW, Strauss R, Zhang H, Johanns J, et al. Pharmacokinetics and Exposure-response Relationship of Golimumab in Patients with Moderately-to-Severely Active Ulcerative Colitis: Results from Phase 2/3 PURSUIT Induction and Maintenance Studies. Journal of Crohn's & colitis. 2017;11(1):35-46.

60. Sandborn WJ, Rutgeerts P, Zhang H, Adedokun OJ, Xu S, Shraim R, et al. P446 Characterization of ulcerative colitis patients in the Golimumab PURSUIT-Maintenance study: post-hoc analyses of patients who maintained and did not maintain clinical response through week 54. Journal of Crohn's and Colitis. 2017;11(suppl_1):S304-S5.

61. Reinisch W, Gibson PR, Sandborn WJ, Feagan BG, Strauss R, Johanns J, et al. Long-Term Benefit of Golimumab for Patients with Moderately to Severely Active Ulcerative Colitis: Results from the PURSUIT-Maintenance Extension. Journal of Crohn's and Colitis. 2018;12(9):1053-66.

62. Adedokun OJ, Gunn GR, Leu JH, Gargano C, Xu Z, Sandborn WJ, et al. Immunogenicity of Golimumab and its Clinical Relevance in Patients With Ulcerative Colitis. Inflamm Bowel Dis. 2019;25(9):1532-40.

63. Sandborn WJ, Feagan BG, Stoinov S, Honiball PJ, Rutgeerts P, Mason D, et al. Certolizumab pegol for the treatment of Crohn's disease. N Engl J Med. 2007;357(3):228-38.

64. Sandborn W, Khanna R, Dubinsky M, Arendt CH, Kosutic G, Oliver RE, et al. The effect of anti-drug antibodies on adverse events profile in patients with crohn's disease treated with certolizumab pegol: Results of an integrated safety analysis from clinical trials. Gastroenterology. 2015;1):S231-S2.

65. Sandborn WJ, Schreiber S, Hanauer SB, Colombel JF, Bloomfield R, Lichtenstein GR, et al. Reinduction with certolizumab pegol in patients with relapsed Crohn's disease: results from the PRECiSE 4 Study. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2010;8(8):696-702 e1.

66. Sandborn W, Dubinsky M, Kosutic G, Parker G, Spearman M, Hasan I, et al. Incidence of anti-drug antibodies in crohn's disease patients during 5 years of certolizumab pegol therapy. Inflammatory Bowel Diseases. 2016;22:S41.

67. Sandborn WJ, Schreiber S, Feagan BG, Rutgeerts P, Younes ZH, Bloomfield R, et al. Certolizumab pegol for active Crohn's disease: a placebo-controlled, randomized trial. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2011;9(8):670-8.e3.

68. Sandborn WJ, Wolf DC, Kosutic G, Parker G, Schreiber S, Lee SD, et al. Effects of Transient and Persistent Anti-drug Antibodies to Certolizumab Pegol: Longitudinal Data from a 7-Year Study in Crohn's Disease. Inflamm Bowel Dis. 2017;23(7):1047-56.

69. Lichtenstein GR, Thomsen OO, Schreiber S, Lawrance IC, Hanauer SB, Bloomfield R, et al. Continuous therapy with certolizumab pegol maintains remission of patients with Crohn's disease for up to 18 months. Clinical Gastroenterology & Hepatology. 2010;8(7):600-9.

70. Schreiber S, Rutgeerts P, Fedorak RN, Khaliq-Kareemi M, Kamm MA, Boivin M, et al. A randomized, placebo-controlled trial of certolizumab pegol (CDP870) for treatment of Crohn's disease. Gastroenterology. 2005;129(3):807-18.

71. Schreiber S, Khaliq-Kareemi M, Lawrance IC, Thomsen OO, Hanauer SB, McColm J, et al. Maintenance therapy with certolizumab pegol for Crohn's disease. N Engl J Med. 2007;357(3):239-50.

72. Feagan BG, Greenberg GR, Wild G, Fedorak RN, Pare P, McDonald JW, et al. Treatment of ulcerative colitis with a humanized antibody to the alpha4beta7 integrin. N Engl J Med. 2005;352(24):2499-507.

73. Feagan BG, Greenberg GR, Wild G, Fedorak RN, Pare P, McDonald JW, et al. Treatment of active Crohn's disease with MLN0002, a humanized antibody to the alpha4beta7 integrin. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2008;6(12):1370-7.

74. Feagan BG, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2013;369(8):699-710.

75. Rosario M, Dirks NL, Gastonguay MR, Fasanmade AA, Wyant T, Parikh A, et al. Population pharmacokinetics-pharmacodynamics of vedolizumab in patients with ulcerative colitis and Crohn's disease.[Erratum appears in Aliment Pharmacol Ther. 2015 Nov;42(9):1135; PMID: 26427757]. Alimentary pharmacology & therapeutics. 2015;42(2):188-202.

76. Feagan B, Siegel CA, Melmed G, Isaacs K, Lasch K, Rosario M, et al. Efficacy of vedolizumab maintenance therapy with and without continued immunosuppressant use in GEMINI 1 and GEMINI 2. American Journal of Gastroenterology. 2015;110:S791.

77. Feagan BG, Rubin DT, Danese S, Vermeire S, Abhyankar B, Sankoh S, et al. Efficacy of Vedolizumab Induction and Maintenance Therapy in Patients With Ulcerative Colitis, Regardless of Prior Exposure to Tumor Necrosis Factor Antagonists. Clinical Gastroenterology & Hepatology. 2017;15(2):229-39.e5.

78. Loftus EV, Colombel JF, Feagan BG, Vermeire S, Sandborn WJ, Sands BE, et al. Long-term efficacy of vedolizumab for ulcerative colitis. Journal of Crohn's and Colitis. 2017;11(4):400-11.

79. Wyant T, Lirio RA, Yang L, Rosario M. Long-Term Immunogenicity of Intravenous Vedolizumab in Ulcerative Colitis and Crohn's Disease (Gemini Program). Gastroenterology. 2019;156 (6 S1):S-1107.

80. Motoya S, Watanabe K, Ogata H, Kanai T, Matsui T, Suzuki Y, et al. Vedolizumab in Japanese patients with ulcerative colitis: a Phase 3, randomized, double-blind, placebo-controlled study. PloS one. 2019;14(2):e0212989.

81. Parikh A, Leach T, Wyant T, Scholz C, Sankoh S, Mould DR, et al. Vedolizumab for the treatment of active ulcerative colitis: a randomized controlled phase 2 dose-ranging study. Inflamm Bowel Dis. 2012;18(8):1470-9.

82. Parikh A, Fox I, Leach T, Xu J, Scholz C, Patella M, et al. Long-term clinical experience with vedolizumab in patients with inflammatory bowel disease. Inflammatory Bowel Diseases. 2013;19(8):1691-9.

83. Sandborn WJ, Feagan BG, Rutgeerts P, Hanauer S, Colombel JF, Sands BE, et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. N Engl J Med. 2013;369(8):711-21.

84. Vermeire S, Loftus EV, Colombel JF, Feagan BG, Sandborn WJ, Sands BE, et al. Long-term Efficacy of Vedolizumab for Crohn's Disease. Journal of Crohn's & colitis. 2017;11(4):412‐24.

85. Sandborn WJ, Baert F, Danese S, Krznaric Z, Kobayashi T, Yao X, et al. Efficacy and Safety of Vedolizumab Subcutaneous Formulation in a Randomized Trial of Patients With Ulcerative Colitis. Gastroenterology. 2020;158(3):562-72.e12.

86. Sandborn WJ, Baert F, Danese S, Krznaric Z, D'Haens G, Kobayashi T, et al. Efficacy and safety of vedolizumab subcutaneous formulation for ulcerative colitis: Results of the visible trial. Gut. 2019;68 (Supplement 2):A60.

87. 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 had failed. Gastroenterology. 2014;147(3):618-27 e3.

88. Watanabe K, Motoya S, Ogata H, Kanai T, Matsui T, Suzuki Y, et al. Effects of vedolizumab in Japanese patients with Crohn's disease: a prospective, multicenter, randomized, placebo-controlled Phase 3 trial with exploratory analyses. Journal of gastroenterology. 2020;55(3):291-306.

89. Ghosh S, Goldin E, Gordon FH, Malchow HA, Rask-Madsen J, Rutgeerts P, et al. Natalizumab for active Crohn's disease. N Engl J Med. 2003;348(1):24-32.

90. Gordon FH, Lai CW, Hamilton MI, Allison MC, Srivastava ED, Fouweather MG, et al. A randomized placebo-controlled trial of a humanized monoclonal antibody to alpha4 integrin in active Crohn's disease. Gastroenterology. 2001;121(2):268-74.

91. Gordon FH, Hamilton MI, Donoghue S, Greenlees C, Palmer T, Rowley-Jones D, et al. A pilot study of treatment of active ulcerative colitis with natalizumab, a humanized monoclonal antibody to alpha-4 integrin. Alimentary pharmacology & therapeutics. 2002;16(4):699-705.

92. Sandborn WJ, Colombel JF, Enns R, Feagan BG, Hanauer SB, Lawrance IC, et al. Natalizumab induction and maintenance therapy for Crohn's disease. N Engl J Med. 2005;353(18):1912-25.

93. Targan SR, Feagan BG, Fedorak RN, Lashner BA, Panaccione R, Present DH, et al. Natalizumab for the treatment of active Crohn's disease: results of the ENCORE Trial. Gastroenterology. 2007;132(5):1672-83.

94. Sands BE, Kozarek R, Spainhour J, Barish CF, Becker S, Goldberg L, et al. Safety and tolerability of concurrent natalizumab treatment for patients with Crohn's disease not in remission whil receiving infliximab. Inflammatory Bowel Disease. 2007;13:2-11.

95. Sands BE, Sandborn WJ, Panaccione R, O'Brien CD, Zhang H, Johanns J, et al. Ustekinumab as induction and maintenance therapy for ulcerative colitis. New England Journal of Medicine. 2019;381(13):1201-14.

96. Adedokun OJ, Xu Z, Marano C, O'Brien C, Szapary P, Zhang H, et al. Ustekinumab Pharmacokinetics and Exposure Response in a Phase 3 Randomized Trial of Patients With Ulcerative Colitis: Ustekinumab PK and exposure-response in UC. Clinical Gastroenterology & Hepatology. 2019;06:06.

97. Adedokun OJ, Xu Z, Marano C, O'Brien C, Szapary P, Zhang H, et al. Pharmacokinetics and exposure-response relationships of ustekinumab in patients with ulcerative colitis: Results from the UNIFI induction and maintenance studies. American Journal of Gastroenterology. 2019;114 (Supplement):S481-S2.

98. Feagan BG, Sandborn WJ, Gasink C, Jacobstein D, Lang Y, Friedman JR, et al. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. New England Journal of Medicine. 2016;375(20):1946-60.

99. Hibi T, Imai Y, Murata Y, Matsushima N, Zheng R, Gasink C. Efficacy and safety of ustekinumab in Japanese patients with moderately to severely active Crohn's disease: a subpopulation analysis of phase 3 induction and maintenance studies. Intestinal research. 2017;15(4):475-86.

100. Sandborn W, Gasink C, Blank M, Lang Y, Johanns J, Gao LL, et al. A multicenter, double-blind, placebo-controlled phase3 study of ustekinumab, a human IL-1<sup>-/-</sup>3p40 mab, in moderate-service Crohn's disease refractory to anti-TFNalpha: UNITI-1. Inflammatory Bowel Diseases. 2016;22:S1.

101. Sandborn WJ, Rutgeerts P, Gasink C, Jacobstein D, Zou B, Johanns J, et al. Long-term efficacy and safety of ustekinumab for Crohn's disease through the second year of therapy. Alimentary pharmacology & therapeutics. 2018;48(1):65-77.

102. Sandborn WJ, Sands BE, Nussbaum J, Oortwijn A, Gasink C, Jacobstein D, et al. Immunogenicity of Ustekinumab in Patients with Crohn's Disease: Results from the Im-Uniti Study. Gastroenterology. 2019;156 (6 S1):S-1097.

103. Ghosh S, Kramer BC, Gasink C, Jacobstein D, Adedokun OJ, Gao LL, et al. Long-term efficacy of ustekinumab with and without concomitant immunosuppressants for Crohn's disease: Results from IM-UNITI long-term extension through 2 years. Journal of Crohn's and Colitis. 2019;13 (Supplement 1):S459-S60.

104. Hanauer SB, Sandborn WJ, Feagan BG, Gasink C, Jacobstein D, Zou B, et al. IM-UNITI: Three-year Efficacy, Safety, and Immunogenicity of Ustekinumab Treatment of Crohn's Disease. Journal of Crohn's & colitis. 2020;14(1):23-32.

105. 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;135(4):1130-41.

106. Sandborn WJ, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C, et al. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. N Engl J Med. 2012;367(16):1519-28.

107. Rutgeerts PJ, Fedorak RN, Hommes DW, Sturm A, Baumgart DC, Bressler B, et al. A randomised phase I study of etrolizumab (rhuMAb beta7) in moderate to severe ulcerative colitis. Gut. 2013;62(8):1122-30.

108. Vermeire S, O'Byrne S, Keir M, Williams M, Lu TT, Mansfield JC, et al. Etrolizumab as induction therapy for ulcerative colitis: a randomised, controlled, phase 2 trial. Lancet. 2014;384(9940):309-18.

109. Sandborn WJ, Abreu MT, D'Haens G, Colombel JF, Vermeire S, Mitchev K, et al. Certolizumab pegol in patients with moderate to severe Crohn's disease and secondary failure to infliximab. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2010;8(8):688-95.e2.

110. Sandborn W, Dubinsky M, Kosutic G, Parker G, Spearman M, Hasan I, et al. P-101 Incidence of Anti-drug Antibodies in Crohn's Disease Patients During 5 Years of Certolizumab Pegol Therapy. Inflammatory Bowel Diseases. 2016;22(suppl_1):S41-S.

# Chapter 9

## Patterns of anti-TNF use and associated treatment outcomes in inflammatory bowel disease patients: results from an analysis of Dutch health insurance claims data

S. Bots*, D. Hoekman*, M. Benninga, C. Ponsioen, G. D'Haens, M. Löwenberg

*contributed equally

*Neth J Med 2017;75(10):432-442*

## Abstract

### Introduction

Real-life patterns of anti-TNF use remain largely unknown. We aimed to investigate: survival rates, clinical outcomes and costs of anti-TNF agents in a large population of IBD patients.

### Methods

Health insurance data from 22,082 IBD patients were provided by Achmea Healthcare. Time to anti-TNF discontinuation, treatment intensification, corticosteroid initiation and hospitalization were analyzed in patients starting with anti-TNF treatment from January 2008 till December 2014. Treatment regimens were analyzed at different time points.

### Results

In this cohort, 855 and 1,199 subjects started infliximab and adalimumab treatment, respectively. The median time to anti-TNF discontinuation was 600 days (IQR 156–1693). The proportion of subjects receiving intensified treatment increased over time (infliximab at 3 vs. 24 months: 22.2% vs. 33.6%, p=0.01; adalimumab at 3 vs. 24 months: 10.5% vs. 19.3%, p<0.001). Cessation of anti-TNF treatment was less common in Crohn's disease patients (HR 0.79, p=0.001) and in patients receiving intensified treatment (HR 0.62, p=0.001). Immunomodulator use was associated with longer time to corticosteroid initiation (HR 0.80, p=0.048), but not with longer drug survival (HR 0.99, p=0.617). Hospitalization was more common in Crohn's disease patients (HR 1.49, p=0.011). Corticosteroid initiation was lower in Crohn's disease patients (HR 0.57, p<0.001) and in patients using infliximab (HR 0.55, p<0.001).

### Conclusions

Discontinuation of anti-TNF therapy occurred earlier than previously reported and was associated with a diagnosis of ulcerative colitis and non-intensified anti-TNF treatment. Immunomodulator use at the start of anti-TNF treatment was associated with longer time to corticosteroid inititiation, but not with longer drug survival.

## Introduction

The introduction of anti-tumor necrosis factor (anti-TNF) antibodies has revolutionized the therapy of Crohn's disease and ulcerative colitis, collectively known as inflammatory bowel disease (IBD). Anti-TNF agents are able to induce and maintain remission in IBD patients.[1–6] Infliximab was registered in the Netherlands for Crohn's disease in 1999 and for ulcerative colitis in 2006 and adalimumab was registered in the Netherlands for Crohn's disease and ulcerative colitis in 2007 and 2012, respectively.

The clinical management of IBD patients with anti-TNF agents is complicated by primary and secondary non-response. Approximately 30% of patients do not respond to anti-TNF induction therapy (primary non-responders)[1–7], and up to half of initial responders will gradually lose response over time (secondary non-responders).[1,8–11] Primary and secondary non-response are related to low serum drug concentrations and the development of anti-drug-antibodies.[12–19] The proportion of IBD patients with a durable response to anti-TNF treatment in a real-life setting has been investigated in relatively small cohorts.[10,11]

Several strategies are used to prevent and treat primary and secondary non-response to anti-TNF agents. Firstly, combination therapy (consisting of an anti-TNF agents combined with an immunomodulator) is more effective compared to anti-TNF monotherapy[20], which can (at least partly) be explained by reduced anti-drug antibody formation.[21] Secondly, loss of response can often be managed by increasing the dose and/or decreasing the dosing interval of anti-TNF agents.[22] Thirdly, loss of response to anti-TNF agents, especially when this is related to anti-drug-antibody formation, can be overcome by switching to another anti-TNF agent[22], or by adding an immunomodulator if a patient receives anti-TNF monotherapy.

It is unknown how many IBD patients receive combination therapy and how often anti-TNF treatment is intensified in daily practice. Furthermore, associated treatment outcomes and drug costs of anti-TNF agents in a large real-life population are relatively unknown. Van der valk et al. studied IBD health care and medication costs in a Dutch cohort of 2252 patients in 201123. Bernstein et al. assessed costs of IBD management in a large real-life Canadian cohort in 2005 and 2006, but they did not specifically focus on anti-TNF use and related treatment outcomes.[24]

The aim of the present study was to investigate: (i) drug survival rates of anti-TNF agents, (ii) clinical outcomes of anti-TNF therapy, and (iii) drug costs of TNF blockers in a large population consisting of approximately 22,000 Dutch IBD patients.

## Methods

*Database*

Health insurance claims data were provided by Achmea Healthcare, the largest health insurance provider in the Netherlands. Data were available from 2008 to 2014 on approximately 2.7 million insured persons in 2008, gradually increasing to approximately 4.2 million insured persons in 2014. This population is a representative sample of the urbanized area of the Netherlands.[25]

*Data collection*

The following data were collected from subjects who received IBD-related health care between 2008 and 2014 (observation period).

Background information: year of birth, gender, number of days insured by Achmea per year, year of death (if applicable), start and stop date of the insured period.

IBD-related health care: diagnosis (Crohn's disease or ulcerative colitis) and treatment setting (inpatient or outpatient).

IBD-related medication use: administration/dispensation date, dose and costs of infliximab, adalimumab, corticosteroids [prednisone or budesonide], thiopurines [azathioprine, 6-mercaptopurine or 6-tioguanine] and methotrexate. Data on infliximab use was available from 2012 to 2014 due to a different reimbursement system before 2012. Prior to 2012, infliximab costs were reimbursed as part of hospital care, thus treatment details were not specified in healthcare claims before 2012. As from 2012, infliximab costs are directly reclaimed by pharmacies based on specific dosages and dispensation dates.

Comorbidity: documented healthcare claims for psoriatic arthritis, ankylosing spondylitis, psoriasis and rheumatoid arthritis.

*Outcomes*

The primary outcome was anti-TNF drug survival (i.e. time from start of anti-TNF therapy to discontinuation). Secondary outcomes included time to anti-TNF dose intensification, time to corticosteroid initiation and time to IBD-related hospitalization in anti-TNF starters and analysis of potential determinants for time to drug discontinuation, treatment intensification, hospitalization, and corticosteroid initiation. Moreover, treatment intervals, dosing regimens and drug costs of anti-TNF therapy were analyzed.

*Classifications, definitions, calculations and selection criteria*

All analyses were performed on patients aged ≥ 18 years at the end of the observation period. Patients who received their first infliximab infusion > 16 weeks after start of the observation period were considered as infliximab starters. Patients who received their first pharmacy dispensation of adalimumab > 6 months after start of the observation period were considered as adalimumab starters. These cut-offs were based on the assumption that infliximab intervals are unlikely to exceed a 16-week period and that the amount of dispensed adalimumab vials is unlikely to cover a treatment period longer than 6 months.

In order to distinguish between patients starting with anti-TNF monotherapy or combination therapy, pharmacy dispensations of immunomodulators and anti-TNF agents were divided into semesters. Anti-TNF starters receiving a prescription for an immunomodulator in the first semester of anti-TNF treatment were defined as patients using combination therapy.

An infliximab dose adaptation was defined as a dose increase or decrease of at least 50 mg and/or an increase or decrease in the treatment interval between two infusions of ≥ 25%. Infliximab discontinuation was defined as a definitive treatment stop or an infusion interval of > 16 weeks. Infliximab restart was defined as at least one infliximab infusion after treatment discontinuation.

Adalimumab dosing regimens were based on the average amount of adalimumab provided at each dispensation (amount dispensed in mg divided by the time until next dispensation). Adalimumab dosing regimens were categorized into < 40 mg every other week, 40 mg every other week, 40 mg every week and > 40 mg every week based on the following cut-offs: < 15 mg per week, 15-30 mg per week, 30-60 mg per week and > 60 mg per week, respectively. Adalimumab dose adaptations were defined as a change in dosing regimen category that was maintained for at least 2 consecutive dispensations. Adalimumab discontinuation was defined as a definite treatment stop or when the average amount of adalimumab that was dispensed by the pharmacy was < 10 mg per week. Adalimumab restart was defined as at least one adalimumab dispensation after discontinuation.

Time to drug discontinuation, treatment intensification, hospitalization, out and in-hospital corticosteroid initiation (prednisone and budesonides) were analyzed in all patients who started with anti-TNF therapy within the observation period. For all survival analyses, patients were censored on December 31th 2014, at time of death or at the time of an interruption of the insured period (i.e. if patients switched to another health insurance provider). In order to analyze time to corticosteroid initiation, hospitalization and treatment intensification, patients were also censored at the time of anti-TNF discontinuation.

Corticosteroid use during anti-TNF induction therapy (4 weeks for adalimumab and 6 weeks for infliximab) was used as a cut-off point for analyzing time to corticosteroid initiation.

Because the definitions of infliximab and adalimumab treatment intensification were not comparable, time to treatment intensification was analyzed for both agents separately.

Average anti-TNF treatment intervals and dosages were determined in patients who started with anti-TNF treatment during the observation period at 3, 6, 12 and 24 months after treatment initiation if they were not censored and still receiving the same anti-TNF agent. Mean infliximab dose relative to body weight was estimated using an average body weight of Dutch men and women of 70 kilogram.[26]

Drug costs of each anti-TNF dispensation were provided by Achmea. Total anti-TNF costs were calculated as the sum of all dispensations within the observation period and for each year separately.

*Statistical analysis*

All analyses were performed using SPSS 23.0 (IBM, Chicago, Illinois). Descriptive statistics were used to study cohorts characteristics. Observed periods are presented in person-years. Comparisons between groups of not normally distributed dichotomous data were performed using Fisher's exact tests. Survival data are presented as Kaplan-Meier curves. Univariate and multivariate analysis of time to event data was performed using Cox proportional hazards regression. The proportional hazards assumption was tested using visual inspection of log minus log survival plots. The threshold for statistical significance was set at $p < 0.05$.

*Ethical approval*

All provided data were completely anonymized. Data were requested and obtained through official procedures. Therefore, no ethical approval was required.

## Results

*Cohort characteristics*

A total of 22,082 patients that received IBD-related care between 2008 and 2014 were identified. The total observation period comprised 131,134 person-years. Cohort characteristics are provided in table 1. From 2008 to 2014, 1498 patients were treated with adalimumab, and 1,671 patients were treated with infliximab between 2012 and 2014. The proportion of patients receiving anti-TNF treatment increased from 17% in 2012 to 19.7% in 2014 (infliximab and adalimumab combined). In this period, 476 out of 2929 (16.3%) patients had received both infliximab and adalimumab. From 2008 to 2014, 24% of IBD patients receiving an anti-TNF agent also received care (indicated by a documented health insurance claim) for at least one other disease for which anti-TNF agents are indicated, such as psoriatic arthritis, ankylosing spondylitis, psoriasis or rheumatoid arthritis.

### Anti-TNF use

*Infliximab*

Of patients receiving IBD-related care, the proportion of patients that was treated with infliximab increased from 10.3% in 2012 to 11.3% in 2014. In these patients, yearly drug costs of infliximab treatment were €17.4 million in 2012, increasing to €19.7 million in 2014. At the start of infliximab therapy, the proportion of patients receiving combination therapy was 60.4%, of whom the vast majority received azathioprine (66.5%) or 6-mercaptopurine (26.6%). The proportion of patients receiving combination therapy was comparable in 2012 (59.0%), 2013 (61.0%) and 2014 (61.4%).

During the observation period, 20,252 infliximab infusions were administered. In total 855 patients (550 Crohn's disease, 305 ulcerative colitis) started with infliximab within the observation period. The distribution of infliximab administration intervals in these patients over time is shown in figure 1. The proportion of patients receiving infliximab maintenance treatment with an infusion interval between 7 and 9 weeks decreased with longer treatment duration (treatment interval between 7 and 9 weeks at 3 months vs. 24 months: 72.3% vs. 60.9%, p=0.02). The proportion of patients receiving infliximab with an infusion interval shorter than 7 weeks increased with longer treatment duration (treatment interval < 7 weeks at 3 months vs. 24 months: 22.2% vs. 33.6%, p=0.01). No clinical factors were significantly associated with time to infliximab intensification (i.e. decreased infusion intervals) in univariable and multivariable analysis (table 2). No change in mean infliximab dose per kg bodyweight was observed over time (3 months vs. 24 months: 5.8 [SD 1.8] vs. 5.7 [SD 2.1], p=0.64).

**Table 1.** *Cohort characteristics.*

| | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 |
|---|---|---|---|---|---|---|---|
| Number of insured patients | 2,747,095 | 3,151,065 | 3,123,626 | 3,074,985 | 3,144,339 | 4,412,107 | 4,248,903 |
| Number of patients receiving IBD-related care | 7732 | 9166 | 9680 | 10090 | 10604 | 11227 | 10666 |
| CD (n,%*) | 3533 (45.7%) | 4218 (46.0%) | 4484 (46.3%) | 4637 (46.0%) | 4926 (46.5%) | 5189 (46.2%) | 4901 (45.9%) |
| UC (n,%*) | 4199 (54.3%) | 4948 (54.0%) | 5196 (53.7%) | 5453 (54.0%) | 5678 (53.5%) | 6038 (53.8%) | 5765 (54.1%) |
| Age* (mean, SD) | 46 (18) | 47 (17) | 47 (17) | 48 (17) | 49 (17) | 50 (17) | 51 (17) |
| Males (n, %*) | 3485 (45.1%) | 4166 (45.5%) | 4337 (44.8%) | 4514 (44.7%) | 4703 (44.4%) | 4992 (44.5%) | 4783 (44.8%) |
| Deceased (n, %*) | 87 (1.1%) | 111 (1.2%) | 119 (1.2%) | 149 (1.5%) | 182 (1.7%) | 198 (1.8%) | 189 (1.8%) |
| Receiving infliximab (n, %*) | N.A. | N.A. | N.A. | N.A. | 1093 (10.3%) | 1223 (10.9%) | 1206 (11.3%) |
| CD (n,%†) | N.A. | N.A. | N.A. | N.A. | 786 (16.0%) | 861 (16.6%) | 846 (17.3%) |
| UC (n,%‡) | N.A. | N.A. | N.A. | N.A. | 307 (5.4%) | 362 (6.0%) | 360 (6.2%) |
| Receiving adalimumab (n, %*) | 248 (3.2%) | 396 (4.3%) | 539 (5.6%) | 621 (6.2%) | 710 (6.7%) | 834 (7.4%) | 896 (8.4%) |
| CD (n,%†) | 221 (6.3%) | 356 (8.4%) | 479 (10.7%) | 534 (11.5%) | 591 (12.0%) | 682 (13.1%) | 720 (14.7%) |
| UC (n,%‡) | 27 (0.6%) | 40 (0.8%) | 60 (1.2%) | 87 (1.6%) | 119 (2.1%) | 152 (2.5%) | 176 (3.1%) |

** = of patients receiving IBD-related care; † = of CD patients; ‡ = of UC patients.*

*IBD: inflammatory bowel disease; CD: Crohn's disease; UC: ulcerative colitis; N.A.: data not available.*

*Adalimumab*

Of patients receiving IBD-related care, the proportion that was treated with adalimumab increased from 3.2% in 2008 to 8.4% in 2014. From 2008 to 2014, 121,406 adalimumab syringes were dispensed with a median of 60 (IQR 28 - 118) syringes per patient. Yearly drug costs of adalimumab treatment increased from €3.2 million in 2008 to €13 million in 2014. At the start of adalimumab treatment, the proportion of patients receiving combination therapy was 52.5%, of whom the vast majority received azathioprine (64.4%) or 6-mercaptopurine (18.1%). The proportion of patients starting adalimumab combined with an immunomodulator increased from 42.1% to 51.5% between 2008 and 2014.

A total of 1,199 subjects (940 Crohn's disease, 259 ulcerative colitis) started with adalimumab treatment within the observation period. The distribution of adalimumab administration intervals among these subjects over time is shown in figure 2. The proportion of patients receiving 40 mg adalimumab every other week decreased with longer treatment duration (at 3 months vs. 24 months: 81.5% vs. 71.2%, p<0.001),whereas the proportion of patients that received intensified adalimumab treatment (i.e. ≥ 40 mg every week) increased with longer treatment duration (at 3 months vs. 24 months: 10.5% vs. 19.3%, p<0.001). No clinical factors were significantly associated with time to adalimumab intensification in univariable and multivariable analysis (table 2).

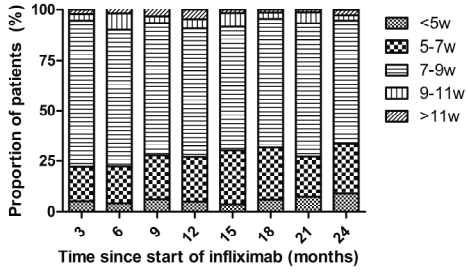



**Figure 1.** *Distribution of infliximab infusion intervals over time.*

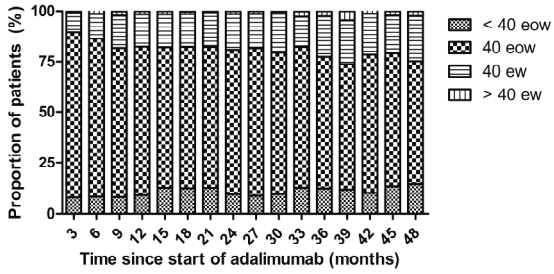



**Figure 2.** *Distribution of adalimumab treatment intervals over time.*

**Table 2.** *Univariable and multivariable Cox proportional hazards regression analysis of time to anti-TNF treatment intensification.*

| | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Adalimumab | | | | |
| Crohn's disease (vs ulcerative colitis) | 0.73 (0.43-1.24) | 0.246 | 0.72 (0.43-1.23) | 0.232 |
| Male gender | 1.06 (0.70-1.59) | 0.779 | 1.07 (0.71-1.61) | 0.743 |
| Combination treatment at initiation | 1.13 (0.75-1.69) | 0.560 | 1.15 (0.76-1.72) | 0.511 |
| Infliximab | | | | |
| Crohn's disease (vs ulcerative colitis) | 1.07 (0.72-1.57) | 0.746 | 1.10 (0.74-1.63) | 0.634 |
| Male gender | 1.39 (0.96-2.00) | 0.081 | 1.4 (0.97-2.03) | 0.073 |
| Combination treatment at initiation | 0.78 (0.54-1.13) | 0.192 | 0.78 (0.54-1.13) | 0.197 |

*HR: Hazard rate; CI: confidence interval*

## Drug survival

Median time to anti-TNF treatment discontinuation was 600 days (IQR 156 – 1693 days). At 6, 12 and 24 months after initiation of anti-TNF treatment, the proportion of patients receiving continuous treatment with anti-TNF agents was 72.5% (95% CI 70.5-74.5), 61.5% (95% CI 59.1-63.9) and 45.6% (95% CI 43.1-48.1), respectively. Univariable and multivariable analysis of factors associated with time to drug discontinuation are shown in table 3. Patients with Crohn's disease were less likely to stop anti-TNF treatment compared to ulcerative colitis patients (hazard ratio [HR] 0.79 [95% CI 0.69-0.91], p=0.001). Patients who received anti-TNF treatment intensification were less likely to discontinue their treatment (HR 0.62 [95% CI 0.47-0.82], p=0.001). A trend was observed towards a higher discontinuation rate in patients receiving infliximab compared to adalimumab (HR 1.14 [95% CI 0.99-1.34], p=0.071). Combination treatment at initiation of anti-TNF therapy was not associated with longer drug survival (HR 0.99 [96%CI 0.87-1.11]. Kaplan-Meier curves of time to anti-TNF discontinuation are shown in figure 3. The proportion of patients that restarted infliximab or adalimumab treatment within 6 months after discontinuation was 19.2% and 21.4%, respectively. The proportion of patients that restarted infliximab and adalimumab within 12 months after cessation of anti-TNF therapy, was 24.3% and 33.4%, respectively (supplementary figure 1 and 2).

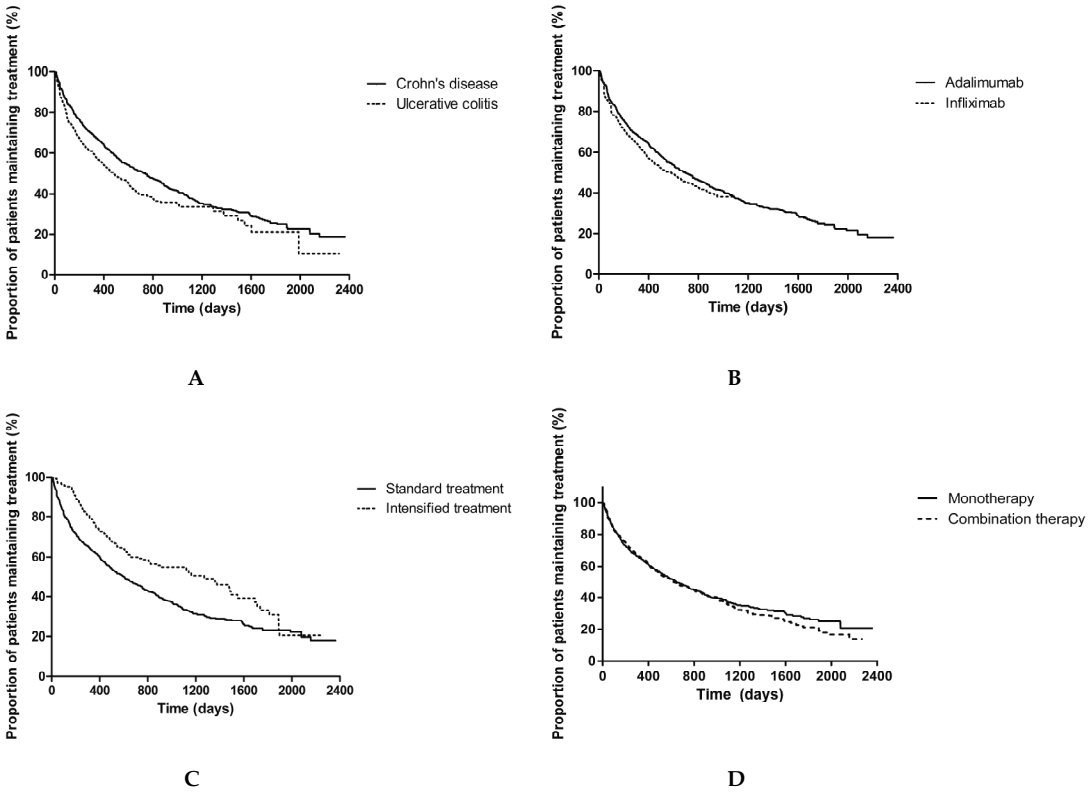



**Figure 3.** *Kaplan-Meier curves of time to anti-TNF treatment discontinuation: a. Crohn's disease vs. ulcerative colitis; b. adalimumab vs. infliximab; c. standard treatment vs. intensified treatment; d. monotherapy vs combinationtherapy.*

**Table 3.** *Univariable and multivariable Cox proportional hazards regression analysis of time to drug discontinuation.*

| | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Crohn's disease (vs ulcerative colitis) | 0.79 (0.68-0.89) | <0.001 | 0.79 (0.69-0.91) | 0.001 |
| Male gender | 0.92 (0.81-1.04) | 0.173 | 0.92 (0.81-1.04) | 0.172 |
| Infliximab (vs adalimumab) | 1.13 (0.99-1.28) | 0.065 | 1.14 (0.99-1.30) | 0.071 |
| Combination treatment at initiation | 1.00 (0.89-1.13) | 0.94 | 0.99 (0.87-1.11) | 0.617 |
| Treatment intensification | 0.61 (0.50-0.75) | <0.001 | 0.62 (0.47-0.82) | 0.001 |

*HR: Hazard rate; CI: confidence interval*

## IBD-related hospitalization

Among patients who started with anti-TNF treatment within the observation period, the cumulative proportion of patients that was hospitalized for IBD-related problems was 9.2% (95% CI 7.8-10.6), 13.7% (95% CI 11.9-15.5) and 19.8% (95% CI 17.3-22.3), at 6, 12 and 24 months, respectively. Univariable and multivariable analysis of factors associated with time to hospitalization is provided in table 4. Crohn's disease was the only factor that was significantly associated with hospitalization (HR 1.49 [95% CI 1.10-2.03], p=0.011). A Kaplan-Meier plot of time to hospitalization is shown in figure 4.

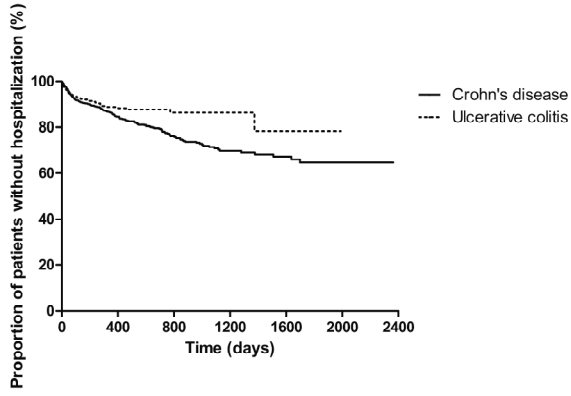



**Figure 4.** *Kaplan-Meier curves of time to hospitalization: Crohn's disease vs. ulcerative colitis.*

### Corticosteroid initiation

The cumulative proportion of patients receiving corticosteroids after initiation of anti-TNF treatment was 14.4% (95% CI 12.4-16.4), 19.2% (95% CI 16.8-21.6) and 27.2% (95%CI 24.1-30.3) at 6, 12 and 24 months, respectively. Univariable and multivariable analysis of factors associated with time to corticosteroid initiation is provided in table 5. Patients with Crohn's disease (HR 0.57 [95% CI 0.45-0.73] p<0.001) and patients receiving infliximab (HR 0.55 [95% CI 0.42-0.72] p<0.001) were less likely to receive treatment with corticosteroids. Patients who received combination therapy at time of initiation of anti-TNF treatment used significantly less corticosteroids as compared to patients receiving anti-TNF monotherapy (HR 0.80 [95% CI 0.64-1.00] p=0.048). Kaplan-Meier plots of time to corticosteroid initiation are depicted in figure 5.

**Table 4.** *Univariable and multivariable Cox proportional hazards regression analysis of time to hospitalization.*

| | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Crohn's disease (vs ulcerative colitis) | 1.46 (1.08-1.98) | 0.014 | 1.49 (1.10-2.03) | 0.011 |
| Male gender | 0.972 (0.77-1.23) | 0.812 | 0.98 (0.77-1.24) | 0.853 |
| Infliximab (vs adalimumab) | 1.04 (0.82-1.33) | 0.733 | 1.11 (0.86-1.43) | 0.419 |
| Combination treatment at initiation | 0.83 (0.66-1.05) | 0.127 | 0.83 (0.65-1.04) | 0.109 |
| Treatment intensification | 1.26 (0.92-1.72) | 0.151 | 1.24 (0.91-1.70) | 0.178 |

*HR: Hazard rate; CI: confidence interval*

**Table 5.** *Univariable and multivariable Cox proportional hazards regression analysis of time to corticosteroid initiation.*

| | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Crohn's disease (vs ulcerative colitis) | 0.61 (0.48-0.77) | <0.001 | 0.57 (0.45-0.73) | <0.001 |
| Male gender | 1.15 (0.92-1.44) | 0.221 | 1.13 (0.91-1.42) | 0.270 |
| Infliximab (vs adalimumab) | 0.59 (0.45-0.76) | <0.001 | 0.55 (0.42-0.72) | <0.001 |
| Combination treatment at initiation | 0.67 (0.51-0.87) | 0.002 | 0.80 (0.64-1.00) | 0.048 |
| Treatment intensification | 1.07 (0.78-1.46) | 0.673 | 1.12 (0.82-1.53) | 0.479 |

*HR: Hazard rate; CI: confidence interval*

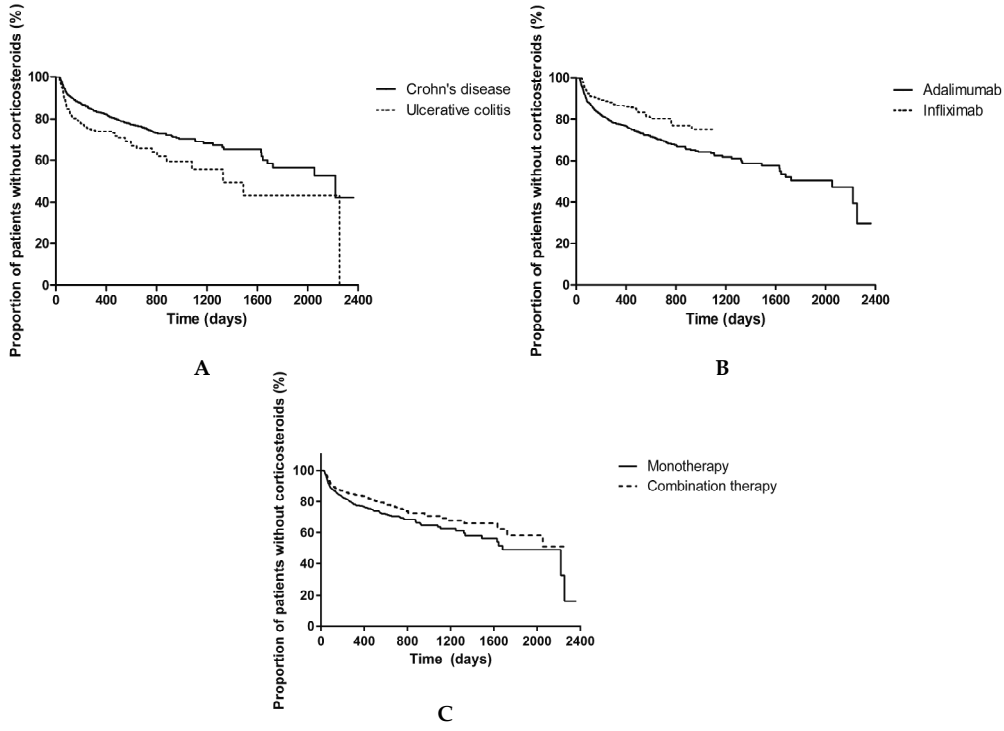



**Figure 5.** *Kaplan-Meier curves of time to corticosteroid use: a. Crohn's disease vs. ulcerative colitis; b. adalimumab vs. infliximab; c. Anti-TNF monotherapy vs combination therapy (i.e. anti-TNF combined with an immunomodulator) at anti-TNF initiation.*

## Discussion

To our knowledge, this study is the first to describe real life patterns of anti-TNF use and associated treatment outcomes in a large IBD population in the Netherlands. In this cohort of more than 22,000 Crohn's disease and ulcerative colitis patients, the proportion of patients receiving infliximab or adalimumab increased to approximately 20% in 2014 which accounted for €32.7 million of drug costs. However, it is important to note that drug costs of anti-TNF therapy decreased substantially after the introduction of biosimilar infliximab in 2015 in The Netherlands. Anti-TNF discontinuation occurred in approximately 40% of patients within the first year after treatment initiation and this was associated with a diagnosis of ulcerative colitis, infliximab use and non-intensified anti-TNF treatment regimens. Thus, we here show that the real-life anti-TNF discontinuation rate is substantially higher than previously reported. Moreover, we demonstrate that the proportion of IBD patients receiving intensified anti-TNF treatment gradually increases over time.

Anti-TNF discontinuation rates range from 5% to 23% at 12 months of follow-up according to different studies.[8,27] There are several potential explanations for these differences. Firstly, most of these studies concern analyses of clinical trials and tertiary care cohorts which may not provide reliable estimates of real-life drug survival.[8,27,10,11] Furthermore, early discontinuation (due to primary non-response or intolerance) may not have been included in these estimates. On the other hand, we may have overestimated the discontinuation rate due to the definitions that were applied. These definitions could not account for poor treatment adherence, short drug holidays or episodic treatment strategies. This may also have contributed to a higher proportion of patients restarting the same anti-TNF agent within 12 months (24% and 33% for infliximab and adalimumab, respectively). However, we presume that the number of patients receiving episodic treatment with TNF blockers is very low in this cohort, since it is well known that scheduled continuous treatment is the preferred treatment strategy.

Strikingly, immunomodulator use at the start of anti-TNF treatment was not associated with a longer drug survival or time to anti-TNF intensification. This is an unexpected finding because combination therapy appears to be more effective than either therapeutic agent alone, explained by reduced immunogenicity, increased anti-TNF serum levels and possible synergistic effects.[20,28] Nevertheless, several previous studies also found no significant association between time to anti-TNF intensification and concomitant immunomodulator use.[29,30,31] We hypothesize that patients with more severe disease are more likely to receive combination therapy. Consequently, the potential beneficial effect of combination therapy may be neutralized by the patients' poorer prognosis. Furthermore, some patients in our cohort could have been misclassified as patients who started with combination therapy. We defined combination therapy at the time of anti-TNF initiation as a pharmacy dispensation of an immunomodulator in the same semester as the first anti-TNF administration. As a result, some patients may have already discontinued the immunomodulator prior to anti-TNF initiation. However, we did find a significantly longer time to corticosteroid initiation in

patients on combination therapy as compared to anti-TNF monotherapy, which reflects the beneficial effect of concomitant immunomodulator use.

A diagnosis of ulcerative colitis was associated with a shorter time to anti-TNF discontinuation and corticosteroid initiation. This could reflect lower response rates to anti-TNF agents in ulcerative colitis compared to Crohn's disease patients. Although head-to-head studies are lacking, it has been suggested that anti-TNF agents may be more effective in Crohn's disease as compared to ulcerative colitis.[32] In line with this notion, previous studies have found higher rates of anti-TNF treatment intensification in ulcerative colitis compared to Crohn's disease patients.[29,30,31] A possible explanation for this difference is a higher inflammatory burden and a higher drug clearance in ulcerative colitis patients.[19,29,33] However, we did not find a significant association between time to treatment intensification and a diagnosis of ulcerative colitis. Furthermore, other studies found no difference in time to infliximab discontinuation between Crohn's disease and ulcerative colitis.[29]

It is currently unclear if infliximab or adalimumab is superior for the treatment of IBD because head to head studies are lacking. Results from meta-analyses and several studies show conflicting results.[34–41] A population study in IBD patients showed no difference in efficacy between these two agents.[40] In our study, infliximab use was associated with a reduced risk of corticosteroid initiation compared to adalimumab. However, the difference in cut-off point between adalimumab and infliximab that we used for time to corticosteroid initiation (an induction period of 4 and 6 weeks, respectively) might have influenced the results. Nevertheless, this finding has also been reported previously in another administrative claims database study that consisted of 1,400 ulcerative colitis patients starting anti-TNF therapy.[35] Furthermore, no difference in time to hospitalization was found, and a trend towards a higher drug discontinuation rate was seen in infliximab users compared to adalimumab. We postulate that the small difference in discontinuation rate could be explained by the fact that IBD patients with severe disease requiring hospitalization are more likely to receive treatment with infliximab.[42] Disease severity at the start of anti-TNF treatment could not be assessed in our database, which can cause potential bias for the comparison of both agents. Hence, based on our results we cannot draw firm conclusions with regard to differences in therapeutic efficacy between infliximab and adalimumab. An ongoing study will determine if higher induction and maintenance doses of adalimumab will improve the outcome in ulcerative colitis patients (NCT02065622).

The present study cohort is a representative sample of the Dutch IBD population, consisting of both second and third line patients. More than 22,000 IBD patients receiving IBD-related care between 2008 and 2014 were included. Long-term data from large population based cohorts allow for robust analyses of patterns of drug use. However, this study has several limitations. Firstly, adalimumab use was based on the amount of drug that was dispensed by pharmacists to patients. Therefore, actual drug use, premature drug discontinuation, therapeutic compliance and variation in drug dispense rates because of logistical reasons (such

as lost drug) could not be assessed. Secondly, the definitions that were used for patient selection and classification (such as selection of anti-TNF starters, anti-TNF discontinuation, combination therapy, treatment intensification and corticosteroid initiation) may have resulted in selection bias. Thirdly, relevant clinical information such as disease location, behaviour and severity or reasons for anti-TNF discontinuation and corticosteroid initiation could not be obtained. In addition, surgical interventions could not be evaluated since detailed data on IBD related surgery was not available. Furthermore, the Dutch health insurance claims system does not allow for a diagnosis of IBD-unspecified. Consequently, all IBD cases were categorized as either Crohn's disease or ulcerative colitis, while approximately 8% of the Dutch IBD population is diagnosed with IBD-unspecified.[43] Despite these limitations, this study contributes to the knowledge on the use of anti-TNF agents and is the first to describe patterns of anti-TNF use in a large real-life population in the Netherlands.

In conclusion, the proportion of IBD patients receiving anti-TNF treatment increased to almost 20% in 2014, which is a major cost driver. Discontinuation of anti-TNF agents appears to occur earlier than previously reported, which was associated with a diagnosis of ulcerative colitis and non-intensified anti-TNF treatment regimens, but not with combination therapy. However, immunomodulator use at the start of anti-TNF treatment was associated with a longer time to corticosteroid initiation.

## References


1. Peyrin-Biroulet L, Deltenre P, de Suray N, et al. Efficacy and safety of tumor necrosis factor antagonists in Crohn's disease: meta-analysis of placebo-controlled trials. Clin Gastroenterol Hepatol 2008; 6: 644–53.

2. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005; 353: 2462–76.

3. Song Y-N, Zheng P, Xiao J-H, et al. Efficacy and safety of adalimumab for the Crohn's disease: a systematic review and meta-analysis of published randomized placebo-controlled trials. Eur J Clin Pharmacol 2014; 70: 907–14.

4. Thorlund K, Druyts E, Mills EJ, et al. Adalimumab versus infliximab for the treatment of moderate to severe ulcerative colitis in adult patients naïve to anti-TNF therapy: an indirect treatment comparison meta-analysis. J Crohns Colitis 2014; 8: 571–81.

5. Löwenberg M, de Boer NK, Hoentjen F. Golimumab for the treatment of ulcerative colitis. Clin Exp Gastroenterol 2014; 7: 53–59.

6. Ben-Bassat O, Iacono A, Irwin SP, et al. Tu1327a Golimumab for Treatment of Moderate to Severe Anti-TNF Refaractory Crohn's Disease: Open Label Experience. Gastroenterology 2012; 142: S-804.

7. Volonaki E, Mutalib M, Kiparissi F, et al. Adalimumab as a second-line biological therapy in children with refractory ulcerative colitis. Eur J Gastroenterol Hepatol 2015; 27: 1425–8.

8. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther 2011; 33: 987–95.

9. Ma C, Huang V, Fedorak DK, et al. Outpatient Ulcerative Colitis Primary Anti-TNF Responders Receiving Adalimumab or Infliximab Maintenance Therapy Have Similar Rates of Secondary Loss of Response. J Clin Gastroenterol 2015; 49: 675–82.

10. Peters CP, Eshuis EJ, Toxopeüs FM, et al. Adalimumab for Crohn's disease: long-term sustained benefit in a population-based cohort of 438 patients. J Crohns Colitis 2014; 8: 866–75.

11. Eshuis EJ, Peters CP, van Bodegraven AA, et al. Ten Years of Infliximab for Crohn's Disease. Inflamm Bowel Dis 2013; 19: 1622–1630.

12. Ainsworth MA, Bendtzen K, Brynskov J. Tumor necrosis factor-alpha binding capacity and anti-infliximab antibodies measured by fluid-phase radioimmunoassays as predictors of clinical efficacy of infliximab in Crohn's disease. Am J Gastroenterol 2008; 103: 944–8.

13. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003; 348: 601–8.

14. Ben-Horin S, Yavzori M, Katz L, et al. The immunogenic part of infliximab is the F(ab')2, but measuring antibodies to the intact infliximab molecule is more clinically useful. Gut 2011; 60: 41–8.

15. Hanauer SB, Wagner CL, Bala M, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. Clin Gastroenterol Hepatol 2004; 2: 542–53.

16. Karmiris K, Paintaud G, Noman M, et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. Gastroenterology 2009; 137: 1628–40.

17. Maser EA, Villela R, Silverberg MS, et al. Association of Trough Serum Infliximab to Clinical Outcome After Scheduled Maintenance Treatment for Crohn's Disease. Clin Gastroenterol Hepatol 2006; 4: 1248–1254.

18. Seow CH, Newman A, Irwin SP, et al. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut 2010; 59: 49–54.

19. Brandse JF, Mathôt RA, van der Kleij D, et al. Pharmacokinetic Features and Presence of Antidrug Antibodies Associate With Response to Infliximab Induction Therapy in Patients With Moderate to Severe Ulcerative Colitis. Clin Gastroenterol Hepatol 2016; 14: 251–258.e2.

20. Colombel JFF, Sandborn WJ, Reinisch W, et al. Infliximab, Azathioprine, or Combination Therapy for Crohn's Disease. N Engl J Med 2010; 362: 1383–1395.

21. Vermeire S, Noman M, Van Assche G, et al. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. Gut 2007; 56: 1226–31.

22. Afif W, Loftus E V, Faubion WA, et al. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. Am J Gastroenterol 2010; 105: 1133–9.

23. van der Valk ME, Mangen M-JJ, Leenders M, et al. Healthcare costs of inflammatory bowel disease have shifted from hospitalisation and surgery towards anti-TNFα therapy: results from the COIN study. Gut 2012; gutjnl-2012-303376-.

24. Bernstein CN, Longobardi T, Finlayson G, et al. Direct medical cost of managing IBD patients: a Canadian population-based study. Inflamm Bowel Dis 2012; 18: 1498–508.

25. Smeets HM, de Wit NJ, Hoes AW. Routine health insurance data for scientific research: potential and limitations of the Agis Health Database. J Clin Epidemiol 2011; 64: 424–30.

26. Statistics Netherlands StatLine. Statistics Netherlandshttp://statline.cbs.nl (2016).

27. van den Reek JMPA, Pijls PARR, Tummers M, et al. Adalimumab drug survival in patients with psoriasis, Crohn's disease, and rheumatoid arthritis: Relevant differences using the same treatment. J Am Acad Dermatol 2016; 74: 177–9.

28. Panaccione R, Ghosh S, Middleton S, et al. Combination Therapy With Infliximab and Azathioprine Is Superior to Monotherapy With Either Agent in Ulcerative Colitis. Gastroenterology 2014; 146: 392–400.e3.

29. O'Donnell S, Stempak JM, Steinhart AH, et al. Higher Rates of Dose Optimisation for Infliximab Responders in Ulcerative Colitis than in Crohn's disease. J Crohns Colitis 2015; 9: 830–6.

30. Magro F, Rodrigues-Pinto E, Lopes S, et al. Earlier need of infliximab intensification in ulcerative colitis than in Crohn's disease. J Crohns Colitis 2014; 8: 1331–2.

31. Taxonera C, Olivares D, Mendoza JL, et al. Need for infliximab dose intensification in Crohn's disease and ulcerative colitis. World J Gastroenterol 2014; 20: 9170–7.

32. Bizzarri B, Fornaroli F, de' Angelis N, et al. Infliximab in a Paediatric Population with Inflammatory Bowel Disease: an Important Achievement? Am J Gastroenterol 2008; 103: S529.

33. Brandse JF, van den Brink GR, Wildenberg ME, et al. Loss of Infliximab Into Feces Is Associated With Lack of Response to Therapy in Patients With Severe Ulcerative Colitis. Gastroenterology 2015; 149: 350–5.e2.

34. Singh S, Heien HC, Sangaralingham LR, et al. Comparative Effectiveness and Safety of Anti-Tumor Necrosis Factor Agents in Biologic-Naïve Patients with Crohn's Disease. Clin Gastroenterol Hepatol. Epub ahead of print 4 April 2016. DOI: 10.1016/j.cgh.2016.03.038.

35. Singh S, Heien HC, Sangaralingham LR, et al. Comparative effectiveness and safety of infliximab and adalimumab in patients with ulcerative colitis. Aliment Pharmacol Ther 2016; 43: 994–1003.

36. Ananthakrishnan AN, Cagan A, Cai T, et al. Comparative Effectiveness of Infliximab and Adalimumab in Crohn's Disease and Ulcerative Colitis. Inflamm Bowel Dis 2016; 22: 880–885.

37. Osterman MT, Haynes K, Delzell E, et al. Comparative Effectiveness of Infliximab and Adalimumab for Crohn's Disease. Clin Gastroenterol Hepatol 2014; 12: 811–817.e3.

38. Danese S, Fiorino G, Peyrin-Biroulet L, et al. Biological Agents for Moderately to Severely Active Ulcerative Colitis. Ann Intern Med 2014; 160: 704.

39. Singh S, Garg SK, Pardi DS, et al. Comparative Efficacy of Biologic Therapy in Biologic-Naïve Patients With Crohn Disease: A Systematic Review and Network Meta-analysis. Mayo Clin Proc 2014; 89: 1621–1635.

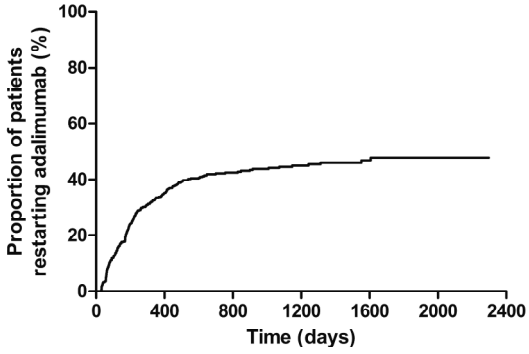
40. Kestens C, van Oijen MGH, Mulder CLJ, et al. Adalimumab and Infliximab Are Equally Effective for Crohn's Disease in Patients Not Previously Treated With Anti–Tumor Necrosis Factor-$\alpha$ Agents. Clin Gastroenterol Hepatol 2013; 11: 826–831.

41. Van Assche G, Vermeire S, Ballet V, et al. Switch to adalimumab in patients with Crohn's disease controlled by maintenance infliximab: prospective randomised SWITCH trial. Gut 2012; 61: 229–34.

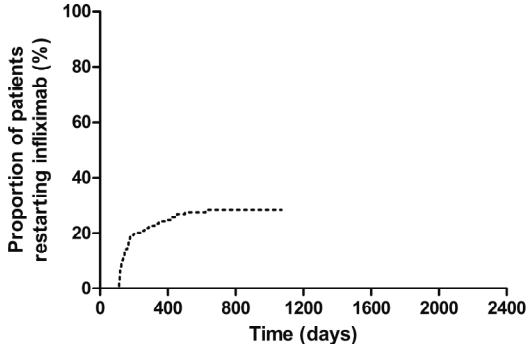
42. Dignass A, Lindsay JO, Sturm A, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. J Crohns Colitis 2012; 6: 991–1030.

43. de Groof EJ, Rossen NGM, van Rhijn BD, et al. Burden of disease and increasing prevalence of inflammatory bowel disease in a population-based cohort in the Netherlands. Eur J Gastroenterol Hepatol. Epub ahead of print 8 June 2016. DOI: 10.1097/MEG.0000000000000660.

## Supplementary material




**Supplemental figure 1.** *Time to restart of adalimumab after discontinuation.*




**Supplemental figure 2.** *Time to restart of infliximab after discontinuation.*

# Chapter 10

## Relapse rates and predictors for relapse in a real-life cohort of IBD patients after discontinuation of anti-TNF therapy

S. Bots, S. Kuin, C. Ponsioen, K. Gecse, M. Duijvestein, G. D'Haens, M. Löwenberg

*Scand J Gastroenterol 2019;54(3):281-288*

## Abstract

### Objective

We investigated relapse rates after anti-TNF withdrawal in inflammatory bowel disease (IBD) patients, response to restart of anti-TNF treatment and predictors for relapse.

### Methods

IBD patients in remission receiving infliximab (IFX) or adalimumab (ADL) treatment for ≥ 1 year who discontinued treatment were included. Relapse rates and predictors for relapse were studied using survival and Cox regression analysis.

### Results

In total, 101 patients were included (77 CD, 24 UC). A total of 56 patients (55%) experienced a relapse (CD 38, UC 18) with a median time to relapse of 32 and 18 months in CD and UC, respectively. Of patients that were retreated with the same anti-TNF agent, 84% responded. A trough serum concentration ≥ 2 μg/ml within one year prior to anti-TNF discontinuation was associated with a higher relapse rate in CD patients (HR 2.89; p=0.018), which was more evident in patients requiring retreatment with biologicals, bowel-related surgery or experimental medication (HR: 4.18; p=0.009). A young age (<17 years) at diagnosis was associated with a higher relapse rate (HR: 2.29; p=0.040) and fecal calprotectin levels < 25μg/g with a lower relapse rate in CD patients (HR: 0.34; p=0.041). Relapse rates, requiring treatment with biologicals or experimental medication, was lower in UC patients who continued immunosuppressive treatment (HR: 0.26; p=0.042).

### Conclusions

Approximately 55% of patients relapsed after anti-TNF withdrawal with a median time to relapse of 32 and 18 months in CD and UC, respectively. Retreatment with the same anti-TNF was successful in 84% of patients.

## Introduction

The introduction of anti-tumor necrosis factor (anti-TNF) agents has revolutionized the treatment of inflammatory bowel disease (IBD) and has resulted in better long-term outcomes.[1-7] Anti-TNF agents are able to induce mucosal healing and decrease the risk of hospitalization and surgery.[6-9] Patients that achieve remission with anti-TNF agents are often treated for many years if they tolerate the treatment.[10, 11] Long-term treatment with anti-TNF agents is considered to be safe[12-16], although side effects include infusion/injection site reactions, infections, skin problems and a small increase in risk for some malignancies in combination with immunosuppressive agents.[13, 15, 17] As a result, the proportion of patients treated with anti-TNF agents is steadily increasing which is associated with high costs [8, 18] in spite of the introduction of biosimilars.[19]

Due to the increasing number of IBD patients treated with anti-TNF agents, elective discontinuation of these agent is of particular interest. Common reasons for anti-TNF discontinuation are loss of efficacy, anti-drug-antibody formation, side-effects, pregnancy, patient preference, disease remission and costs. The decision to stop anti-TNF treatment in patients with primary non-response, secondary loss of response or severe side effects is usually simple. However, the decision to stop treatment in patients in remission or in patients with mild side effects, is more difficult and is influenced by factors such as doctor and patient preference, treatment duration, treatment adherence and costs. It is known that adherence to long-term treatment is generally low, which is associated with suboptimal treatment outcomes and this also might be a reason to stop treatment.[20, 21]

Several studies have been conducted to investigate the effect of anti-TNF withdrawal and retreatment.[22-30] These studies showed that retreatment with the same anti-TNF agent was effective in the majority of patients. Nevertheless, it still remains uncertain when and how to stop anti-TNF treatment in IBD patients who are in remission and which patients are likely to have successful retreatment. Therefore, guidelines on elective anti-TNF discontinuation in IBD patients are still lacking.

In this study, we aimed to evaluate the frequency of relapse, predictive factors associated with relapse and effect of retreatment after discontinuation of anti-TNF treatment in a tertiary cohort of IBD patients in corticosteroid-free clinical remission. These real life findings might be useful for the development of future prediction models and guidelines.

## Methods

*Study design and patient population*

This was a single-center observational cohort study performed at the Department of Gastroenterology and Hepatology of the Amsterdam UMC. Consecutive adult CD and UC patients that discontinued infliximab (IFX) or adalimumab (ADL) treatment between November 2012 and February 2018 were included. Patients were in corticosteroid free clinical remission and treated with IFX or ADL for at least 1 year. All patients were prospectively followed on the outpatient clinic by IBD experts with regular appointments. Clinical, biochemical, endoscopic and radiological evaluation were performed during follow-up at physician's discretion. All patients were followed until the end of the observation period (September 2018) or until relapse. Patients with a relapse that were retreated with an anti-TNF agent, were followed until the end of the observation period or when retreatment failed.

*Definitions*

Remission was determined by physician's global assessment and was based on absence of clinical symptoms and/or normal biochemistry (i.e. fecal calprotectin < 250 μg/g and C-reactive protein (CRP) < 5 mg/l) and/or endoscopic/radiologic remission (no signs of active inflammation) in the year prior to discontinuation. The cut-off for fecal calprotectin of 250 μg/g was based on data showing that it correlates well with endoscopic lesions.[31] In case endoscopic, radiological or biochemical evaluations were not available in the year prior to discontinuation, inclusion was based on absence of clinical symptoms alone. Relapse was defined as the requirement for (re)treatment with IBD medication (i.e. corticosteroids, immunosuppressives, biologicals or experimental medication), dose increase of IBD medication in follow-up period or IBD-related surgical interventions (i.e. bowel resections, deviating ileo/colostomy). In case of a relapse, treatment decisions were made at physician's discretion. Response to retreatment with the same anti-TNF agent was determined by physician's global assessment based on clinical, biochemical, endoscopic and/or radiological assessments. Intensified IFX schedules were defined as treatment intervals < 7 weeks or a dose of 10mg/kg and intensified ADL schedule were defined as doses of 40mg every week.

*Data collection*

Data were collected from medical records. The following data were collected: gender, diagnosis, date of diagnosis, Montreal classification, smoking habits, IBD-related surgical history, previous and current medical treatment, anti-TNF start and stop date, anti-TNF treatment schedule and reasons for anti-TNF discontinuation. At time of anti-TNF discontinuation, we reviewed age, duration of anti-TNF treatment, continued IBD medication, laboratory results (CRP, albumin, hemoglobin, leukocytes, thrombocytes and fecal calprotectin), endoscopic and radiologic results in the year prior to discontinuation of TNF inhibitors. Duration of follow-up and occurrence of relapse was documented. At time of relapse and after anti-TNF retreatment, clinical, biochemical, endoscopic and radiological data were collected.

*Statistical analysis*

Descriptive statistics were used to characterize the patient population. Results were provided as numbers (percentages) for discrete variables and median (range) for continuous variables and as frequencies and percentages for categorical variables. Differences in not normally distributed paired parameter were tested with the Wilcoxon signed rank test. Time to relapse and relapse rates were assessed using Kaplan Meier analysis. Univariable Cox regression analysis was used to study potential predictors for relapse. Multivariable Cox proportional hazard analysis was not performed since data was collected retrospectively resulting in occasionally missing data. The proportional hazards assumption was tested by testing the product of time to relapse and each variable for interaction within the model with the same variable alone. A p-value of $<0.05$ was considered to be statistically significant. All statistical analyses were performed using SPSS 24.0 software (IBM Corporation, Armonk, NY, USA).

*Ethical approval and patient consent*

This study was approved by the ethical committee of the Amsterdam UMC. Since this was a retrospective study, included patients provided consent with an opt-out procedure.

## Results

*Patient population*

In total, 101 patients who discontinued anti-TNF treatment were included (77 CD, 24 UC). Patient characteristics are shown in table 1. The median follow-up time of all patients was 47 months (IQR 33-51). The median treatment duration prior to anti-TNF discontinuation was 53 months (IQR 24.5-86.5). At time of anti-TNF discontinuation, 51% of patients did not continue any medical treatment and 37% continued treatment with immunosuppressive agents (table 2).

**Table 1**. *Patient characteristics in CD and UC patients.*

| **CD patients** | **n = 77** |
|---|---|
| **Characteristic** | |
| Male; n (%) | 27 (35%) |
| Age; median (range), years | 43 (34-53) |
| Montreal classification | |
| A1 (<16 years) | 11 (14%) |
| A2 (17-40 years) | 64 (83%) |
| A3 (>40 years) | 2 (3%) |
| L1 (ileum) | 24 (31%) |
| L2 (colon) | 20 (26%) |
| L3 (ileocolonic) | 33 (43%) |
| + L4 (upper GI) | 9 (12%) |
| B1 (non stricturing, non-penetrating) | 48 (62%) |
| B2 (stricturing) | 20 (26%) |
| B3 (penetrating) | 9 (12%) |
| P (Perianal disease) | 29 (38%) |
| Median months anti-TNF use (IQR) | 60 (29-91) |
| Endoscopy available <1year | 44 (57%) |
| Fecal calprotectin available <1 year | 49 (64%) |
| Radiology (MRI, US) available <1 year | 3 (4%) |
| Discontinued anti-TNF type and schedules | |
| Infliximab | 37 (48%) |
| Normal | 33 (89%) |
| Intensified | 4 (11%) |
| Adalimumab | 40 (52%) |
| Normal | 31 (78%) |
| Intensified | 9 (12%) |
| Previous surgical resection | |
| 1 resection | 19 (25%) |
| 2 resections | 11 (14%) |
| >2 resections | 3 (4%) |

**Table 1**. *(Continued).*

| | |
|---|---|
| Previous medication use | |
| Infliximab | 28 (36%) |
| Adalimumab | 9 (12%) |
| Thiopurines | 72 (94%) |
| Methotrexate | 27 (35%) |
| Corticosteroids | 63 (82%) |
| Budesonide | 36 (47%) |
| Disease duration median years (IQR) | 19 (13-27) |
| **UC patients** | n = 24 |
| **Characteristic** | |
| Male | 8 (33%) |
| Age; median (range), years | 48 (33-57) |
| Montreal classification | |
| E1 (proctitis) | 1 (4%) |
| E2 (left-sided) | 10 (42%) |
| E3 (pancolitis) | 13 (54%) |
| S1 (mild) | 0 (0%) |
| S2 (moderate) | 6 (25%) |
| S3 (severe) | 18 (75%) |
| Median months anti-TNF use (IQR) | 29 (16-73) |
| Endoscopy available <1year | 16 (67%) |
| Fecal calprotectin available <1 year | 17 (71%) |
| Discontinued anti-TNF type and schedules | |
| Infliximab | 20 (83%) |
| Normal | 16 (80%) |
| Intensified | 4 (20%) |
| Adalimumab | 4 (17%) |
| Normal | 4 (100%) |
| Intensified | 0 (0%) |
| Previous surgical resection | |
| 1 resection | 1 (4%) |
| Previous medication use | |
| Infliximab | 4 (17%) |
| Adalimumab | 1 (4%) |
| Thiopurines | 21 (88%) |
| Methotrexate | 4 (17%) |
| Corticosteroids | 23 (96%) |
| Budesonide | 3 (13%) |
| Disease duration median years (IQR) | 14 (7-31) |

**Table 2**. *Continued treatment at time of anti-TNF discontinuation in CD and UC patients.*

| **CD patients** | n = 77 |
|---|---|
| None | 49 (64%) |
| Immunomodulators | 26 (34%) |
| Thiopurines | 22 (29%) |
| Methotrexate | 4 (5%) |
| 5-ASA | 2 (3%) |

| **UC patients** | n = 24 |
|---|---|
| None | 2 (8%) |
| Immunomodulators | 11 (46%) |
| Thiopurines | 11 (46%) |
| Methotrexate | 0 (0%) |
| 5-ASA | 19 (79%) |

*Anti-TNF discontinuation*

IFX and ADL treatment was discontinued in 57/101 (56%) and in 44/101 (44%) of patients, respectively. Endoscopy results showing quiescent disease within 1 year from anti-TNF discontinuation were available in 60/101 patients. Fecal calprotectin levels <250 μg/g in the year prior to discontinuation were available in 66/101 and CRP concentrations < 5mg/l were available in 94/101. Thirty three patients had both normal endoscopy and fecal calprotectin levels within 1 year prior to anti-TNF discontinuation and 7/101 had normal radiological findings in combination with normal fecal calprotectin levels. In 8/101 patients remission was based on clinical symptoms alone. Out of these 8 patients, 1 patient had a normal endoscopy (performed 21 months prior to discontinuation) and 2 patients had normal fecal calprotectin levels (measured at 13 months and 23 months prior to discontinuation) during anti-TNF treatment. Six patients had measurable anti-drug antibodies prior to anti-TNF discontinuation. Table 3 shows additional reasons besides disease remission why it was decided to discontinue anti-TNF treatment.

*Relapse rates*

In total, 56/101 (55%) patients relapsed during the observation period. The relapse rate for CD patients was 49% (38/77) and 75% (18/24) for UC patients (figure 1). The median time to relapse was 28 months for all patients (figure 2a). The median time to relapse was 32 months in CD and 18 months in UC patients (figures 2b and c). Time to relapse was not significantly different between CD and UC patients (p=0.143). Within 1 year after anti-TNF discontinuation, 22/77 (29%) CD and 8/24 (33%) UC patients relapsed and corresponding numbers within 2 years were 32/77 (42%) and 12/24 (50%). In 45/101 (45%) patients (33 CD, 12 UC), treatment with biologicals (i.e. anti-TNF, vedolizumab), IBD-related surgery or experimental medication (clinical trials) was required at the time of relapse. The

median time to relapse for this subgroup was 41 months for all patients (44 months for CD and 38 months for UC patients (figures 3 a,b,c).

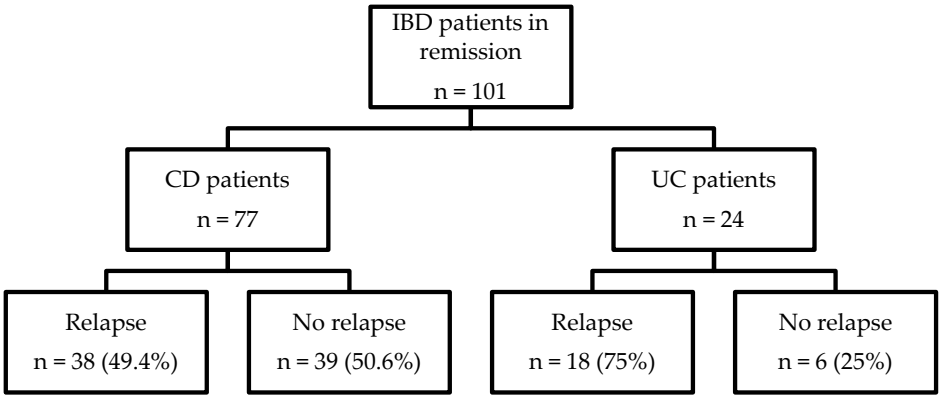



***Figure 1**. Flowchart of included patients.*

**Table 3.** *Reasons for discontinuing anti-TNF treatment.*

| **Reasons for discontinuation** | **n (%)** |
|---|---|
| Side effects | 24 (24%) |
| Infections | 7 (7%) |
| Low trough levels | 2 (2%) |
| Anti-drug antibodies | 6 (6%) |
| Patient preference | 14 (14%) |
| Malignancy | 2 (2%) |
| Pregnancy | 2 (2%) |

*All patients (n=101) were in remission at time of anti-TNF discontinuation. This table shows additional reasons that contributed to the decision to stop anti-TNF treatment.*

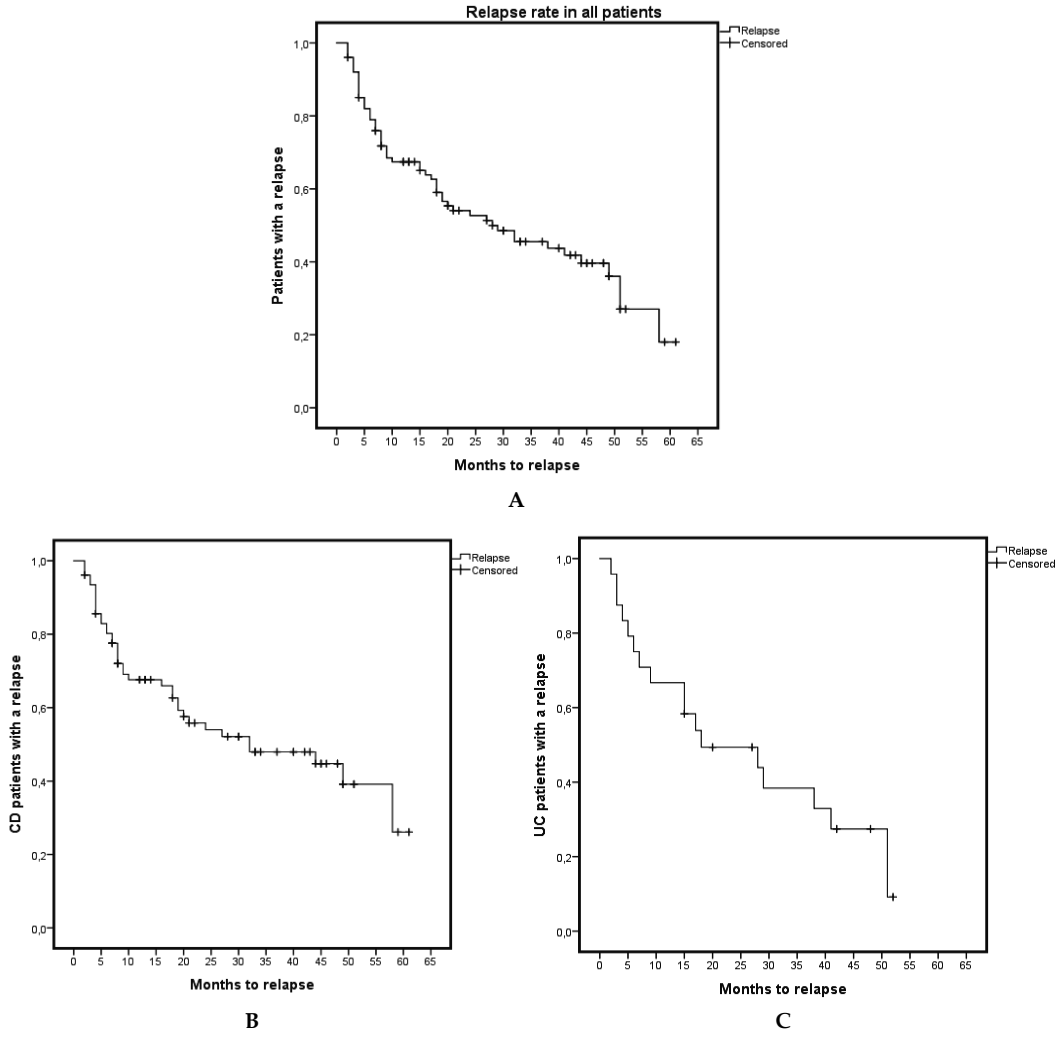
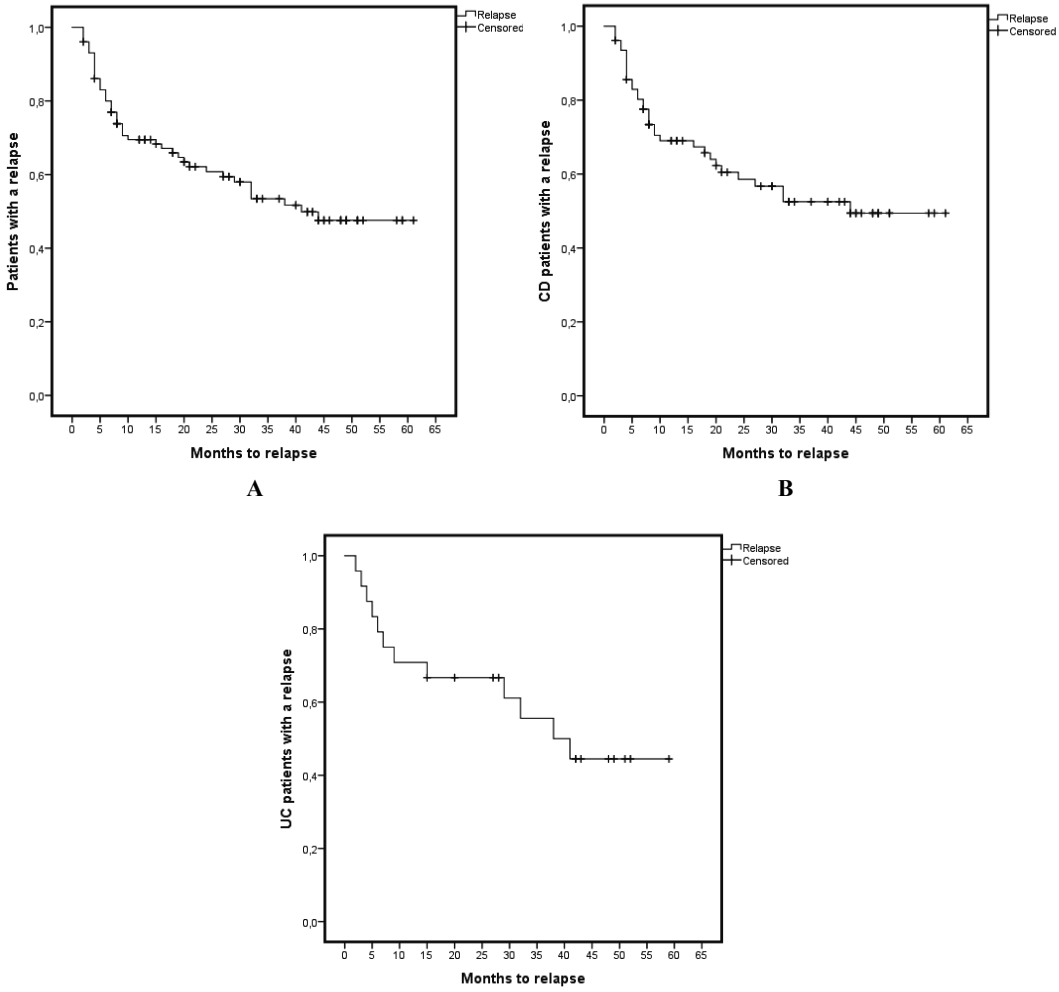



**Figure 2 a,b,c,.** *Time to relapse in all patients (a), CD patients (b) and UC patients (c).*




**Figures 3a,b,c**. *Time to relapse requiring treatment with biologicals, surgery or experimental medication in all patients (a), CD patients (b) and UC patients (c).*

**Table 4.** *Treatment started at relapse in CD and UC patients.*

| | CD patients | UC patients |
|---|---|---|
| **Patients with relapse** | n = 38 | n = 18 |
| Infliximab | 12 (32%) | 8 (47%) |
| Adalimumab | 14 (18%) | 1 (6%) |
| Golimumab | - | 2 (8%) |
| Vedolizumab | 3 (4%) | - |
| Experimental medication | 4 (5%) | - |
| Thiopurines | 3 (8%) | 2 (11%) |
| Methotrexate | 3 (8%) | 2 (11%) |
| Corticosteroids (oral or topical) | 4 (11%) | 4 (17%) |
| Budesonide (oral or topical) | 5 (13%) | 1 (4%) |
| 5-ASA (oral or topical) | - | 8 (33%) |
| Cyclosporine | - | 1 (4%) |

*All treatments started at relapse in CD and UC patients. Patients could start a combination of these treatments.*

*Efficacy of anti-TNF retreatment*

Anti-TNF treatment was re-started in 37 patients (20 infliximab, 15 adalimumab, 2 golimumab). Thirty three patients were re-treated with the same anti-TNF agent that was discontinued earlier (19 IFX, 14 ADL). The median follow-up time for patients retreated with the same anti-TNF agent was 38 months (IQR 29-26). Retreatment with any anti-TNF agent was successful in 30/37 (81%) patients (22CD, 8 UC). Retreatment with the same anti-TNF agent was successful in 28/33 (84%) of which 21 were CD and 7 were UC patients. In 21/33 patients fecal calprotectin levels were available and improved significantly after a median of 4 (IQR 2-11) months (mean 1384 μg/ml vs 196 μg/ml; $p<0.001$). CRP levels were available in 24 patients showing a significant decrease after a median of 11 months (IQR 8-21) (mean 13.6 mg/ml vs 2.5 mg/ml; $p=0.001$). Insufficient endoscopic and radiological data were available to reliably assess retreatment effects.

Three patients did not respond to retreatment with the same anti-TNF. One patient had side effects (allergic reaction and skin rash) and in 1 patient the follow-up time after retreatment was only 1 month. One patient lost response to retreatment after an initial response after 17months, 1 patient stopped the anti-TNF agent at 18 months after retreatment because of achieving clinical and biochemical remission after 8 months and 1 patient stopped the anti-TNF agent after retreatment because of anti-TNF induced lupus after a favorable initial response.

*Predictors for relapse in patients requiring IBD treatment*

Variables included in the univariable cox proportional hazards analysis are shown in table 5. The assumption of proportionality was met for all tested variables. A trough serum drug concentration >2 μg/ml, irrespective of the anti-TNF agent in the year prior to discontinuation, was associated with

a higher relapse risk in CD patients (HR: 2.89; p = 0.018), but not in UC patients (HR: 1.14; p=0.829) with serum drug concentrations >2 μg/ml. Fecal calprotectin values <50 μg/g were not associated with a lower relapse risk in CD and UC patients (HR: 0.53; p=0.114 and HR: 1.06; p=0.93). However, fecal calprotectin values <25 μg/g before discontinuation were associated with a lower relapse risk in CD (HR: 0.34; p=0.041), but not in UC patients (HR: 0.79; p=0.697). Continuation of immunosuppressive agents was not associated with a lower relapse risk in all IBD patients, but a trend towards a lower relapse rate was observed in UC patients (HR: 0.37; p=0.071). A younger age at diagnosis in CD patients (<17 years) was associated with a higher relapse risk (HR: 2.29; p=0.040). A trend towards a higher relapse risk was seen in CD patients that received prior intensified anti-TNF treatment (HR: 1.96; p=0.081). Due to incomplete data in several variables, multivariate analysis was not performed using variables p<0.1.

*Predictors for relapse in patients requiring biologicals, surgery or experimental medication*

Subgroup analysis was performed in patients who relapsed requiring treatment with biologicals (n= 40), IBD-related surgery (n=1) or experimental medication in the setting of a clinical trial (n=4). In this group, continuing treatment with immunosuppressive agents was associated with a lower relapse risk in UC (HR: 0.60; p=0.042), but not in CD patients (HR: 0.83; p=0.405). A trough level > 2 μg/ml was associated with a higher relapse rate in all patients (HR: 3.31; p=0.008) and in CD (HR: 4.18; p=0.009), but not in UC patients (HR: 1.14; p=0.829). A younger age at diagnosis in CD patients (<17 years) was associated with a higher relapse risk (HR: 2.68; p=0.017). A trend towards a higher relapse risk was observed in CD patients requiring prior intensified anti-TNF treatment (HR: 2.00; p=0.089) and a trend towards a lower relapse risk was seen in patients with fecal calprotectin values <25 μg/g (HR:0.45; p=0.069). Due to incomplete data in several variables, multivariate analysis was not performed with variables p<0.1.

**Table 5.** *Univariate Cox proportional hazard analysis of potential predictors for relapse.*

| Predictors for relapse in patients requiring any kind of IBD treatment (n=56) | | | | | | |
|---|---|---|---|---|---|---|
| | All patients (n=101) | | CD patients (n=77) | | UC patients (n=24) | |
| **Factor** | **HR (95%CI)** | **p-value** | **HR (95%CI)** | **p-value** | **HR (95%CI)** | **p-value** |
| Diagnosis | 1.51 (0.86-2.66) | 0.15 | - | - | - | - |
| Gender | 0.90 (0.52-1.58) | 0.722 | 0.98 (0.50-1.91) | 0.94 | 0.68 (0.25-1.89) | 0.464 |
| Previous anti-TNF use | 1.22 (0.70-2.10) | 0.484 | 1.40 (0.74-2.65) | 0.305 | 1.26 (0.35-4.48) | 0.72 |
| Intensified anti-TNF | 1.37 (0.72-2.60) | 0.340 | **1.96 (0.92-4.16)** | **0.081** | 0.43 (0.12-1.59) | 0.206 |
| IM continuation at stop | 0.69 (0.39-1.21) | 0.195 | 0.79 (0.40-1.57) | 0.504 | **0.37 (0.13-1.09)** | **0.071** |
| Calprotectin <50* | 0.68 (0.38-1.24) | 0.208 | 0.53 (0.25-1.14) | 0.114 | 1.05 (0.37-3.00) | 0.93 |
| Calprotectin <25* | **0.49 (0.24-1.03)** | **0.059** | **0.34 (0.12-0.96)** | **0.041** | 0.79 (0.25-2.54) | 0.697 |
| Trough level >2* | **2.27 (1.13-4.53)** | **0.021** | **2.89 (1.20-6.98)** | **0.018** | 1.14 (0.35-3.65) | 0.829 |
| Disease duration (years) | 1.003 (0.98 – 1.03) | 0.844 | 1.008 (0.97-1.04) | 0.665 | 1.002 (0.97-1.04) | 0.917 |
| Anti-TNF duration (months) | 0.997 (0.989-1.005) | 0.411 | 0.998 (0.988-1.008) | 0.708 | 1.00 (0.99-1.02) | 0.968 |
| Surgical resection CD | - | - | 1.35 (0.72-2.56) | 0.353 | - | - |
| Age diagnosis <17 CD | - | - | **2.29 (1.04-5.07)** | **0.040** | - | - |
| Colonic involvement CD | - | - | 0.82 (0.42-1.62) | 0.566 | - | - |
| Complicated disease CD | - | - | 0.75 (0.39-1.42) | 0.370 | - | - |
| Perianal disease CD | - | - | 0.91 (0.47-1.76) | 0.777 | - | - |

**Table 5.** *(Continued).*

| Predictors for relapse in patients requiring treatment with biologicals, surgery or experimental medication (n=45) | | | | | | |
|---|---|---|---|---|---|---|
| | All patients (n=101) | | CD patients (n=77) | | (UC patients) n=24 | |
| Diagnosis | 1.06 (0.54-2.04) | 0.875 | - | - | - | - |
| Gender | 0.94 (0.51-1.73) | | 0.98 (0.50-1.91) | 0.943 | 0.68 (0.25-1.89) | 0.464 |
| Previous anti-TNF use | 1.45 (0.80-2.64) | 0.220 | 1.38 (0.70-2.74) | 0.356 | 2.30 (0.62-8.57) | 0.216 |
| Intensified anti-TNF | 1.37 (0.67-2.84) | 0.405 | **2.00 (0.90-4.45)** | **0.089** | 0.32 (0.04-2.50) | 0.278 |
| IM continuation at stop | 0.62 (0.33-1.16) | 0.132 | 0.83 (0.40-1.71) | 0.60 | **0.26 (0.07-0.95)** | **0.042** |
| Calprotectin <50 | 0.66 (0.34-1.27) | 0.207 | 0.68 (0.31-1.47) | 0.323 | 0.61 (0.17-2.17) | 0.609 |
| Calprotectin <25 | **0.45 (0.18-1.07)** | **0.069** | 0.43 (0.15-1.22) | 0.110 | 0.54 (0.12-2.57) | 0.411 |
| **Trough level >2** | **3.31 (1.37-8.00)** | **0.008** | **4.18 (1.42-12.30)** | **0.009** | 1.59 (0.34-7.53) | 0.561 |
| Disease duration (years) | 1.01 (0.99-1.04) | 0.363 | 1.01 (0.98-1.05) | 0.585 | 1.02 (0.98-1.06) | 0.426 |
| Anti-TNF durations (months) | 1.00 (0.99-1.01) | 0.58 | 1.00 (0.99-1.01) | 0.716 | 1.01 (0.99-1.02) | 0.551 |
| Surgical resection CD | - | - | 0.99 (0.49-1.97) | 0.968 | - | - |
| Age diagnosis <17 CD | - | - | **2.68 (1.19-6.01)** | **0.017** | - | - |
| Colonic involvement CD | - | - | 1.08 (0.52-2.28) | 0.832 | - | - |
| Complicated CD | - | - | 0.60 (0.30-1.18) | 0.139 | - | - |
| Perianal disease CD | - | - | 0.88 (0.43-1.78) | 0.716 | - | - |

*Hazard ratios (HR), 95% confidence intervals (CI) and p values for all variables tested with univariate Cox proportional hazard analysis.*

## Discussion

In this observational cohort study we observed an overall relapse rate after anti-TNF discontinuation of 55% out of 101 consecutive IBD patients, with a median follow-up of 47 months. Of CD patients, 49% and of UC patients 75% relapsed. Retreatment with anti-TNF therapy was effective in 84% of patients. The relatively long follow-up period before and after relapse (median 47 and 38 months, respectively) in our cohort, allows for good estimations of relapse rate and retreatment success.

The outcomes in our study are comparable with relapse rates reported previously.[22, 23, 26-28, 30, 32] However, follow-up periods vary considerably between the different studies and most of them lack long-term outcomes. Long-term data from the STORI trial in CD patients after IFX withdrawal showed that approximately 20% of patients remained without biological treatment, surgery or perianal disease with a 7 year follow-up period.[30] In our study, half of CD patients remained relapse free with a median follow-up of 32 months. The success percentage of retreatment of 84% in our cohort is in line with other studies ranging between 71% and 94%.[22, 23, 27-30, 32-35] An exception was the study performed by Fiorino et al. In their cohort of 193 UC patients only 51% achieved remission after anti-TNF retreatment.[33] In the STORI trial, anti-TNF retreatment was safe and effective in the 88% of patients.[30] The small differences in reported relapse rates after anti-TNF cessation as well as in efficacy outcomes after retreatment between these studies could be explained by heterogeneity in study populations, different definitions of response and remission and variable follow-up periods after discontinuation and retreatment. Overall, it seems evident that a substantial proportion of IBD patients that discontinue anti-TNF treatment are prone to experience a relapse within several years and that retreatment with the same anti-TNF agent is effective and safe in the majority of patients.

Predicting which patients have an increased relapse risk is of great value for the decision making process when considering elective anti-TNF discontinuation. We identified several potential predictors for relapse in our cohort. Low trough levels at the time of anti-TNF discontinuation were associated with a lower relapse risk in CD patients, but not in UC patients. This predictor has also been identified by Ben Horin et al. and by the STORI investigators.[26, 30] Thus, a significant proportion of patients that remain in remission with low anti-TNF serum concentrations probably do not need the drug, hence they might be over-treated. Only 6 patients had measurable anti-drug antibodies prior to anti-TNF discontinuation. Therefore, we were unable to assess the effect of anti-drug antibody formation on relapse risk. Continuation of immunosuppressive agents was associated with a lower relapse risk in UC patients, but not in CD patients in our study. This was also shown in a cohort of UC patients by Fiorino et al.[33] Casanova et al showed a minor advantage of continuation of immunosuppressive agents in CD patients after anti-TNF discontinuation.[22] In addition, disease onset at a younger age in CD patients was associated with an increased relapse risk in our cohort, which has also been reported in 3 other studies.[22, 23, 36] Low fecal

calprotectin levels (<25 μg/ml) before anti-TNF discontinuation were associated with a lower relapse rate, which was mainly observed in CD patients. The frequently used cut-off of 50 μg/g was not associated with a lower relapse rate in this study. Therefore, we decided to investigate the 25 μg/g cut-off as potential predictor for relapse since unpublished data from our group suggest that lower fecal calprotectin values could predict histological remission more accurately. Other studies have also shown that normal biochemistry (i.e. CRP < 5mg/ml and fecal calprotectin < 250 μg/g) at the time of anti-TNF discontinuation is associated with a lower relapse risk.[32] Therefore, it seems appropriate to at least confirm that fecal calprotectin levels are within the normal range before electively stopping anti-TNF treatment. Finally, we observed a trend towards a higher relapse risk in CD patients who received prior intensified anti-TNF treatment. To our knowledge this association, although not statistically significant, has not yet been observed in other studies.

Several other predictors for an increased relapse risk have been identified by others, including treatment with ADL, anti-TNF discontinuation because of adverse events, stricturing disease, perianal disease, gender, disease duration, ileocolonic or colonic disease, previous anti-TNF use and previous failure to immunosuppressives.[22, 28, 30, 32, 35-37] However, in our study none of these factors were identified as potential risk factors. Variability in relapse predictors between different studies could be explained by heterogeneity in study populations, study design, follow-up time and statistical methods. Nevertheless, several risk factors and protective factors have been identified in various independent cohorts which should be confirmed in larger prospective studies.

Identification of patients that are likely to have a favorable response to retreatment is also important for elective discontinuation of anti-TNF therapy. To our knowledge, only one study by Baert et al. has addressed this issue so far.[34] They showed that absence of anti-drug antibodies and re-initiation with immunosuppressive agents were predictive for safe and effective infliximab retreatment. These findings are not surprising since it is well known that combination therapy and absence of anti-drug antibodies are important factors for successful anti-TNF treatment.[38, 39] In our cohort, we were unable to assess predictors for successful retreatment due to the relative low number of patients that were retreated with anti-TNF agents. Larger cohorts are needed to identify additional predictors for successful retreatment.

Our study has several limitations. Firstly, this was not a controlled study and evaluations were performed at physician's discretion. However, patients were followed up with regular appointments on the outpatient clinic, hence occurrence of relapses could be accurately estimated. Additionally, data from real-life experience could be considered a strength, since it reflects the normal clinical setting. Secondly, patient data were collected retrospectively. Therefore, biochemical, endoscopic and/or radiological data were not always available. Results from the univariable Cox regression analysis for the identification of potential relapse predictors should thus be interpreted with caution and should be considered as an explorative analysis. For this reason we decided not to perform a multivariable Cox regression analysis.

To our opinion, predictive models using multiple variables should be based on large prospective datasets. Finally, our cohort consists of 77 CD and 24 UC patients. Especially the UC patient number might be too small for analyzing relapse predictors. Despite this, we identified several predictors that could be associated with a lower or higher relapse risk, confirming earlier work. These predictors might be used in future algorithms in order to discontinue anti-TNF treatment in patients with a low relapse risk.

In conclusion, this real life IBD cohort shows a relapse rate of 55% and retreatment success of 84% confirming previous studies. The most important predictors for an increased relapse risk after stopping anti-TNF therapy in our cohort were a young age (< 17 years), trough levels >2μg/ml and elevated fecal calprotectin levels in CD patients. Continuation of immunosuppressive agents was associated with a decreased relapse risk in UC patients. Prospective studies are needed to confirm these predictors for relapse and to identify factors that could predict successful retreatment with anti-TNF agents.

## References


1. Reinisch W, Sandborn WJ, Rutgeerts P, Feagan BG, Rachmilewitz D, Hanauer SB, et al. Long-term infliximab maintenance therapy for ulcerative colitis: the ACT-1 and -2 extension studies. Inflammatory bowel diseases. 2012;18(2):201-11.

2. Papamichael K, Chachu KA, Vajravelu RK, Vaughn BP, Ni J, Osterman MT, et al. Improved Long-term Outcomes of Patients With Inflammatory Bowel Disease Receiving Proactive Compared With Reactive Monitoring of Serum Concentrations of Infliximab. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2017;15(10):1580-8.e3.

3. Van Assche G, Vermeire S, Rutgeerts P. Infliximab therapy for patients with inflammatory bowel disease: 10 years on. European journal of pharmacology. 2009;623 Suppl 1:S17-25.

4. Sands BE, Blank MA, Patel K, van Deventer SJ. Long-term treatment of rectovaginal fistulas in Crohn's disease: response to infliximab in the ACCENT II Study. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2004;2(10):912-20.

5. Fidder H, Schnitzler F, Ferrante M, Noman M, Katsanos K, Segaert S, et al. Long-term safety of infliximab for the treatment of inflammatory bowel disease: a single-centre cohort study. Gut. 2009;58(4):501-8.

6. Schnitzler F, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, et al. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. Inflammatory bowel diseases. 2009;15(9):1295-301.

7. Rutgeerts P, Van Assche G, Sandborn WJ, Wolf DC, Geboes K, Colombel JF, et al. Adalimumab induces and maintains mucosal healing in patients with Crohn's disease: data from the EXTEND trial. Gastroenterology. 2012;142(5):1102-11.e2.

8. van der Valk ME, Mangen MJ, Leenders M, Dijkstra G, van Bodegraven AA, Fidder HH, et al. Healthcare costs of inflammatory bowel disease have shifted from hospitalisation and surgery towards anti-TNFalpha therapy: results from the COIN study. Gut. 2014;63(1):72-9.

9. Colombel JF, Rutgeerts PJ, Sandborn WJ, Yang M, Camez A, Pollack PF, et al. Adalimumab induces deep remission in patients with Crohn's disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2014;12(3):414-22.e5.

10. Peters CP, Eshuis EJ, Toxopeus FM, Hellemons ME, Jansen JM, D'Haens GR, et al. Adalimumab for Crohn's disease: long-term sustained benefit in a population-based cohort of 438 patients. Journal of Crohn's & colitis. 2014;8(8):866-75.

11. Eshuis EJ, Peters CP, van Bodegraven AA, Bartelsman JF, Bemelman W, Fockens P, et al. Ten years of infliximab for Crohn's disease: outcome in 469 patients from 2 tertiary referral centers. Inflammatory bowel diseases. 2013;19(8):1622-30.

12. Minozzi S, Bonovas S, Lytras T, Pecoraro V, Gonzalez-Lorenzo M, Bastiampillai AJ, et al. Risk of infections using anti-TNF agents in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis: a systematic review and meta-analysis. Expert opinion on drug safety. 2016;15(sup1):11-34.

13. Lichtenstein GR, Rutgeerts P, Sandborn WJ, Sands BE, Diamond RH, Blank M, et al. A pooled analysis of infections, malignancy, and mortality in infliximab- and immunomodulator-treated adult patients with inflammatory bowel disease. The American journal of gastroenterology. 2012;107(7):1051-63.

14. Lichtenstein GR, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Langholff W, et al. Drug therapies and the risk of malignancy in Crohn's disease: results from the TREAT Registry. The American journal of gastroenterology. 2014;109(2):212-23.

15. D'Haens G, Reinisch W, Colombel JF, Panes J, Ghosh S, Prantera C, et al. Five-year Safety Data From ENCORE, a European Observational Safety Registry for Adults With Crohn's Disease Treated With Infliximab [Remicade(R)] or Conventional Therapy. Journal of Crohn's & colitis. 2017;11(6):680-9.

16. Mercer LK, Askling J, Raaschou P, Dixon WG, Dreyer L, Hetland ML, et al. Risk of invasive melanoma in patients with rheumatoid arthritis treated with biologics: results from a collaborative project of 11 European biologic registers. Annals of the rheumatic diseases. 2017;76(2):386-91.

17. Osterman MT, Sandborn WJ, Colombel JF, Robinson AM, Lau W, Huang B, et al. Increased risk of malignancy with adalimumab combination therapy, compared with monotherapy, for Crohn's disease. Gastroenterology. 2014;146(4):941-9.

18. Bots SJA, Hoekman DR, Benninga MA, Ponsioen CY, D'Haens GR, Lowenberg M. Patterns of anti-TNF use and associated treatment outcomes in inflammatory bowel disease patients: results from an analysis of Dutch health insurance claims data. The Netherlands journal of medicine. 2017;75(10):432-42.

19. CADTH Rapid Response Reports. Switching from Innovator to Biosimilar (Subsequent Entry) Infliximab: An Updated Review of the Clinical Effectiveness, Cost-Effectiveness, and Guidelines. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2017.

20. Severs M, Mangen MJ, Fidder HH, van der Valk ME, van der Have M, van Bodegraven AA, et al. Clinical Predictors of Future Nonadherence in Inflammatory Bowel Disease. Inflammatory bowel diseases. 2017;23(9):1568-76.

21. Testa A, Castiglione F, Nardone OM, Colombo GL. Adherence in ulcerative colitis: an overview. Patient preference and adherence. 2017;11:297-303.

22. Casanova MJ, Chaparro M, Garcia-Sanchez V, Nantes O, Leo E, Rojas-Feria M, et al. Evolution After Anti-TNF Discontinuation in Patients With Inflammatory Bowel Disease: A Multicenter Long-Term Follow-Up Study. The American journal of gastroenterology. 2017;112(1):120-31.

23. Kennedy NA, Warner B, Johnston EL, Flanders L, Hendy P, Ding NS, et al. Relapse after withdrawal from anti-TNF therapy for inflammatory bowel disease: an observational study, plus systematic review and meta-analysis. Alimentary pharmacology & therapeutics. 2016.

24. Torres J, Boyapati RK, Kennedy NA, Louis E, Colombel JF, Satsangi J. Systematic Review of Effects of Withdrawal of Immunomodulators or Biologic Agents From Patients With Inflammatory Bowel Disease. Gastroenterology. 2015;149(7):1716-30.

25. Papamichael K, Vermeire S. Withdrawal of anti-tumour necrosis factor alpha therapy in inflammatory bowel disease. World journal of gastroenterology : WJG. 2015;21(16):4773-8.

26. Ben-Horin S, Chowers Y, Ungar B, Kopylov U, Loebstein R, Weiss B, et al. Undetectable anti-TNF drug levels in patients with long-term remission predict successful drug withdrawal. Alimentary pharmacology & therapeutics. 2015.

27. Molander P, Farkkila M, Salminen K, Kemppainen H, Blomster T, Koskela R, et al. Outcome after discontinuation of TNFalpha-blocking therapy in patients with inflammatory bowel disease in deep remission. Inflammatory bowel diseases. 2014;20(6):1021-8.

28. Steenholdt C, Molazahi A, Ainsworth MA, Brynskov J, Ostergaard Thomsen O, Seidelin JB. Outcome after discontinuation of infliximab in patients with inflammatory bowel disease in clinical remission: an observational Danish single center study. Scandinavian journal of gastroenterology. 2012;47(5):518-27.

29. Louis E, Mary JY, Vernier-Massouille G, Grimaud JC, Bouhnik Y, Laharie D, et al. Maintenance of remission among patients with Crohn's disease on antimetabolite therapy after infliximab therapy is stopped. Gastroenterology. 2012;142(1):63-70.e5; quiz e31.

30. Reenaers C, Mary JY, Nachury M, Bouhnik Y, Laharie D, Allez M, et al. Outcomes 7 Years After Infliximab Withdrawal for Patients With Crohn's Disease in Sustained Remission. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2018;16(2):234-43.e2.

31. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflammatory bowel diseases. 2012;18(12):2218-24.

32. Brooks AJ, Sebastian S, Cross SS, Robinson K, Warren L, Wright A, et al. Outcome of elective withdrawal of anti-tumour necrosis factor-alpha therapy in patients with Crohn's disease in established remission. Journal of Crohn's & colitis. 2017;11(12):1456-62.

33. Fiorino G, Cortes PN, Ellul P, Felice C, Karatzas P, Silva M, et al. Discontinuation of Infliximab in Patients With Ulcerative Colitis Is Associated With Increased Risk of Relapse: A Multinational Retrospective Cohort Study. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2016;14(10):1426-32.e1.

34. Baert F, Drobne D, Gils A, Vande Casteele N, Hauenstein S, Singh S, et al. Early trough levels and antibodies to infliximab predict safety and success of reinitiation of infliximab therapy. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2014;12(9):1474-81.e2; quiz e91.

35. Farkas K, Lakatos PL, Nagy F, Szepes Z, Miheller P, Papp M, et al. Predictors of relapse in patients with ulcerative colitis in remission after one-year of infliximab therapy. Scandinavian journal of gastroenterology. 2013;48(12):1394-8.

36. Gisbert JP, Marin AC, Chaparro M. Systematic review: factors associated with relapse of inflammatory bowel disease after discontinuation of anti-TNF therapy. Alimentary pharmacology & therapeutics. 2015;42(4):391-405.

37. Gisbert JP, Marin AC, Chaparro M. The Risk of Relapse after Anti-TNF Discontinuation in Inflammatory Bowel Disease: Systematic Review and Meta-Analysis. The American journal of gastroenterology. 2016;111(5):632-47.

38. 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;146(2):392-400.e3.

39. Colombel JF, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. The New England journal of medicine. 2010;362(15):1383-95.

# Chapter 11

## Thesis summary

## Part I: Implementation of intestinal ultrasound

**Part I** of this thesis focuses on optimal implementation of intestinal ultrasound (IUS) for inflammatory bowel disease (IBD) patients. A crucial aspect when treating IBD patients is grading of the severity of inflammation, since this influences the treatment choice. Existing IUS indices for grading disease severity were never thoroughly reviewed. In **chapter 2** we identified 21 studies with an IUS index through literature search of which 11 (7 Crohn's disease [CD] and 4 ulcerative colitis [UC]) met the inclusion criteria for further review. Several indices that used clinical disease activity as reference standard were excluded from the analysis. Study quality for risk of bias was graded with a modified version of the QUADAS-2 tool and scored as high in 5 studies, moderate in 3 studies and low in 3 studies. The IUS parameters used in the indices included bowel wall thickness (BWT), colour Doppler signal (CDS), wall layer stratification(WLS), compressibility, peristalsis, haustrations, fatty wrapping, contrast enhancement (CE), and strain pattern. Only one CD index was validated in a second cohort. We concluded that that the methodology for the developed indices was suboptimal in most studies and that more stringent methodology was required for development of future indices.

Based on the results of **chapter 2**, we developed a novel UC-IUS index in **chapter 3**. Several IUS parameters (BWT, CDS, haustrations, fat wrapping, enlarged lymphnodes and WLS) were compared with endoscopy in 60 UC patients in different endoscopic categories of disease severity. Additionally inter- and intra-rater agreement were tested for most parameters by assessment of recorded cine loops. With ROC analysis, BWT cut-offs of 2.1, 3.2 and 3.9 mm were determined to detect mild, moderate and severe (i.e. eMayo 1, 2 and 3) endoscopic disease activity, respectively. Spots of CDS were associated with active disease and stretches of CDS with moderate to severe endoscopic disease. Disrupted haustrations were strongly associated with active disease and presence of fat wrapping with severe disease. Inter – and intra-rater were substantial for BWT and CDS. For haustrations inter- and intra-rater agreement varied from slight to substantial, indicating that this parameter was more difficult to agree on. However, since it seemed a parameter of importance, it was included in the final index. For fat wrapping inter- and intra-rater agreement were not assessed since the recorded cine loops were not suitable for this purpose. A point-based index was constructed including BWT, CDS, haustrations and fat wrapping as parameters. The index will be validated and tested for sensitivity to change in UC patients treated with anti-inflammatory agents. The index showed strong correlation with endoscopy through internal validation and the correlation between observers was also strong.

An important purpose of the index developed in **chapter 3**, would be for implementation of point-of-care (POC) assessment of IBD patients. In **chapter 4**, we studied the impact of POC IUS on treatment decisions in our clinic in 345 IUS examinations from 2 different cohorts (250 cohort 1; 195 cohort 2). Cohort 1 was collected between January 2016 and July 2018 and cohort 2 between October 2019 and December 2019. IUS outcomes were compared with clinical symptoms, biomarkers, endoscopy and MRI. POC IUS had an impact on treatment decisions

in approximately 60% of cases and medication use was changed in approximately 50% of cases. IUS showed inflammation or complications in many asymptomatic CD patients, whereas symptoms in UC patients were more reliable. The IUS results showed good correlation with endoscopy and MRI. Furthermore, we showed change in IUS implementation between the 2 cohorts, with a shifting paradigm towards more treatment follow-up and more use of IUS over the years. Based on the results we proposed a POC IUS algorithm for follow-up of IBD patients.

In **chapter 5** we studied the use of conventional IUS and contrast enhanced ultrasound (CEUS) parameters for follow-up of anti-TNF treatment in 40 CD patients. Our aim was to assess if early changes in IUS parameters after treatment initiation could be used to predict endoscopic treatment response later on. Patients received IUS at baseline (T0), 4-8 weeks (T1) and 12-34 weeks (T3). Per segment analysis (sigmoid, descending, transverse, ascending colon and TI) showed good correlation between endoscopy and IUS. The inter-observer agreement was also good. A BWT decrease of 18% after 4-8 weeks and 29% after approximately 6 months predicted endoscopic response at 6 months. The presence of CDS also decreased significantly at T1 and T2 in patients with endoscopic response. Most other conventional IUS parameters also improved in patients with endoscopic response but were less or not predictive at T1. For the CEUS parameters, the percentage decrease in wash out rate (WoR) at T1 was significantly different between endoscopic responders versus non-responders. Furthermore, decrease in percentage for peak enhancement (PE), wash in rate (WiR), wash in perfusion index (WiPI) and WoR at T2 was significantly more pronounced in endoscopic responders. When constructing a multivariable regression model BWT and CDS were most useful to predict endoscopic remission and response. Addition of other IUS or clinical parameters did not improve the model. WoR was the only CEUS parameter that slightly improved the model to predict endoscopic remission at T2, but not at T1. Therefore we concluded that measurement of BWT and CDS might be sufficient to assess treatment response in CD patients in an early stage after treatment initiation.

### Part II: Optimal use of biologics

**Part II** of this thesis includes several studies on biologic treatment in IBD patients. In **chapter 6 & 7** optimization of anti-TNF therapy and combination immunosuppression are discussed. We highlighted the importance of patient selection, screening, combination treatment, dosing strategies, managing loss of response, TDM, individualized treatment and stopping and reinitiating treatment in **chapter 6**. In **chapter 7** we further zoomed in on combination immunosuppression (anti-TNF and immunomodulators), discussing mechanism of action, efficacy, safety and effect on withdrawal of anti-TNF therapy. Both chapters include useful recommendations for clinical practice.

Since anti-drug antibody (ADA) formation is one of the most important clinical problems in treatment with biologics (mainly anti-TNF agents), we further focused on this topic in **Chapter 8.** Literature regarding ADA formation against all the biologics for treatment of IBD was

systematically reviewed. Data from 68 studies were analyzed and 33 studies were included in the meta-analysis. Pooled ADA rates differed substantially between agents and were higher for the older (anti-TNF) agents. However, the use of drug sensitive assays in most studies hampered comparison of true ADA rates. Furthermore, we showed that combination therapy with immunosuppressives reduces ADA positivity for most biologics. ADA formation was also associated with lower clinical response rates and higher rates of infusion reactions in patients treated with infliximab.

Patterns of anti-TNF use in the Netherlands were studied in **chapter 9** using anonymous data from a Dutch healthcare provider. We studied approximately 22.000 IBD patients of which 855 patients started infliximab between 2011 and 2014 and 1199 patients started adalimumab between 2008 and 2014. Anti-TNF usage increased significantly over the studied years and the proportion of patients receiving intensified treatment increased over time. Immunomodulator use was associated with longer time to corticosteroid initiation but not with longer drug survival in this cohort.

In **chapter 10** we studied relapse rates and predictors for relapse after anti-TNF discontinuation in a real-life cohort of 101 IBD patients (77 CD and 24 UC). All patients were in clinical remission at time of anti-TNF discontinuation. In our cohort approximately 55% of patients relapsed after anti-TNF withdrawal with a median time to relapse of 32 and 18 months in CD and UC, respectively. Retreatment with the same anti-TNF agent was successful in 84% of patients. We found several predictors associated with a higher relapse rates which were higher trough levels prior to cessation and a young age at diagnosis. Low fecal calprotectin levels were associated with a lower relapse rate in CD patients and continuing immunomodulators was protective in UC patients.

# Chapter 12

## General discussion and future perspectives

Our knowledge regarding IBD has increased significantly in the last decades. Over the years, the number of therapeutic options have increased with the introduction of several biologics and a small molecule (tofacitinib). It is to be expected that the number of treatment options will only increase in the future, with many ongoing clinical trials.

As a result of the expanding pool of treatment options, treatment paradigms in IBD are evolving. Doctors are not satisfied with improvement of symptoms alone, but strive for complete resolution of inflammation. However, this goal can sometimes be hard to reach and we are still a long way from curing IBD.

Nevertheless, ambitious treatment targets require for tight monitoring and optimization of current treatment options. In this thesis we aimed to further contribute to these important pillars in the management of IBD patients.

**Part I: Implementation of intestinal ultrasound**

In **part I** of this thesis, our goal was to further optimize the implementation of IUS for the assessment of disease activity, follow-up of treatment and for point-of-care (POC) assessment of IBD patients.

There are several advantages to POC testing such as less outpatients visits, facilitation of quick decision making, less treatment delay and potentially lower costs. For these reasons POC management of IBD patients is becoming of increasing interest.[1, 2] IUS is the only imaging modality suitable for POC follow-up of IBD patients.[1-3] Only a few studies have been performed on this topic which have shown promising results.[1, 4] We showed that POC IUS significantly impacts clinical decision making and that it has the potential to reduce additional endoscopies and MRI scans. When we first started to perform IUS in our clinic, we mainly focused on confirming disease flare-ups. Over the years, the paradigm expanded to treatment monitoring. We proposed a POC IUS algorithm for monitoring of IBD patients which should be tested and optimized in clinical practice. Mainly the best timing for scheduled IUS in CD patients who are in clinical remission is unknown. However, it seems reasonable to perform IUS more often in CD patients with a complex disease history while the frequency in monitoring can probably be reduced in patients with long-term transmural remission, although there is no evident data backing this statement. Nevertheless, it has been stated that transmural healing is associated with better long-term outcomes.[5] Large cohorts/registries with long-term follow up of IBD patients monitored with IUS should be collected to determine the best intervals for monitoring with IUS, especially in CD patients.

With the implementation of POC IUS in our clinic from the ground up, we believe to have set an example for what this can lead to. To date this is still evolving with more of our physicians and specialized nurses implementing the technique. We hope that more IBD clinics in the Netherlands will follow our example and to our knowledge, several have begun to do so.

For optimal POC IUS, accurate assessment of disease severity is essential. Immediate knowledge on the outpatient clinic regarding severity has the potential to reduce development of severe complications when patients are assessed on a frequent basis. Additionally, it can support clinicians when to act quickly and to treat more aggressively without the need to wait for further investigation with endoscopy or MRI.[6, 7]

The definitions for grading of disease severity with IUS are evolving.[6] By systematically reviewing available IUS indices in **chapter 2,** we highlighted problems with the existing IUS indices for grading of disease severity and our findings were confirmed by another group 2 years later.[8]

Especially in UC patients, IUS for grading of disease severity has been understudied. We showed that many UC patients with a bowel wall thickness (BWT) below 3mm may have active mucosal inflammation, which is usually mild (i.e. Mayo 1). In other studies a BWT cut-off of 3 or 4 mm is often used to classify active disease.[9] We also showed that a combination of FCP and IUS results in higher sensitivity for detection of disease activity which can be of particular use in patients with proctitis and mild disease activity. Other available UC indices appear to be less sensitive for the detection of mild disease activity and have used an endoscopic Mayo score of 1 as cut-off for mucosal healing.[8-10] Although this definition is often used in clinical trials, mounting evidence suggests that complete endoscopic remission (i.e. eMayo 0) or even histological healing are preferable long-term outcomes.[7, 11] Therefore, more sensitive IUS criteria are needed and we took one step further towards defining these criteria.

However, it is important to note that detection of mild disease with IUS will remain challenging. For instance, many UC patients with BWT between 2-3 mm or even above 3 mm do not have active inflammation. This can be a result of factors such as wrong measurements (e.g. measuring a collapsed bowel, measuring oblique), tissue fibrosis, measurement of other abnormalities such as (pseudo)polyps or just variations in normal anatomy. These potential confounders should always be taken into account when performing IUS and addition of other IUS parameters in combination with clinical and biochemical parameters can aid to determine if there is indeed active inflammation in UC patients.

In this thesis we did not focus on detection of disease activity with IUS in CD patients since the available data for this purpose were already quite convincing.[12] Many IUS indices for disease activity in CD patients had already been developed (albeit mostly with suboptimal methodology) and several new ones have become available in recent years [13, 14] Saevik et al. developed a simple ultrasound activity score (SUS-CD score).[13] This index uses different degrees of BWT and CDS and showed good correlation with the SES-CD. We are currently working on a joint effort to externally validate this index by using cohorts from different clinics, including our own. The IBUS-SAS score is a segmental activity score developed by Novak et al. and includes BWT, hyperaemia, WLS and inflammatory mesenteric fat as parameters.[14] This index was developed on a theoretical basis through expert consensus but

should be tested in clinical practice using endoscopy as the reference standard. With all these initiatives it is clear that there are currently multiple options to assess disease severity with IUS in IBD patients. Furthermore there are ongoing initiatives for development of new indices. All the newly developed indices have strengths and weaknesses and there is no general consensus on which one to use in clinical practice. In the future we should focus on optimizing and harmonizing current indices and develop uniform standards that can be used in clinics around the globe and in clinical trials.

Another important factor in this regard is agreement of measurements between observers. Ideally, a good IUS index show good reproducibility between observers. Some studies have already been performed on this topic and future studies should further focus on optimizing inter-observer agreement of the most important IUS parameters.[14, 15] Furthermore, technical differences between US devices have never been investigated, which can also influence the reliability of IUS indices. This is for instance of importance when assessing parameters such as Colour Doppler Signal (CDS) and contrast enhanced ultrasound (CEUS). These parameters are more dependent on processing of ultrasound machines and it may very well be that there are differences in sensitivity for these parameters between different US machines. Studies comparing equipment have never been performed and are therefore of particular interest in the future.

Besides detecting and grading disease severity, the second important pillar of POC IUS in IBD patients is monitoring treatment effect. In **chapter 5** we concluded that conventional parameters BWT and CDS might be sufficient to predict endoscopic response in an early phase after treatment initiation and that CEUS may not add much for this purpose. Some other studies have shown that improvement of CEUS parameters could be associated with endoscopic response.[16-19] However, since CEUS is time consuming, more prone to investigator variability and difficult to analyze on the spot, it is probably not of much added value in the POC setting. The most important parameters seem to be a sufficient decrease in BWT and CDS after treatment initiation. Recently, it has been proposed by a panel of experts that reduction in BWT 25% or>2.0 mm or >1.0 mm and 1 CDS reduction could be used to assess response.[20] These recommendations were based on existing literature and should be tested in clinical practice.

In this thesis we did not focus on monitoring treatment in UC patients. Several studies have shown good accuracy of IUS for this purpose.[10, 21] Preliminary data from our group shows that also in UC patients BWT and CDS are sufficient to predict endoscopic response in an early phase after initiation of anti-inflammatory treatment. Thus it seems reasonable to conclude that improvement in BWT and CDS are sufficient when measuring treatment response in both CD and UC patients. Other IUS parameters also improve after treatment but tend to do so in a later stage. Complete transmural healing and normalization of all IUS parameters are probably associated with the best long-term outcomes.[5, 22, 23] However, this statement should be further investigated in long-term prospective registries.

Still, there are several other knowledge gaps remaining. One of the most intriguing questions is if IUS could be used to distinguish inflammation from fibrosis. It has been proposed that CEUS, elastography and even conventional CDS may be useful for this purpose in CD patients.[24-28] Knowledge on the presence of fibrosis could be of great value for clinical decision making, especially in patients with stricturing disease. Predominantly inflammatory strictures could be treated with anti-inflammatory medication while predominantly fibrotic strictures would require endoscopic dilation or surgery. Although several studies on the assessment of fibrosis in IBD patients have been performed, the data are conflicting.[26, 28-33] An important factor is that there are very few data comparing resection specimens with IUS findings.[34] In an ongoing study at our clinic we are comparing IUS findings with presence of fibrosis resection specimens (Netherlands Trial Register: NL9105). We hope to further elucidate the potential of CEUS and elastography for assessment of fibrosis in IBD patients which could potentially greatly improve clinical decision making.

In conclusion, the implementation of IUS for management of IBD patients has improved significantly in recent years but there is still room for further optimization. Future research should focus on harmonizing IUS indices for grading of severity, optimization of POC IUS implementation, monitoring treatment effect and the assessment of intestinal fibrosis.

**Part II: Optimal use of biologics**

In **part** II of this thesis we focused on optimal use of biologics. Biologics are being used increasingly for treatment of IBD patients. In **chapter 9** we showed the increasing trend of anti-TNF use in the Netherlands. It is likely that this trend has continued in recent years and with the introduction of newer biologics the proportion of IBD patients on biologic treatment is only increasing. This increasing use is associated with significant costs, although the costs for anti-TNF agents have reduced with the introduction of biosimilars.[35] Nevertheless, management of treatment costs is one of the most important reasons to optimize biologic treatment. In other words, only patients that really need the treatment should receive biologics, treatment should be discontinued timely when it's ineffective and dosing and co-medication should be optimized for optimal results with the lowest possible costs.

*Anti-drug antibody formation*

Prevention of anti-drug antibody (ADA) formation, which is mostly of concern for the anti-TNF agents, is one of the most important factors in treatment optimization. In **chapter 8** we studied the effect of combination treatment on ADA formation in a large and comprehensive systematic review. Combination treatment significantly reduces ADA formation for infliximab and to a lesser extent for several anti-TNF agents. For this reason (besides others), infliximab in combination with an immunomodulator is the currently the most effective treatment available for moderate to severe disease. Furthermore it costs less than the newer therapeutic agents (e.g. vedolizumab, ustekinumab and tofacitinib).

Combination therapy is therefore preferred when starting infliximab and could also be considered when initiating other anti-TNF agents. However, immunomodulators (just like anti-TNF agents) are not always tolerated which reduces the number of patients receiving combination therapy, leading to suboptimal outcomes.[36, 37] An understudied question in this regard is what doses of immunomodulators actually suffice for ADA reduction. It's conceivable that lower doses of immunomodulators are sufficient for prevention of ADA formation which would potentially lead to a better side-effect profile. We could not answer this question in this thesis. Future studies should therefore focus on anti-TNF treatment in combination with lower doses of immunomodulators.

A similar statement can be made for addition of immunomodulators in patients on anti-TNF monotherapy (which is often adalimumab) that develop ADA There is some evidence from retrospective studies that addition of an immunomodulator suppresses ADA formation with therapeutic drug levels as a result.[38, 39] This strategy should therefore be considered in patients on anti-TNF monotherapy that develop ADA and that have the time to await its effect. However, prospective studies are needed to further study this concept. Questions such as for what ADA titers would such a strategy be viable, in what proportion of patients would this strategy have effect and how long would it take for ADA to disappear require answers.

When considering anti-TNF dosing in relation to ADA development, there is also room for improvement. Currently, in many patients that lose response with sub-therapeutic anti-TNF serum levels, the dose of the drug is increased or the treatment interval is decreased.[40] In clinical practice, ADA are usually measured with drug sensitive assays. However, there is increasing evidence that many patients may have lower drug levels due to ADA that are not measured presence of the drug, leading to sub-therapeutic levels.[41, 42] Since ADA formation can lead to accelerated clearance, it may play an important role in patients with sub-therapeutic levels.[43] Instead of increasing the dose or decreasing the interval, addition of an immunomodulator could potentially have a similar effect on drug serum levels and thus on regaining efficacy these patients. Future studies should focus on the use of drug-tolerant assays in clinical practices for this purpose.

*Biologic discontinuation*

Another topic regarding optimal use of biologics which was addressed in this thesis, is discontinuation of anti-TNF agents in patients who achieved remission. Our data and data from other studies have shown that many patients relapse after anti-TNF discontinuation.[44-50] In a recent systematic review all the available studies on CD patients were evaluated and a relapse prediction model was developed which showed moderate discriminative ability.[44] Predictive factors for relapse included clinical symptoms at discontinuation, a young age at diagnosis, no use of concomitant immunomodulators, smoking, second line anti-TNF, upper gastrointestinal involvement, adalimumab vs infliximab, age at cessation, C-reactive protein, longer disease duration and fecal calprotectin. None of the factors showed high

discriminability. Furthermore, the model has not been validated yet. In a Cochrane systematic review, RCTs addressing anti-TNF discontinuation in CD patients with quiescent disease were reviewed.[50] The authors concluded that outcomes after discontinuation are uncertain and that more adequate studies are needed. Continuation of an immunomodulator could maybe prevent relapse, but current data are not convincing. For UC patients data are very limited and no prediction model exists to date. Future studies focusing on UC patients that discontinue anti-TNF treatment would therefore be of particular interest.

Nevertheless, it seems difficult to adequately identify patients that have a low relapse chance after anti-TNF discontinuation. When using common sense, elective anti-TNF discontinuation is probably not wise in patients that were treated for severe penetrating disease or that have a complex disease history with multiple surgeries. One should probably also be careful in patients that developed ADA at some point during treatment since this may later influence the effect of retreatment (e.g. higher chance treatment failure due to ADA development). Anti-TNF discontinuation should therefore mainly be considered in patients with relatively uncomplicated disease since most studies have shown good effect of retreatment in more than 80% of patients. However, this still means that approximately 1 in 5 patients cannot be successfully retreated. Studies focusing on identifying patients that have the highest chance of successful retreatment are therefore of particular interest and should be conducted. Furthermore, discontinuation and retreatment studies for the newer biologics are lacking and should be conducted in the future.

If it is decided to discontinue biologic treatment in patients in remission, it seems sensible to tightly monitor patients thereafter. As stated in **part I** of this thesis, IUS would be the best imaging tool for this purpose. Prospective studies in patients that discontinue anti-inflammatory treatment that are in transmural remission and are tightly monitored with IUS thereafter would be of great interest. Identifying patients with a pre-clinical relapse with IUS would allow for quick re-initiation of treatment before more bowel damage has occurred and this could potentially increase the efficacy of retreatment.

**Towards a personalized treatment approach**

To conclude this thesis we want to spend a few words envisioning the future of treatment of IBD patients. Currently, the treatment paradigm consists of starting with anti-inflammatory drugs such as corticosteroids, immunomodulators and mesalazine. If these don't work treatment is upscaled tot anti-TNF therapy and if this fails treatment is escalated to the newer biologics such as vedolizumab or ustekinumab or a Jak inhibitor (tofacitinib). There are several new drugs becoming available in the future which target different molecules in different inflammatory pathways. Furthermore, the field of fecal transplantation for treatment of IBD is progressing[51] and surgical approaches are also evolving with an important place for optimally timed surgery.[52, 53] All these treatment options potentially pave the way to a more personalized treatment approach. The main question in this regard is what inflammatory pathways drive

inflammation and could this guide the decision process? We won't further elaborate since this is beyond the scope of this thesis. Nevertheless, we envision a future in which patients receive optimally tailored treatment regimens with tight monitoring with non-invasive tools (IUS and biomarkers) allowing for timely treatment optimization and for safe 'drug holidays'. There are still so many things to improve for IBD patients. We hope to have contributed a bit further with the work described in this thesis.

## References


1. Sathananthan D, Rajagopalan A, Van De Ven L, et al. Point-of-care gastrointestinal ultrasound in inflammatory bowel disease: An accurate alternative for disease monitoring. JGH Open. 2020;4(2):273-9. Epub 2020/04/14.

2. Bryant RV, Friedman A, Wright EK, et al. Gastrointestinal ultrasound in inflammatory bowel disease: an underused resource with potential paradigm-changing application. Gut. 2018. Epub 2018/02/14.

3. de Voogd FAE, Verstockt B, Maaser C, et al. Point-of-care intestinal ultrasonography in inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2021. Epub 2021/01/29.

4. Novak K, Tanyingoh D, Petersen F, et al. Clinic-based Point of Care Transabdominal Ultrasound for Monitoring Crohn's Disease: Impact on Clinical Decision Making. Journal of Crohn's & colitis. 2015;9(9):795-801. Epub 2015/06/17.

5. Geyl S, Guillo L, Laurent V, et al. Transmural healing as a therapeutic goal in Crohn's disease: a systematic review. Lancet Gastroenterol Hepatol. 2021;6(8):659-67. Epub 2021/06/07.

6. Maaser C, Sturm A, Vavricka SR, et al. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. Journal of Crohn's & colitis. 2019;13(2):144-64. Epub 2018/08/24.

7. Turner D, Ricciuto A, Lewis A, et al. STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): Determining Therapeutic Goals for Treat-to-Target strategies in IBD. Gastroenterology. 2021;160(5):1570-83. Epub 2020/12/29.

8. Goodsall TM, Nguyen TM, Parker CE, et al. Systematic Review: Gastrointestinal Ultrasound Scoring Indices for Inflammatory Bowel Disease. Journal of Crohn's & colitis. 2021;15(1):125-42. Epub 2020/07/03.

9. Allocca M, Fiorino G, Bonovas S, et al. Accuracy of Humanitas Ultrasound Criteria in Assessing Disease Activity and Severity in Ulcerative Colitis: A Prospective Study. Journal of Crohn's & colitis. 2018;12(12):1385-91. Epub 2018/08/08.

10. Parente F, Molteni M, Marino B, et al. Are colonoscopy and bowel ultrasound useful for assessing response to short-term therapy and predicting disease outcome of moderate-to-severe forms of ulcerative colitis?: a prospective study. The American journal of gastroenterology. 2010;105(5):1150-7. Epub 2009/12/10.

11. Bryant RV, Winer S, Travis SP, et al. Systematic review: histological remission in inflammatory bowel disease. Is 'complete' remission the new treatment paradigm? An IOIBD initiative. Journal of Crohn's & colitis. 2014;8(12):1582-97. Epub 2014/10/01.

12. Panes J, Bouzas R, Chaparro M, et al. Systematic review: the use of ultrasonography, computed tomography and magnetic resonance imaging for the diagnosis, assessment of activity and abdominal complications of Crohn's disease. Alimentary pharmacology & therapeutics. 2011;34(2):125-45. Epub 2011/05/28.

13. Sævik F, Eriksen R, Eide GE, et al. Development and Validation of a Simple Ultrasound Activity Score for Crohn's Disease. Journal of Crohn's & colitis. 2021;15(1):115-24. Epub 2020/06/07.

14. Novak KL, Nylund K, Maaser C, et al. Expert consensus on optimal acquisition and development of the International Bowel Ultrasound Segmental Activity Score (IBUS-SAS): a reliability and inter-rater variability study on intestinal ultrasonography in Crohn's Disease. Journal of Crohn's & colitis. 2020. Epub 2020/10/25.

15. De Voogd F, Wilkens R, Gecse K, et al. A reliability study - strong inter-observer agreement of an expert panel for intestinal ultrasound in ulcerative colitis. Journal of Crohn's & colitis. 2021. Epub 2021/01/10.

16. Quaia E, Gennari AG, Cova MA. Early Predictors of the Long-term Response to Therapy in Patients With Crohn Disease Derived From a Time-Intensity Curve Analysis After Microbubble Contrast Agent Injection. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine. 2019;38(4):947-58. Epub 2018/09/13.

17. Ripolles T, Martinez MJ, Paredes JM, et al. Crohn disease: correlation of findings at contrast-enhanced US with severity at endoscopy. Radiology. 2009;253(1):241-8. Epub 2009/07/29.

18. Saevik F, Nylund K, Hausken T, et al. Bowel perfusion measured with dynamic contrast-enhanced ultrasound predicts treatment outcome in patients with Crohn's disease. Inflammatory bowel diseases. 2014;20(11):2029-37. Epub 2014/09/05.

19. Laterza L, Ainora ME, Garcovich M, et al. Bowel contrast-enhanced ultrasound perfusion imaging in the evaluation of Crohn's disease patients undergoing anti-TNFα therapy. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2021;53(6):729-37. Epub 2020/09/10.

20. Ilvemark J, Hansen T, Goodsall TM, et al. Defining transabdominal Intestinal Ultrasound treatment response and remission in Inflammatory Bowel Disease: Systematic review and expert consensus statement. Journal of Crohn's & colitis. 2021. Epub 2021/10/07.

21. Maaser C, Petersen F, Helwig U, et al. Intestinal ultrasound for monitoring therapeutic response in patients with ulcerative colitis: results from the TRUST&UC study. Gut. 2020;69(9):1629-36. Epub 2019/12/22.

22. Castiglione F, Testa A, Rea M, et al. Transmural healing evaluated by bowel sonography in patients with Crohn's disease on maintenance treatment with biologics. Inflammatory bowel diseases. 2013;19(9):1928-34. Epub 2013/07/10.

23. Helwig U, Fischer I, Hammer L, et al. Transmural Response and Transmural Healing Defined by Intestinal Ultrasound - New Potential Therapeutic Targets? Journal of Crohn's & colitis. 2021. Epub 2021/06/30.

24. Wilkens R, Hagemann-Madsen RH, Peters DA, et al. Validity of Contrast-enhanced Ultrasonography and Dynamic Contrast-enhanced MR Enterography in the Assessment of Transmural Activity and Fibrosis in Crohn's Disease. Journal of Crohn's & colitis. 2018;12(1):48-56. Epub 2017/10/06.

25. Coelho R, Ribeiro H, Maconi G. Bowel Thickening in Crohn's Disease: Fibrosis or Inflammation? Diagnostic Ultrasound Imaging Tools. Inflammatory bowel diseases. 2017;23(1):23-34. Epub 2016/12/22.

26. Fraquelli M, Branchi F, Cribiu FM, et al. The Role of Ultrasound Elasticity Imaging in Predicting Ileal Fibrosis in Crohn's Disease Patients. Inflammatory bowel diseases. 2015;21(11):2605-12. Epub 2015/08/01.

27. Stidham RW, Xu J, Johnson LA, et al. Ultrasound elasticity imaging for detecting intestinal fibrosis and inflammation in rats and humans with Crohn's disease. Gastroenterology. 2011;141(3):819-26.e1. Epub 2011/07/26.

28. Nylund K, Jirik R, Mezl M, et al. Quantitative contrast-enhanced ultrasound comparison between inflammatory and fibrotic lesions in patients with Crohn's disease. Ultrasound in medicine & biology. 2013;39(7):1197-206. Epub 2013/05/07.

29. Bettenworth D, Bokemeyer A, Baker M, et al. Assessment of Crohn's disease-associated small bowel strictures and fibrosis on cross-sectional imaging: a systematic review. Gut. 2019;68(6):1115-26. Epub 2019/04/05.

30. Quaia E, Gennari AG, van Beek EJR. Differentiation of Inflammatory from Fibrotic Ileal Strictures among Patients with Crohn's Disease through Analysis of Time-Intensity Curves Obtained after Microbubble Contrast Agent Injection. Ultrasound in medicine & biology. 2017;43(6):1171-8. Epub 2017/04/08.

31. Higgins PD. Measurement of Fibrosis in Crohn's Disease Strictures with Imaging and Blood Biomarkers to Inform Clinical Decisions. Digestive diseases (Basel, Switzerland). 2017;35(1-2):32-7. Epub 2017/02/02.

32. Baumgart DC, Muller HP, Grittner U, et al. US-based Real-time Elastography for the Detection of Fibrotic Gut Tissue in Patients with Stricturing Crohn Disease. Radiology. 2015;275(3):889-99. Epub 2015/02/11.

33. Lenze F, Wessling J, Bremer J, et al. Detection and differentiation of inflammatory versus fibromatous Crohn's disease strictures: prospective comparison of 18F-FDG-PET/CT, MR-enteroclysis, and transabdominal ultrasound versus endoscopic/histologic evaluation. Inflammatory bowel diseases. 2012;18(12):2252-60. Epub 2012/02/24.

34. Lu C, Gui X, Chen W, et al. Ultrasound Shear Wave Elastography and Contrast Enhancement: Effective Biomarkers in Crohn's Disease Strictures. Inflammatory bowel diseases. 2017;23(3):421-30. Epub 2017/01/28.

35. Solitano V, D'Amico F, Fiorino G, et al. Biosimilar switching in inflammatory bowel disease: from evidence to clinical practice. Expert review of clinical immunology. 2020;16(10):1019-28. Epub 2020/09/22.

36. Targownik LE, Leung S, Lix LM, et al. Persistence With Immunomodulator Monotherapy Use And Incidence of Therapeutic Ineffectiveness Among Users of Immunomodulator Monotherapy in IBD. The American journal of gastroenterology. 2018;113(8):1206-16. Epub 2018/06/22.

37. Ward MG, Irving PM, Sparrow MP. How should immunomodulators be optimized when used as combination therapy with anti-tumor necrosis factor agents in the management of inflammatory bowel disease? World journal of gastroenterology : WJG. 2015;21(40):11331-42. Epub 2015/11/04.

38. Strik AS, van den Brink GR, Ponsioen C, et al. Suppression of anti-drug antibodies to infliximab or adalimumab with the addition of an immunomodulator in patients with inflammatory bowel disease. Alimentary pharmacology & therapeutics. 2017;45(8):1128-34. Epub 2017/02/24.

39. Ben-Horin S, Waterman M, Kopylov U, et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2013;11(4):444-7. Epub 2012/10/30.

40. Mattoo VY, Basnayake C, Connell WR, et al. Systematic review: efficacy of escalated maintenance anti-tumour necrosis factor therapy in Crohn's disease. Alimentary pharmacology & therapeutics. 2021;54(3):249-66. Epub 2021/06/22.

41. Van Stappen T, Vande Casteele N, Van Assche G, et al. Clinical relevance of detecting anti-infliximab antibodies with a drug-tolerant assay: post hoc analysis of the TAXIT trial. Gut. 2018;67(5):818-26.

42. Dreesen E, Van Stappen T, Ballet V, et al. Anti-infliximab antibody concentrations can guide treatment intensification in patients with Crohn's disease who lose clinical response. Alimentary pharmacology & therapeutics. 2018;47(3):346-55. Epub 2017/12/12.

43. Brandse JF, Mould D, Smeekes O, et al. A Real-life Population Pharmacokinetic Study Reveals Factors Associated with Clearance and Immunogenicity of Infliximab in Inflammatory Bowel Disease. Inflammatory bowel diseases. 2017;23(4):650-60. Epub 2017/02/15.

44. Pauwels RWM, van der Woude CJ, Nieboer D, et al. Prediction of Relapse After Anti-Tumor Necrosis Factor Cessation in Crohn's Disease: Individual Participant Data Meta-analysis of 1317 Patients From 14

Studies. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2021. Epub 2021/05/03.

45. Casanova MJ, Chaparro M, Garcia-Sanchez V, et al. Evolution After Anti-TNF Discontinuation in Patients With Inflammatory Bowel Disease: A Multicenter Long-Term Follow-Up Study. The American journal of gastroenterology. 2017;112(1):120-31. Epub 2016/12/14.

46. Gisbert JP, Marin AC, Chaparro M. The Risk of Relapse after Anti-TNF Discontinuation in Inflammatory Bowel Disease: Systematic Review and Meta-Analysis. The American journal of gastroenterology. 2016;111(5):632-47. Epub 2016/03/24.

47. Fiorino G, Cortes PN, Ellul P, et al. Discontinuation of Infliximab in Patients With Ulcerative Colitis Is Associated With Increased Risk of Relapse: A Multinational Retrospective Cohort Study. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2016;14(10):1426-32.e1. Epub 2016/06/19.

48. Gisbert JP, Marin AC, Chaparro M. Systematic review: factors associated with relapse of inflammatory bowel disease after discontinuation of anti-TNF therapy. Alimentary pharmacology & therapeutics. 2015;42(4):391-405. Epub 2015/06/16.

49. Molander P, Farkkila M, Salminen K, et al. Outcome after discontinuation of TNFalpha-blocking therapy in patients with inflammatory bowel disease in deep remission. Inflammatory bowel diseases. 2014;20(6):1021-8. Epub 2014/05/07.

50. Boyapati RK, Torres J, Palmela C, et al. Withdrawal of immunosuppressant or biologic therapy for patients with quiescent Crohn's disease. The Cochrane database of systematic reviews. 2018;5(5):Cd012540. Epub 2018/05/15.

51. Mocanu V, Rajaruban S, Dang J, et al. Repeated Fecal Microbial Transplantations and Antibiotic Pre-Treatment Are Linked to Improved Clinical Response and Remission in Inflammatory Bowel Disease: A Systematic Review and Pooled Proportion Meta-Analysis. J Clin Med. 2021;10(5). Epub 2021/04/04.

52. de Groof EJ, Stevens TW, Eshuis EJ, et al. Cost-effectiveness of laparoscopic ileocaecal resection versus infliximab treatment of terminal ileitis in Crohn's disease: the LIR!C Trial. Gut. 2019;68(10):1774-80. Epub 2019/06/25.

53. Stevens TW, Haasnoot ML, D'Haens GR, et al. Laparoscopic ileocaecal resection versus infliximab for terminal ileitis in Crohn's disease: retrospective long-term follow-up of the LIR!C trial. Lancet Gastroenterol Hepatol. 2020;5(10):900-7. Epub 2020/07/04.

# Appendix

# Nederlandse samenvatting

## Deel I: Implementatie van intestinale echografie

**Deel I** van dit proefschrift richt zich op optimale implementatie van intestinale echografie voor patiënten met een inflammatoire darmziekte (IBD). Cruciaal voor de behandeling van IBD patiënten is kennis over de ernst van darminflammatie omdat dit de behandelkeuze beïnvloedt. Bestaande echografische scores voor het graderen van ziekteactiviteit bij IBD patiënten waren niet eerder systematisch geëvalueerd. In **hoofdstuk 2** hebben we 21 studies die een echografische score voor IBD beschreven geïdentificeerd middels literatuuronderzoek, waarvan 11 studies (7 ziekte van Crohn en 4 colitis ulcerosa) voldeden aan onze inclusiecriteria voor nadere analyse. Echografische scores die waren ontwikkeld met klinische ziekteactiviteit als referentie standaard werden bijvoorbeeld niet geïncludeerd, omdat klinische ziekteactiviteit niet altijd betrouwbaar is en bovendien niet bruikbaar is voor bepaling van o.a. de ziektelokalisatie. De kwaliteit van de studies werd beoordeeld met een gemodificeerde versie van de QUADAS-2 tool. Vijf studies werden gescoord als hoge kwaliteit, 3 als matige en 3 als slechte kwaliteit. De echografische parameters gebruikt in de verschillende scores waren darmwanddikte, kleuren Doppler signaal, comprimeerbaarheid van de darm, peristaltiek, haustraties, vetinfiltratie, contrast aankleuring en strain elastografisch patroon. Slechts 1 score voor de ziekte van Crohn was gevalideerd in een tweede cohort. We concludeerden daarom dat de methodologie voor de meeste scores suboptimaal was en dat meer stringente methodologie vereist was voor de ontwikkeling van nieuwe echografische scores.

In navolging op onze conclusies uit **hoofdstuk 2** hebben we een nieuwe echografische score ontwikkeld voor colitis ulcerosa patiënten in **hoofdstuk 3** (UC-IUS index). Verschillende echografische parameters (darmwanddikte, kleuren Doppler signaal, haustraties, vetinfiltratie, vergrote lymfklieren, stratificatie van de darmwand lagen) werden vergeleken met 60 colitis ulcerosa patiënten met verschillende categorieën van endoscopische ziekteactiviteit. Tevens werden inter- en intra-beoordelaarsbetrouwbaarheid getest voor de meeste parameters met gebruik van opgenomen cineloops. Darmwanddikte afkap punten van 2.1, 3.2 en 3.9 mm bleken het meest predictief voor het detecteren van respectievelijk Mayo 1, 2, en 3 endoscopische ziekteactiviteit. Stipjes van kleuren Doppler signaal waren geassocieerd met enige vorm van ziekteactiviteit en uitgestrekt kleuren Doppler signaal was meer geassocieerd met matig tot ernstige endoscopische ziekteactiviteit. Een verstoord haustratiepatroon was sterk geassocieerd met actieve ziekte en de aanwezigheid van vetinfiltratie was geassocieerd met ernstige ziekteactiviteit. De overeenkomst tussen beoordelaars was goed voor darmwanddikte en kleuren Doppler signaal. Voor haustratie patroon varieerde de overeenkomst van matig tot goed, hetgeen aantoont dat deze parameter lastiger consistent te beoordelen is. Desalniettemin werd de parameter geïncludeerd in de index vanwege de sterke associatie met ziekteactiviteit. Betrouwbaarheid tussen beoordelaars

voor de parameter vetinfiltratie werd niet onderzocht omdat de opgenomen cineloops hier niet geschikt voor waren. Op basis van de resultaten werd een punten scoringssysteem geconstrueerd met de parameters darmwanddikte, kleuren Doppler signaal, haustratrie patroon en vetinfiltratie. Het scoresysteem zal worden gevalideerd op nieuwe cohorten van colitis ulcerosa patiënten, voor en na behandeling met anti-inflammatoire medicatie. Het scoresysteem toonde sterke correlatie met endoscopische ziekte activiteit op basis van interne validatie en de betrouwbaarheid tussen beoordelaars was ook sterk.

Een belangrijk doel van het scoresysteem ontwikkeld in **hoofdstuk 3** is de implementatie voor point-of-care beoordeling van IBD patiënten. In **hoofdstuk 4** hebben we de impact van point-of-care echografie op behandelbeslissingen in onze kliniek onderzocht op basis van 345 echografische onderzoeken in 2 verschillende cohorten (250 cohort 1; 195 cohort 2). Cohort 1 werd verzameld in de periode van januari 2016 tot en met juli 2018 en cohort 2 van oktober 2019 tot en met december 2019. Echografische uitkomsten werden vergeleken met symptomen, biomarkers, endoscopie en MRI. Point-of-care echografie beïnvloedde behandelbeslissingen in ongeveer 60% van de gevallen en medicatie gebruik werd aangepast in ongeveer 50% van de gevallen. Echografie toonde inflammatie of complicaties in menig patiënt met de ziekte van Crohn zonder symptomen, terwijl de aanwezigheid van symptomen bij colitis ulcerosa patiënten meestal betrouwbaar was voor de aanwezigheid van inflammatie. De echografische uitkomsten correleerden sterk met endoscopie en MRI. Daarnaast toonden we een verandering in implementatie in tussen de 2 cohorten met een verschuivend paradigma naar meer monitoring van behandeling en toegenomen toepassing van echografie sinds begin van implementatie. Gebaseerd op de resultaten hebben we een point-of-care echografie algoritme voor opvolging van IBD patiënten voorgesteld.

In **hoofdstuk 5** hebben we het gebruik van conventionele echografie en contrast echografie onderzocht voor het vervolgen van behandeling met anti-TNF therapie in 40 patiënten met de ziekte van Crohn. Het doel was om te onderzoeken of vroege verandering in echografische parameters kunnen worden gebruikt voor het voorspellen van endoscopische respons later in de behandeling. Patiënten ondergingen echografie voor start behandeling (T0), na 4 tot 8 weken (T1) en na 12 tot 34 weken (T3). Analyse per darm segment (colon sigmoideum, descendens, transversum, ascendens en terminaal ileum) toonde goede correlatie tussen endoscopie en echografie. De betrouwbaarheid tussen beoordelaars was tevens goed. Een afname in darmwanddikte van 18% na 4 tot 8 weken en 29% na ongeveer 6 maanden voorspelde endoscopische respons na ongeveer 6 maanden. Het kleuren Doppler signaal nam ook significant af op zowel T1 als T2 in patiënten met endoscopische respons. De meeste andere conventionele echografische parameters verbeterden ook bij patiënten met endoscopische respons maar waren minder of niet voorspellend op T1. Wat betreft de contrast echografische parameters was de procentuele afname in 'wash out rate' opT1 significant verschillend tussen endoscopische responders versus non-responders. Daarnaast was de procentuele afname in 'peak enhancement', 'wash in rate', 'wash in perfusion index'en 'wash

out rate' op T2 groter in endoscopische responders. In een multivariabel regressie model waren vroege afname in darmwanddikte en kleuren Doppler signaal voorspellend voor het bereiken van endoscopische respons. Toevoeging van andere conventionele echografische parameters verbeterde het model niet. 'Wash out rate'was de enige contrast echografische parameter die het model op T2 iets verbeterde, maar niet op T1. Daarom concludeerden we dat metingen van darmwanddikte en kleuren Doppler signaal waarschijnlijk voldoende zijn voor vroege beoordeling van behandel effect in patiënten met de ziekte van Crohn.

## Deel II: Optimaal gebruik van biologicals

**Deel II** van dit proefschrift behelst enkele studies over behandeling met biologicals (met name anti-TNF therapie) in IBD patiënten. In **hoofdstuk 6 & 7** bespreken we optimalisatie van anti-TNF therapie en inzichten omtrent combinatie therapie. We bediscussiëren het belang van patiënt selectie, screening, geïndividualiseerde behandeling, stoppen en herstarten van behandeling in **hoofdstuk 6**. In **hoofdstuk 7** verdiepen we ons in combinatie immunosuppressie (anti-TNF en immunomodulators), gericht op manier van werking, effectiviteit, veiligheid en effect op stoppen van anti-TNF therapie. Beide hoofdstukken bevatten bruikbare aanbevelingen voor de klinische praktijk.

Omdat anti-drug antilichaam (ADA) vorming tegen biologicals (voornamelijk de anti-TNF middelen) een belangrijke oorzaak is voor het falen van therapie, hebben we ons nader gericht op dit probleem in **hoofdstuk 8**. We hebben systematisch literatuur onderzoek verricht naar ADA vorming tegen alle beschikbare biologicals voor de behandeling van IBD. Data van 68 studies werden geanalyseerd en 33 studies werden geïncludeerd in de meta-analyse. Gepoolde incidentie van ADA formatie verschilde sterk per biological en was hoger voor de oudere (anti-TNF) middelen. Het gebruik van 'drug sensitieve' assays in de meeste studies belemmerde echter het bestuderen en vergelijken van ware ADA incidentie. Combinatietherapie met immunomodulatoren verminderde ADA detectie voor de meeste biologicals. ADA ontwikkeling was bovendien geassocieerd met een slechtere klinische respons en meer infusie reacties in patiënten behandeld met infliximab.

In **hoofdstuk 9** bestudeerden we anti-TNF gebruik in Nederland op basis van anonieme zorgverzekeringsdata van ongeveer 22.000 IBD patiënten. Tussen 2011 en 2014 werd behandeling met infliximab gestart in 855 patiënten en tussen 2008 en 2014 werd behandeling met adalimumab gestart in 1199 patiënten. Het anti-TNF gebruik in Nederland steeg significant over de jaren en de proportie patiënten die een geïntensifieerde dosis kreeg nam toe over de tijd. Het gebruik van een immunomodulator was geassocieerd met langere tijd tot corticosteroïd initiatie maar niet met langer anti-TNF gebruik in het bestudeerde cohort.

In **hoofdstuk 10** hebben we de incidentie van ziekte opvlamming na staken van anti-TNF therapie en voorspellers daarvoor bestudeerd in een levensgetrouw cohort van 101 IBD patiënten (77 ziekte van Crohn, 24 colitis ulcerosa). Alle patiënten waren in klinische remissie ten tijde van staken van anti-TNF therapie. Ongeveer 55% van de patiënten kreeg een

opvlamming met een mediane tijd tot opvlamming van 32 maanden in patiënten met de ziekte van Crohn en 18 maanden in patiënten met colitis ulcerosa. Herbehandeling met dezelfde anti-TNF remmer was succesvol in 84% van de patiënten. Hogere dalspiegels voor het staken en jonge leeftijd bij diagnose waren voospellers voor een grotere kans op opvlamming. Lage fecaal calprotectine waarden waren geassocieerd met een lagere kans op opvlamming in patiënten met de ziekte van Crohn en doorgebruiken van een immunomodulator was beschermend in patiënten met colitis ulcerosa.

# List of publications

## Publications in this thesis

Ultrasound for assessing disease activity in IBD: a systematic review of activity scores. **S Bots**, K Nylund, M Löwenberg, K Gecse, OH Gilja, G D'Haens. *J Crohns Colitis. 2018;12(8):920-929. Systematic review.*

Intestinal Ultrasound to Assess Disease Activity in Ulcerative Colitis: Development of a novel UC-Ultrasound index. **S Bots**, K Nylund, M Löwenberg, K Gecse, G D'Haens. *J Crohns Colitis. 2021;15(8):1264-1271. Cross-sectional study.*

Point-of-care intestinal ultrasound in IBD patients: disease management and diagnostic yield in a real-world cohort and proposal of a point-of-care algorithm. **S Bots***, F de Voogd*, V Ligtvoet, M de Jong. M Löwenberg, G D'Haens, K Gecse. *J Crohns Colitis. 2021. Online ahead of print. Cohort study.*

Optimization of anti-TNF therapy in patients with inflammatory bowel disease. A Strik, **S Bots** , G D'Haens , M Löwenberg. *Expert Rev Clin Pharmacol. 2016;9(3):429-39. Expert review.*

Combination immunosuppression in IBD. **S Bots**, K Gecse, M Barclay G D'Haens. *Inflamm Bowel Dis. 2018;24(3):539-545. Expert review.*

Anti-drug antibody formation against biologic agents in inflammatory bowel disease: a Systematic Review and Meta-analysis. **S Bots**, C Parker, J Brandse, M Löwenberg, B Faegan, W Sandborn, V Jairath, G D'Haens, N Vande Casteele. *BioDrugs 2021;35(6):715-733. Systematic review.*

Patterns of anti-TNF Use and Associated Treatment Outcomes in Inflammatory Bowel Disease Patients: Results From an Analysis of Dutch Health Insurance Claims Data. **S Bots***, D Hoekman*, M Benninga, C Ponsioen, G D'Haens, M Löwenberg. *Neth J Med. 2017;75(10):432-442. Cohort study.*

Relapse Rates and Predictors for Relapse in a Real-Life Cohort of IBD Patients After Discontinuation of anti-TNF Therapy. **S Bots**, S Kuin, C Ponsioen, K Gecse, M Duijvestein, G D'Haens, M Löwenberg. *Scand J Gastroenterol. 2019;54(3):281-288. Cohort study.*

## Other publications

Intestinal Ultrasound to Evaluate Treatment Response During Pregnancy in Patients With Inflammatory Bowel Disease. De Voogd F, Joshi H, Van Wassenaer E, **Bots S**, D'Haens G, Gecse K. *Inflamm Bowel Dis. 2021 Sep 15:izab216. doi: 10.1093/ibd/izab216. Online ahead of print.*

Response to letter to the editor "Intestinal Ultrasound to Assess Disease Activity in Ulcerative Colitis: Development of a novel UC-Ultrasound index". **Bots S**, Nylund K, Gecse K. *J Crohns Colitis. 2021 Aug 14:jjab148. doi: 10.1093/ecco-jcc/jjab148. Online ahead of print*

Prediction of Relapse After Anti-Tumor Necrosis Factor Cessation in Crohn's Disease: Individual Participant Data Meta-analysis of 1317 Patients From 14 Studies. Pauwels RWM, van der Woude CJ, Nieboer D, Steyerberg EW, Casanova MJ, Gisbert JP, Kennedy NA, Lees CW, Louis E, Molnár T, Szántó K, Leo E, **Bots S**, Downey R, Lukas M, Lin WC, Amiot A, Lu C, Roblin X, Farkas K, Seidelin JB, Duijvestein M, D'Haens GR, de Vries AC; CEASE Study Group. *Clin Gastroenterol Hepatol. 2021 Apr 30:S1542-3565(21)00347-5. doi: 10.1016/j.cgh.2021.03.037. Online ahead of print.*

SMAD4 exerts a tumor-promoting role in hepatocellular carcinoma. Hernanda PY, Chen K, Das AM, Sideras K, Wang W, Li J, Cao W, **Bots SJ**, Kodach LL, de Man RA, Ijzermans JN, Janssen HL, Stubbs AP, Sprengers D, Bruno MJ, Metselaar HJ, ten Hagen TL, Kwekkeboom J, Peppelenbosch MP, Pan Q. Oncogene. 2015 Sep 24;34(39):5055-68. doi: 10.1038/onc.2014.425. Epub 2014 Dec 22

Tumour antigen expression in hepatocellular carcinoma in a low-endemic western area. Sideras K, **Bots SJ**, Biermann K, Sprengers D, Polak WG, IJzermans JN, de Man RA, Pan Q, Sleijfer S, Bruno MJ, Kwekkeboom J. *Br J Cancer. 2015 Jun 9;112(12):1911-20. doi: 10.1038/bjc.2015.92.*

## Contributing authors

M. Barclay
Department of gastroenterology
Christchurch Hospital
Christchurch, New Zealand

M.A. Benninga
Department of paediatric gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

J.F. Brandse
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

N. vande Casteele
Department of medicine
University of California San Diego
La Jolla, United States of America

G.R. D'Haens
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

M. Duijvestein
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

B.G. Feagan
Department of medicine
University of Western Ontario
London, Canada

K.B. Gecse
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

O.H. Gilja
Department of gastroenterology
Haukeland University Hospital
Bergen, Norway

D.R. Hoekman
Department of paediatric gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

V. Jairath
Department of medicine
University of Western Ontario
London, Canada

M. de Jong
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

S. Kuin
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

V. Ligtvoet
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

M. Löwenberg
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

K. Nylund
Department of gastroenterology
Haukeland university
Bergen, Norway

C.E. Parker
Robarts clinical trials Inc
London, Canada

A.S. Strik
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

C.Y. Ponsioen
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

F. de Voogd
Department of gastroenterology
Amsterdam UMC, University of Amsterdam
Amsterdam, the Netherlands

W.J. Sandborn
Department of medicine
University of California San Diego
La Jolla, United States of America

# Portfolio

Name: Steven J. A. Bots

PhD period: August 2014 – December 2021

Promotor: Prof. dr. G.R.A.M. D'Haens

Copromotors: dr. M. Löwenberg & dr. K.B. Gecse

| | Year | Workload (ECTS) |
|---|---|---|
| **General courses (graduate school)** | | |
| Basic course legislation & organization of clinical research (BROK) | 2014 | 1.0 |
| Practical biostatistics | 2014 | 1.1 |
| The AMC world of science | 2014 | 0.7 |
| Clinical epidemiology: randomized controlled trials | 2014 | 0.6 |
| Clinical data management | 2015 | 0.3 |
| **Specific courses** | | |
| Transabdominal Ultrasound training Haukeland Hospital, Bergen, Norway (3 months) | 2015 | 10.0 |
| Non-invasive monitoring in IBD (Berlin) | 2016 | 0.5 |
| **Seminars, workshops and masterclasses** | | |
| Weekly department seminars | 2014-2018 | 2.0 |
| Bi-weekly IBD journal club | 2017-2018 | 2.0 |
| Bi-weekly IBD lunch & learn | 2014-2018 | 2.0 |
| Gut club | 2014-2018 | 1.0 |
| AG&M PhD retreat | 2016-2018 | 1.5 |
| **Oral presentations** | | |
| European Crohn's and Colitis Organisation | 2017 | 0.5 |
| Digestive Disease Week (DDW) | 2017 | 0.5 |
| Digestive Disease Days (DDD) | 2017, 2018 | 1.0 |
| AG&M PhD retreat | 2017 | 0.5 |
| CCUVN IBD patientday | 2016 | 0.5 |
| Young ICC symposium | 2017 | 0.5 |
| Pfizer symposium | 2016 | 0.5 |

| | | |
|---|---|---|
| Tillots symposium | 2015-2018 | 2.0 |
| MSD symposium | 2015, 2016 | 1.0 |
| Takeda symposium | 2014 | 0.5 |
| Abbvie symposium | 2015 | 0.5 |
| IBD Lunch & Learn | 2017, 2018 | 0.6 |
| **Poster presentations** | | |
| European Crohn's and Colitis Organisation | 2016, 2017, 2018 (2x) | 2.0 |
| United European Gastroenterology Week | 2016 | 0.5 |
| Digestive Disease Week (DDW) | 2016, 2018 | 1.0 |
| **Attended (inter)national conferences** | | |
| European Crohn's and Colitis Organisation | 2016-2018 | 1.5 |
| United European Gastroenterology Week | 2015, 2016, 2018 | 3.75 |
| Digestive Disease Week (DDW) | 2016, 2017 | 2.5 |
| Digestive Disease Days | 2017, 2018 | 1.0 |
| Young-ICC symposium | 2015-2018 | 1.5 |
| National ICC day | 2014-2017 | 1.5 |
| **Teaching** | | |
| *Lecturing* | | |
| ECCO Ultrasound workshop | 2016 | 0.5 |
| IBUS Ultrasound module 1, Amsterdam | 2021 | 0.5 |
| *Tutoring* | | |
| Bachelorthesis student: Man-Wai Chan | 2015 | 1.0 |
| Bachelorthesis student: Juri Smit | 2016 | 1.0 |
| Masterthesis student: Sabine Kuin | 2016 | 1.5 |
| Several ultrasound trainees | 2016-2018 | 2.0 |
| **Parameters of esteem** | | |
| ECCO travel grant | 2014 | |
| IOIBD travel grant | 2014 | |
| AMC innovation impulse: Point-of-care ultrasound in gastroenterology & hepatology | 2016 | |
| **Other** | | |
| *Committee* | | |
| IBD ultrasound workshop ECCO congress | 2016 | |

| | |
|---|---|
| Public relations workgroup IBUS | 2018 |
| AMC fieldhockey tournament | 2017 |
| AMC football tournament | 2016, 2017, 2018 |

## Dankwoord

In dit proefschrift heb ik mij kunnen richten op toepassing van echografie voor inflammatoire darmziekten en enkele onderwerpen gericht op optimalisatie van medicamenteuze therapie met biologicals. Echografie voor inflammatoire darmziekten is een sterk onderbelicht onderwerp in Nederland. Gedurende mijn promotie traject heb ik de kans gekregen om hiermee een start te maken in Nederland. Door het feit dat alles van de grond af moest worden opgebouwd, was het een lange weg met pieken en dalen. Inmiddels groeit de interesse voor het onderwerp en is er toenemend enthousiasme onder Nederlandse MDL artsen om dit toe te passen. Ik ben erg trots en dankbaar dat ik hiervoor de eerste stappen heb kunnen zetten maar ik had dit alles uiteraard niet kunnen bewerkstelligen zonder de ondersteuning van velen!

Allereerst wil ik alle patiënten bedanken die aan mijn studies hebben deelgenomen. Zonder jullie interesse en enthousiasme was de realisatie van dit proefschrift niet mogelijk geweest!

Beste Geert, ik weet nog goed dat we samen naar Bergen gingen om kennis te maken met de onderzoeksgroep aldaar. Dit was de start van ons echografie project. Door jouw visie om de IBD echografie naar Nederland te halen is het allemaal begonnen. Ik ben je enorm dankbaar dat ik het vertrouwen van je heb gekregen om hiervoor de kar te trekken. Daarnaast heb ik veel respect voor jouw tomeloze inzet voor het vakgebied en de gave om altijd mogelijkheden te zien en te creëren!

Beste Mark, ik heb genoten van onze samenwerking. Ik kon altijd laagdrempelig met je sparren en kon mijn ei kwijt als dat nodig was. Je bijdragen aan de verschillende studies en manuscripten waren altijd zeer waardevol!

Beste Krisztina, jij bent later in mijn promotie traject in het AUMC komen werken. Samen met Floris heb je de IBD echografie in het AMC verder doorontwikkeld. Ik ben trots om te zien waar het nu staat. Ik waardeer onze prettige samenwerking en je vriendelijkheid enorm. Geniet van je pas gevonden moederschap!

Geachte leden van de promotiecommissie, prof. dr J. Stoker, prof. dr. W.A. Bemelman, prof. dr. C.Y. Ponsioen, prof. dr. B. Oldenburg, dr. P.C.F. Stokkers, prof. dr. C. Maaser. Veel dank voor jullie zitting in mijn promotiecommissie en de moeite om de wetenschappelijke waarde van mijn proefschrift te beoordelen.

Dear colleagues from Bergen, Norway and especially Kim, Trygve and Odd Helge. Thank you for inviting me at your clinic to learn intestinal ultrasound and for the fruitful cooperation thereafter. I had a blast!

Lieve kamergenoten van A3, het 'zweetkamertje'. Wat was het gezellig met jullie op de kamer! Frank, jij hebt me als A3 founding father met open armen ontvangen toen ik startte in het

AMC. Annieke, ik heb met je meegeleefd toen je in je promotietraject te maken kreeg met talloze hordes. Ik bewonder je doorzettingsvermogen enorm! Leonie, zowel kamer als onderzoeksgroep genootje. Door jou voelde ik me direct welkom bij de IBD groep en wat hebben we het mooi gehad op verschillende feesten en congressen. Jasper, de bourgondische internist. Knap dat je het hebt aangedurfd je traject te wijzigen en internist te worden. Je was altijd een fijn klankbord! Thomas, 'dat trekt echt op niets' is een uitspraak die ik nooit meer zal vergeten. Je hebt je PhD in record tijd afgerond, respect! Snel een rondje golf! Anouk, gezellig dat je een tijd bij ons bent geweest! Bizar hoe snel en goed jij Nederlands sprak als Zwitserse. Weggebonjourd van kamer A3 en neergedaald op kamer C2. Bente, respect voor jouw keuze om iets anders te gaan doen. Paul, ook jij hebt voor een ander pad gekozen. Hopelijk heeft dat jou gebracht wat je zocht. Esther, onze tijd samen op de kamer was kort, krachtig en gezellig!

Lieve collega onderzoekers van de IBD onderzoeksgroep, ik heb genoten met jullie! Sara, misschien wel de meest goedlachse persoon die ik ken. Altijd in voor gezelligheid. We hebben er allebei een handje van om tot het einde door te gaan. Geniet van je prachtige dochter! Toer, ik voel me vereerd dat ik mag komen draaien op je bruiloft! In antwoord op jouw dankwoord: we gaan zeker weer eens muziek maken! Anne, danskoningin, je was een fijne collega en wat heb ik een respect voor jouw tomeloze ambitie. Floris, het was top om met je samen te werken. Je hebt de echografie projecten met verve overgenomen en het geheel weer naar een hoger plan getild. Mijntje, onze paden kruisten elkaar van korte duur. Ik wens je alle succes op je nieuwe carrière pad.

Loebje, uitmuntende paranimf! We go way back. Ik vind het prachtig dat we nog steeds zo goed bevriend zijn. Japie D, DJ Dantez, wat heb je een strakke cover gemaakt. Nu dit boek af is moeten we weer vaker muziek gaan maken! Henk-Jan, mr. Fuzzy, dank voor al je hulp bij het afronden van mijn proefschrift. Snel een muzieksessie in studio Weesp! Len, Tony, Kyle, Jopie, mijn goede maten. Door de drukte van alle dag is het moeilijk om elkaar regelmatig te blijven zien. Ik koester menig memorabel moment met jullie. Dat er nog vele moge komen!

Vrienden en vriendinnen van vroeger, jaarclubmaten, (oud) huisgenoten, studie vrienden, hockeyvrienden, burners etc. etc. Dank voor alle mooie gezamenlijke momenten en jullie vriendschap!

Lieve Westerveldjes en aanhang, Dick, Lily, Marjo, Roel, Eelco, Liset en Esmee. Wat zijn jullie een fijne mensen. Ik ben bij jullie in een warm bad beland. Dat we samen nog maar vele feestglaasjes mogen drinken!

Lieve Nispjes en Hesjes, Constant, Marianne, Louise, Michiel, Stijn, Philip en Emma. Familie is het allerbelangrijkste en jullie zijn me ontzettend dierbaar. Cons jongen, je wordt gemist… Wat had ik het mooi gevonden als jij erbij was geweest. Je had voor nog meer leven in de brouwerij gezorgd (pun intended).

Lieve oma Hetty, wat een leven heeft u al achter de rug. U bent uw tijd altijd ver vooruit geweest. Ik heb uw gedrevenheid en vastberadenheid altijd als voorbeeld gezien.

Lieve oma Thea, wat vind ik het leuk dat u bij de verdediging kan zijn en ik voel me vereerd dat ik opa mag opvolgen als promovendus. Ik hoop dat deze dag mooie herinneringen bij u oprakelt. Ik heb tijdens het schrijven van mijn proefschrift vaak aan jullie gedacht en aan de heerlijke tijd die ik in mijn jeugd met jullie heb beleefd.

Eva, andere uitmuntende paranimf en natuurlijk mijn lieve zusje. Ik heb grote bewondering voor hoe ogenschijnlijk gemakkelijk jij door het leven laveert. Wat heb je al veel bereikt op jonge leeftijd! Die effectiviteit en doelgerichtheid van jou, daar kan ik nog een puntje aan zuigen. Waar ik soms probeer te scoren met een omhaal en een hakje, hou jij het liever bij een droge knal. Mike, je weet met wat voor topper je samen bent. Eef, had zich geen betere gozer kunnen wensen. Die dikke met die lange haren bleek voor haar vanaf het begin de ware. Lucas, Guus, Jan en t.b.a., met jullie is het altijd keten. Ik vind het heel bijzonder dat ik jullie oom mag zijn!

Caroline, mijn lieve grote zus. Ik ben waanzinnig trots op je. Je hebt meerdere tegenslagen gekend in je leven maar je krabbelt altijd weer bovenop. Ik heb veel bewondering voor jouw weerbaarheid en je bent de liefste tante die Yara zich kan wensen. Gerco, het is super om je in de familie te hebben. Zorg maar goed voor mijn grote zus!

Pap, rots in de branding. Jouw behulpzaamheid gedurende mijn levenspad is zo essentieel geweest. Zie dit boek als de culminatie daarvan. Zonder jou was ik er nooit gekomen! Zonder jou was ik waarschijnlijk ook nooit arts geworden. Dank voor alles! Mam, wat lijken wij in veel opzichten op elkaar. Soms botst dat een beetje, maar nooit voor lang. Ik kan me geen lievere en betere moeder wensen. Altijd heb je achter me gestaan en heb je me ondersteund. Jij bent degene die al mijn passies vanaf begin af aan heeft gevoed en je hebt er voor gezorgd dat ik heb doorgezet als ik het zelf even niet meer zag zitten.

Lieve Pippa, our firstborn daughter from another (canine) mother. Met jouw komst en onvoorwaardelijke liefde heb je ons leven verrijkt!

Noukie, mijn schatje, wat hou ik veel van jou. Wat ben je lief en loyaal. Ik voel me altijd gesteund door jou. We hebben al zoveel mooie en soms ook moeilijke momenten samen beleefd. In sommige opzichten zijn we elkaars tegenpolen en in andere juist niet. Dat maakt dat we zo goed samen gaan. Ik heb enorm veel bewondering voor je en ik prijs mezelf elke dag gelukkig met jou! Lieve Yara, jouw geboorte heeft ons leven op zijn kop gezet. Wat ben je toch een heerlijk lief meisje met nu al een sterk eigen willetje. Je brengt me dagelijks aan het schateren en ik kan me geen leven meer indenken zonder jou!

## About the author

Steven Joannes Alexander Bots was born on oktober 2nd, 1984 in Leidschendam. In 2003 he graduated from high school 'Gymnasium Haganum'. Thereafter, he started his studies on biomedical sciences at the university of Utrecht at which he received his bachelor's degree. In 2007 he started medical school at the Erasmus Medical Center in Rotterdam, after being selected through the decentralized selection procedure. During his internships he decided to pursue a career in gastroenterology and hepatology. His graduation research focused on tumor antigens in hepatocellular carcinoma and he took his senior internship at the department of gastroenterology and hepatology of the Erasmus Medical Center . After graduation he started working as a PhD fellow at the AMC, supervised by Geert D'Haens, Mark Löwenberg and Krisztina Gecse. His research focused on the implementation of intestinal ultrasound and optimal use of biologics in IBD. From august 2019 he started his training in Gastroenterology and Hepatology at the Noordwest Ziekenhuis Groep and the Amsterdam University Medical Center. During his studies and career, Steven has always been passionate about music (piano, DJ) and fieldhockey.