Ph.D. Thesis

CRUCIAL STEPS FOR TREATMENT OPTIMIZATION AND DECISION MAKING IN INFLAMMATORY BOWEL DISEASE MANAGEMENT

Dr. Kata Judit Szántó

Doctoral School of Theoretical Medicine

Supervisor: Dr. habil. Klaudia Farkas Ph.D.

Department of Medicine

Albert Szent-Györgyi Medical School

University of Szeged

2022

TABLE OF CONTENTS

LIST OF FULL PAPERS RELATED TO THE SUBJECT OF THE THESIS:

I. **Szántó, Kata** ; Nyári, Tibor ; Bálint, Anita ; Bor, Renáta ; Milassin, Ágnes ; Rutka, Mariann ; Fábián, Anna ; Szepes, Zoltán ; Nagy, Ferenc ; Molnár, Tamás et al. Biological therapy and surgery rates in inflammatory bowel diseases - Data analysis of almost 1000 patients from a Hungarian tertiary IBD center PLOS ONE 13: 7 Paper: e0200824 , 8 p. (2018) *Q1, IF: 2.776*

II. Kunovszki, Péter* ; **Szántó, Kata Judit*** ; Gimesi-Országh, Judit ; Takács, Péter ; Borsi, András ; Bálint, Anita ; Farkas, Klaudia ; Milassin, Ágnes ; Lakatos, Péter L. ; Szamosi, Tamás et al. Epidemiological data and utilization patterns of anti-TNF alpha therapy in the Hungarian ulcerative colitis population between 2012-2016 EXPERT OPINION ON BIOLOGICAL THERAPY 20 : 4 pp. 443-449. , 7 p. (2020) *Q1, IF: 4.388* (authors contributed equally to this work)

III. **Szántó, Kata Judit*** ; Madácsy, Tamara* ; Kata, Diána ; Ferenci, Tamás ; Rutka, Mariann ; Bálint, Anita ; Bor, Renáta ; Fábián, Anna ; Milassin, Ágnes ; Jójárt, Boldizsár et al. Advances in the optimisation of therapeutic drug monitoring using serum, tissue and faecal anti-tumour necrosis factor concentration in patients with inflammatory bowel disease treated with TNF-α antagonists EXPERT OPINION ON BIOLOGICAL THERAPY 21 : 4 pp. 539-548. , 10 p. (2021) *Q1, IF: 5.589* (authors contributed equally to this work)

LIST OF FULL PAPERS NOT RELATED TO THE SUBJECT OF THE THESIS:

I. Gönczi, Lóránt ; **Szántó, Kata** ; Farkas, Klaudia ; Molnár, Tamás ; Szamosi, Tamás ; Schafer, Eszter ; Golovics, Petra A. ; Barkai, László ; Lontai, Lívia ; Lovász, Barbara et al. Clinical efficacy, drug sustainability and serum drug levels in Crohn's disease patients treated with ustekinumab – A prospective, multicenter cohort from Hungary DIGESTIVE AND LIVER DISEASE 54 : 2 pp. 207-213. , 7 p. (2022) *Q2, IF: 5.156*

II. Resál, Tamás ; Lupas, Dániel ; Szűcs, Mónika ; **Szántó, Kata Judit** ; Rutka, Mariann ; Farkas, Klaudia ; Varga, Márta ; Molnár, Tamás. A vashiányos anémia gyakorisága gyulladásos bélbetegségben [Frequency of iron deficiency anemia in inflammatory bowel disease] CENTRAL EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY / GASZTROENTEROLÓGIAI ÉS HEPATOLÓGIAI SZEMLE 7 : 1 pp. 2-6. , 5 p. (2021)

III. **Szántó, Kata Judit** ; Mezei, Zoltán András ; Kata, Diána ; Földesi, Imre ; Nyári, Tibor ; Fábián, Anna ; Rutka, Mariann ; Bor, Renáta ; Bálint, Anita ; Milassin, Ágnes et al. Combination therapy with anti-TNFs and thiopurines does affect drug metabolite levels but it is not associated with body composition in inflammatory bowel disease patients: A cross-sectional study. CENTRAL EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY / GASZTROENTEROLÓGIAI ÉS HEPATOLÓGIAI SZEMLE 7 : 4 pp. 164-170. , 7 p. (2021)

IV. **Szántó, Kata Judit** ; Balázs, Tamás ; Schrempf, Dóra Mihonné ; Farkas, Klaudia ; Molnár, Tamás. Does inflammatory bowel disease have different characteristics according to stage of adolescence? THERAPEUTIC ADVANCES IN GASTROENTEROLOGY 14 p. 1756284820986670 , 10 p. (2021) *Q1, IF: 4.802*

V. Resál, Tamás ; Bor, Renáta ; **Szántó, Kata** ; Fábián, Anna ; Rutka, Mariann ; Sacco, Marco ; Ribaldone, Davide Guiseppe ; Molander, Pauliina ; Nancey, Stephane ; Kopylov, Uri et al. Effect of COVID-19 pandemic on the workflow of endoscopy units: an international survey. THERAPEUTIC ADVANCES IN GASTROENTEROLOGY 14 Paper: 17562848211006678 , 9 p. (2021) *Q1, IF: 4.802*

VI. Bor, Renáta ; **Szántó, Kata Judit** ; Fábián, Anna ; Farkas, Klaudia ; Szűcs, Mónika ; Rutka, Mariann ; Tóth, Tibor ; Bálint, Anita ; Milassin, Ágnes ; Dubravcsik, Zsolt et al. Effect of COVID-19 pandemic on workflows and infection prevention strategies of endoscopy units in Hungary: a cross-sectional survey BMC GASTROENTEROLOGY 21 : 1 Paper: 98 , 9 p. (2021) *Q2, IF: 2.847*

VII. Bor, Renáta ; Matuz, Mária ; Fábián, Anna ; Szepes, Zoltán ; **Szántó, Kata** ; Farkas, Klaudia ; Rutka, Mariann ; Milassin, Ágnes ; Pigniczki, Daniella ; Bálint, Anita et al. Efficacy, tolerability and safety of split-dose bowel cleansing regimen of magnesium citrate with sodium picosulfate - a phase IV clinical observational study REVISTA ESPANOLA DE ENFERMEDADES DIGESTIVAS 113 : 9 pp. 635-642. , 8 p. (2021) *Q3, IF: 2.389*

VIII. Fábián, Anna ; Bor, Renáta ; Szabó, Ella ; Kardos, Viktor ; Bálint, Anita ; Farkas, Klaudia ; Milassin, Ágnes ; Rutka, Mariann ; **Szántó, Kata** ; Molnár, Tamás et al. Endoscopic treatment of gastric antral vascular ectasia in real-life settings: argon plasma coagulation or endoscopic band ligation? JOURNAL OF DIGESTIVE DISEASES 22 : 1 pp. 23-30. , 8 p. (2021) *Q3, IF: 3.366*

IX. Milassin, Ágnes ; Rutka, Mariann ; Szűcs, Mónika ; Farkas, Klaudia ; Marcus, Mark ; Bor, Renáta ; **Szántó, Kata Judit** ; Nagy, Ferenc ; Szepes, Zoltán ; Lakatos, László et

al. IBD-related malignancies observed in 2015–2019 CENTRAL EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY / GASZTROENTEROLÓGIAI ÉS HEPATOLÓGIAI SZEMLE 7 : 4 pp. 171-178. , 8 p. (2021)

X. Pauwels, Renske W.M. ; van, der Woude C.Janneke ; Nieboer, Daan ; Steyerberg, Ewout W. ; Casanova, Maria J. ; Gisbert, Javier P. ; Kennedy, Nick A. ; Lees, Charlie W. ; Louis, Edouard ; Molnár, Tamás ; **Szántó, Kata** 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 (2021) *Q1, IF: 13.576*

XI. Farkas, Klaudia ; Pigniczki, Daniella* ; Rutka, Mariann ; **Szántó, Kata Judit** ; Resál, Tamás ; Bor, Renáta ; Fábián, Anna ; Szepes, Zoltán ; Lázár, György ; Molnár, Tamás. The complex relationship between viruses and inflammatory bowel disease - review and practical advices for the daily clinical decision-making during the SARS-CoV-2 pandemic THERAPEUTIC ADVANCES IN GASTROENTEROLOGY 14 Paper: 1756284820988198 , 13 p. (2021) *Q1, IF: 4.802*

XII. Resál, Tamás ; Rutka, Mariann ; **Szántó, Kata** ; Farkas, Klaudia ; Molnár, Tamás. A gyulladásos bélbetegségek kezelése a SARS-CoV-2-járvány idején - gyakorlati javaslatok [The treatment of inflammatory bowel disease during the SARS-CoV-2 epidemic - practical advices]: (A COVID–19-pandémia orvosszakmai kérdései) ORVOSI HETILAP 161 : 25 pp. 1022-1027. , 6 p. (2020) *Q4, IF: 0.54*

XIII. Bacsur, Péter ; Skribanek, Soma ; Milassin, Ágnes ; Farkas, Klaudia ; Bor, Renáta ; Fábián, Anna ; Rutka, Mariann ; Bálint, Anita ; **Szántó, Kata Judit** ; Tóth, Tibor et al. Anti-TNFα-terápiában részesülő gyulladásos bélbetegek hosszú távú utánkövetése [Longterm follow-up of inflammatory bowel disease patients receiving anti-tumor necrosis factor alpha therapy] ORVOSI HETILAP 161 : 47 pp. 1989-1996. , 8 p. (2020) *Q4, IF: 0.54*

XIV. Pigniczki, Daniella ; **Szántó, Kata** ; Rutka, Mariann ; Farkas, Klaudia ; Zsilák-Urbán, Mihály ; Szepes, Zoltán ; Molnár, Tamás. A tápláltsági állapot jelentősége gyulIadásos bélbetegségekben [The importance of nutritional status in inflammatory bowel disease] CENTRAL EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY / GASZTROENTEROLÓGIAI ÉS HEPATOLÓGIAI SZEMLE 6 : 2 pp. 55-61. , 7 p. (2020)

XV. Sánta, Anett ; **Szántó, Kata Judit** ; Miheller, Pál ; Sarlós, Patrícia ; Juhász, Anita ; Hamvas, Edina ; Szegedi-Hallgató, Emese ; Farkas, Klaudia ; Rafael, Beatrix ; Molnár, Tamás. Depresszív tüneteket és öngyilkossági gondolatokat befolyásoló tényezők gyulladásos bélbetegségben szenvedők körében: Multicentrikus tanulmány [Influencing factors on depressive symptoms and suicidal ideation among inflammatory bowel disease patients: multicenter study] ORVOSI HETILAP 161 : 42 pp. 1797-1805. , 9 p. (2020) *Q4, IF: 0.54*

XVI. Kunovszki, Péter ; Milassin, Ágnes* ; Gimesi-Országh, Judit ; Takács, Péter ; **Szántó, Kata** ; Bálint, Anita ; Farkas, Klaudia ; Borsi, András ; Lakatos, Péter L. ; Szamosi, Tamás et al. Epidemiology, mortality and prevalence of colorectal cancer in ulcerative colitis patients between 2010-2016 in Hungary – a population-based study PLOS ONE 15 : 5 Paper: e0233238 , 13 p. (2020) *D1, IF: 3.24*

XVII. Bálint, Anita ; Farkas, Klaudia ; Méhi, Orsolya ; Kintses, Bálint ; Vásárhelyi, Bálint Márk ; Ari, Eszter ; Pál, Csaba ; Madácsy, Tamara ; Maléth, József ; **Szántó, Kata Judit** et al. Functional anatomical changes in ulcerative colitis patients determine their gut microbiota composition and consequently the possible treatment outcome. PHARMACEUTICALS 13 : 11 Paper: 346 , 16 p. (2020) *Q2, IF: 5.863*

XVIII. Bor, Renáta ; Fábián, Anna ; Matuz, Mária ; Szepes, Zoltán ; Farkas, Klaudia ; Miheller, Pál ; Szamosi, Tamás ; Vincze, Áron ; Rutka, Mariann ; **Szántó, Kata** et al. Real-life efficacy of vedolizumab on endoscopic healing in inflammatory bowel disease - A nationwide Hungarian cohort study EXPERT OPINION ON BIOLOGICAL THERAPY 20 : 2 pp. 205-213. , 9 p. (2020) *Q1, IF: 4.388*

XIX. **Szántó, Kata Judit** ; Rutka, Mariann ; Pigniczki, Daniella ; Farkas, Klaudia ; Burián, Katalin ; Terhes, Gabriella ; Molnár, Tamás. Serological Status of Inflammatory Bowel Disease Patients Before Starting Biological Therapy - Data From a Tertiary Centre of the Best Vaccined Country. INFLAMMATORY BOWEL DISEASES 26 : 4 Paper: e28 , 1 p. (2020) *Q1, IF: 5.325*

XX. Bor, Renáta ; Vasas, Béla ; Fábián, Anna ; Szűcs, Mónika ; Füredi, Árpád ; Czakó, László ; Rutka, Mariann ; Farkas, Klaudia ; Molnár, Tamás ; Milassin, Ágnes et al. Slow-pull technique yields better quality smears: prospective comparison of slow-pull and standard suction techniques of endoscopic ultrasound-guided fine-needle aspiration SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY 55 : 11 pp. 1369-1376. , 8 p. (2020) *Q2, IF: 2.423*

XXI. Bor, Renáta; Fábián, Anna ; Farkas, Klaudia ; Bálint, Anita ; Szántó, Kata ; Rutka, Mariann ; Milassin, Ágnes ; Nagy, Ferenc ; Tóth, Tibor ; Molnár, Tamás et al. A mesenchymalis őssejt-transzplantáció hatásmechanizmusa és alkalmazása perianalis Crohn betegség kezelésében [Mechanism of action and application of mesenchymal stem cell transplantation in the treatment of perianal Crohn's disease] CENTRAL EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY / GASZTROENTEROLÓGIAI ÉS HEPATOLÓGIAI SZEMLE 5 : 3 pp. 861-867. , 7 p. (2019)

XXII. Iliás, Ákos ; **Szántó, Kata*** ; Gönczi, Lóránt ; Kürti, Zsuzsanna ; Golovics, Petra Anna ; Farkas, Klaudia ; Schafer, Eszter ; Szepes, Zoltán ; Szalay, Balázs ; Vincze, Áron et al. Outcomes of Patients With Inflammatory Bowel Diseases Switched from Maintenance Therapy with a Biosimilar to Remicade CLINICAL GASTROENTEROLOGY AND HEPATOLOGY 17 : 12 pp. 2506-2513.e2. , 6 p. (2019) *Q1, IF: 8.549*

XXIII. **Szántó, Kata** ; Molnár, Tamás ; Farkas, Klaudia. A gyulladásos bélbetegségek bélrendszeren kívüli tüneteinek kezelési lehetőségei [Treatment options for the extraintestinal symptoms of inflammatory bowel diseases] MAGYAR BELORVOSI ARCHIVUM 71 : 3 pp. 142-146. , 5 p. (2018)

XXIV. **Szántó, Kata** ; Szűcs, Dániel ; Vass, Noémi ; Várkonyi, Ágnes ; Bálint, Anita ; Bor, Renáta ; Fábián, Anna ; Milassin, Ágnes ; Rutka, Mariann ; Szepes, Zoltán et al. Gyulladásos bélbetegségben szenvedő gyermekek kezelésének átadása felnőttszakrendelésre. 10 éves tapasztalataink összegzése [Transfer of care of adolescent inflammatory bowel disease patients without longitudinal transition. Lesson from 10 years experience] ORVOSI HETILAP 159 : 44 pp. 1789-1793. , 5 p. (2018) *Q3, IF: 0.564*

XXV. **Szántó, Kata** ; Rutka, Mariann* ; Milassin, Ágnes ; Farkas, Klaudia ; Fábián, Anna ; Bor, Renáta ; Szepes, Zoltán ; Nyári, Tibor ; Szijártó, András Lajos ; Rácz, István et al. How do you like the pen? Patients' opinion about switching adalimumab from prefilled syringe to pen in inflammatory bowel disease CENTRAL EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY / GASZTROENTEROLÓGIAI ÉS HEPATOLÓGIAI SZEMLE 4 : 1 pp. 22-26. , 5 p. (2018)

XXVI. Bálint, Anita ; Rutka, Mariann ; Kolar, Martin ; Bortlik, Martin ; Duricova, Dana ; Hruba, Veronika ; Lukas, Martin ; Mitrova, Katarina ; Malickova, Karin ; Lukas, Milan et al. Infliximab biosimilar CT-P13 therapy is effective in maintaining endoscopic

remission in ulcerative colitis - results from multicenter observational cohort EXPERT OPINION ON BIOLOGICAL THERAPY 18 : 11 pp. 1181-1187. , 7 p. (2018) *Q1, IF: 3.585*

XXVII. **Szántó, Kata** ; Palatka, Károly ; Molnár, Tamás. Infliximab dóziselemzés colitis ulcerosában. Infliximab dose escalation - contradiction between financing protocol and real life data CENTRAL EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY / GASZTROENTEROLÓGIAI ÉS HEPATOLÓGIAI SZEMLE 4 : 2 pp. 82-84. , 3 p. (2018)

XXVIII. Rutka, Mariann; Farkas, Klaudia ; Bor, Renáta ; Szepes, Zoltán ; Bálint, Anita ; Milassin, Ágnes ; Fábián, Anna ; Szántó, Kata ; Matúz, Mária ; Nyári, Tibor et al. Szérum és endoszkópos markerek diagnosztikus és prognosztikus szerepe gyulladásos bélbetegségekben [Diagnostic and prognostic role of serum and endoscopic markers in inflammatory bowel diseases] CENTRAL EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY / GASZTROENTEROLÓGIAI ÉS HEPATOLÓGIAI SZEMLE 4 : 2 pp. 109-115. , 7 p. (2018)

XXIX. Fábián, Anna ; Rutka, Mariann ; Ferenci, Tamás ; Bor, Renáta ; Bálint, Anita ; Farkas, Klaudia ; Milassin, Ágnes ; Szántó, Kata ; Lénárt, Zsuzsanna ; Nagy, Ferenc et al. The Use of Complementary and Alternative Medicine Is Less Frequent in Patients with Inflammatory Bowel Disease Than in Patients with Other Chronic Gastrointestinal Disorders GASTROENTEROLOGY RESEARCH & PRACTICE 2018 Paper: 9137805 , 8 p. (2018) *Q3, IF: 1.825*

XXX. Farkas, Klaudia ; Rutka, Mariann ; Ferenci, Tamás ; Nagy, Ferenc ; Bálint, Anita ; Bor, Renáta ; Milassin, Ágnes ; Fábián, Anna ; **Szántó, Kata** ; Végh, Zsuzsanna et al. Infliximab biosimilar CT-P13 therapy is effective and safe in maintaining remission in Crohn’s disease and ulcerative colitis – experiences from a single center EXPERT OPINION ON BIOLOGICAL THERAPY 17 : 11 pp. 1325-1332. , 8 p. (2017) *Q2, IF: 3.974*

SCIENTOMETRICS:

Number of full publications: 33
Cumulative impact factor: 77.1

LIST OF ABBREVIATIONS

ADA: adalimumab

anti-TNF-α: anti-tumor necrosis alpha

ATC: Anatomical Therapeutic Chemical Classification System

CD: Crohn's disease

CDAI: Crohn's disease activity index

CI: confidence interval

CRP: C-reactive protein

DE: dose escalation/ dose escalated

ECCO: European Crohn's and Colitis Organisation

EDTA: ethylenediaminetetraacetic acid

EGTA: egtazic acid

ELISA: enzyme-linked immunoassay

GI: gastrointestinal

IBD: inflammatory bowel disease

ICD-10: International Classification of Diseases

ICHI: International Classification of Health Interventions

IFX: infliximab

LOD: limit of detection

LOR: loss of response

NEAK: Nemzeti Egészségbiztosítási Alapkezelő (National Health Insurance Fund)

PFA-PBS: paraformaldehyde-phosphate buffer saline

PNR: primary non response

RCT: randomized controlled trial

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

TDM: therapeutic drug monitoring

UC: ulcerative colitis

SUMMARY

Background: The incidence and prevalence of inflammatory bowel disease (IBD) are increasing worldwide. Lifelong medical therapy is essential in most patients, which places a very significant burden on healthcare and financial systems. During the past decades, treatment paradigms and treatment goals have changed; biological therapies have become more wildly available. However, primary or secondary non-response to biological therapy poses a significant challenge for clinicians. **Aims:** In this thesis, we aimed to evaluate the demographic, clinical and treatment characteristics of IBD patients in our clinic and also at the national level. Moreover, we aimed to broaden the potential methods of therapeutic drug monitoring (TDM) with the determination of serum, tissue and fecal concentrations of anti-TNF-α agents. **Methods:** In the first study, a prospectively maintained database of IBD patients was analyzed, and the demographic, clinical and treatment patterns of the enrolled IBD patients were assessed. The second study was an observational, non-interventional, retrospective, epidemiological study using the National Health Insurance Fund social security database. We have analyzed the treatment characteristics of the Hungarian, anti-TNF-α treated ulcerative colitis (UC) patients, focusing on treatment length of the biological therapy, need for dose escalation or therapy switch and corticosteroid dispensings. In the third study, consecutive IBD patients receiving maintenance anti-TNF-α therapy were enrolled. Anti-TNF-α concentrations in the serum, tissue and feces, as well as the mucosal expression of TNF-α were measured and we have compared the relationship of drug levels in biological samples with endoscopic activity, clinical activity, and body composition of the patients. **Results:** In the first study, 911 IBD patients were enrolled in our prospective database between January 2007 and April 2015. Forty% of the patients received biological therapy. Three hundred and one patients underwent surgery, which was required more frequently in Crohn's disease (CD) than in UC. In UC, more severe disease onset predicted an unfavorable disease course. A higher proportion of surgery was shown in patients above 40 years of age in both CD and UC. The rate of surgery proved to be significantly higher in CD vs. UC ($p \leq 0.001$). No relationship was found between smoking, appendectomy, positive family history of IBD and the presence of extraintestinal manifestations and the need for surgery or biological therapy. The use of oral contraceptives was protective regarding the need for surgery and biologicals in both diseases. The rates of surgery and biological therapy together were significantly higher in patients diagnosed more than 1 year after the onset of symptoms in CD and UC as well ($p=0.012$ and $p=0.002$). In the second study, 568 UC patients were identified. Approximately 70-80% of the patients reached maintenance therapy. A large

proportion of patients stopped therapy after 10 to 12 months due to the reimbursement policy. Corticosteroid use decreased significantly after the initiation of biological therapy. The dose-escalation rate was 19.8% for adalimumab (ADA) and 10.9% for infliximab (IFX), respectively, and DE was performed earlier along the treatment timeline in patients receiving ADA. The rate of primary non-response was 11.6% and the rate of secondary loss of response was 36.5%. The data of 50 patients were analyzed in the third study. The number TNF-α positive cells was significantly higher in the inflamed part of the colon than in the uninflamed part. Tissue and fecal drug levels did not show any association with serum drug levels; moreover, serum anti-TNF-α concentration did not correlate with endoscopic activity. Mucosal anti-TNF-α levels were higher only in IFX-treated patients in remission, and IFX-treated patients with detectable fecal anti-TNF-α had lower tissue drug levels. The presence of the drug in the feces was significantly different according to disease activity. **Conclusions:** The first follow-up study on almost 1000 IBD patients revealed that at the diagnosis of IBD in a referral center, factors predictive of surgery were age below 40, although age above 40 proved to be protective regarding the need for biologicals in both diseases, and the same is true for the use of oral contraceptives. However, more than 1 year of diagnostic delay, disease activity at diagnosis in UC, and CD phenotype are also important factors that may influence disease outcome. Based on the second study, in most cases the treatment lasted for nearly a year, when the majority of patients stopped therapy because reimbursement and medical protocols required them to do so. In a large proportion of UC patients – based on the decision of the treating physician and the medical need – the treatment was continued past this time point, which might suggest that the firm one year stop policy is not optimal for UC patients requiring biological therapy. It was found that with adequately long biological therapy corticosteroid use was significantly reduced after the initiation of biological therapy. Based on the third study, we suggested that the measurement of fecal anti-TNF-α concentration would increase the benefit of therapeutic drug monitoring, while the simultaneous determination of fecal drug concentration and fecal calprotectin may be more accurate for evaluating therapeutic response in the future.

INTRODUCTION

1. Incidence, prevalence and prognostic factors in inflammatory bowel disease

Inflammatory bowel disease (IBD: Crohn's disease [CD], ulcerative colitis [UC], inflammatory bowel disease unclassified [IBD-U]) is a chronic, relapsing, immune-mediated inflammatory disorder of the gastrointestinal tract with unknown etiology. IBD develops as a result of complex interactions between genetic factors, immune response, environmental, and microbial factors[1].

The incidence and prevalence of IBD are increasing worldwide; however, they show significant differences depending on geographical location. Based on a recent systematic review which evaluated a period between 1990 and 2016, the incidence of IBD in North-America and Europe is stabilizing or decreasing; however, the incidence in developing countries is stabilizing or increasing[2]. The estimates of prevalence are in the range of 2.5-3 million people in Europe[3]; the incidence of UC varies between 0.9-24/100 000 person per year, and in CD it is between 0.0-11.5/100 000 person per year[4,5]. The prevalence of UC in Europe varies between 2.4- 294/100 000, while the prevalence of CD varies between 1.5-213/100 000[6–8].

The course of IBD consists of periods of remission and relapse, therefore lifelong medical therapy is essential in most of the patients, which places a very significant burden on healthcare and financial systems. Since IBD is a progressive disease, optimal treatment early in the disease course is paramount to prevent complications. Treatment has been revolutionized by novel therapeutic options, such as biological therapy, introduced in the past decades. Before the era of biologicals, the goal of the treatment was mainly symptomatic relief and the maintenance of clinical and corticosteroid-free remission. However, with the introduction of newer therapeutic options more rigorous endpoints have been identified. The traditional step-up algorithm has shifted to the top-down or to the accelerated step-up algorithm. The current treatment paradigm of IBD is to use immunosuppressive and/or biological therapy early to achieve clinical remission and mucosal healing in „high risk" patients, who will presumably need interventions to improve their medical outcome. The clinical approach to the management of these patients will ultimately decrease the risk of corticosteroid use, hospitalizations, surgeries and will improve their quality of life[9]. The introduction of biological agents occurs earlier among patients with a more aggressive disease phenotype based on clinical data and prognostic factors. These prognostic factors are as follows with no claim of completeness: CD location and behavior of the disease (ileal and upper gastrointestinal location, penetrating phenotype), smoking, deep ulcers on diagnostic endoscopy, presence of extraintestinal manifestation and

the need for early corticosteroid therapy or surgery[10]. In UC, young age at diagnosis, disease extent, disease activity at diagnosis, concomitant primary sclerosing cholangitis have been identified as important risk factors[11,12]. Besides, numerous serological markers, early postoperative recurrence of CD and various genetic markers have been identified as prognostic factors; however, the detailed description of these goes beyond the scope of the present thesis[10–12].

2. Description, regulation and challenges of anti-tumor necrosis factor-α therapy in IBD

The first biological agent approved by the European Medicines Agency in 1999 to treat CD was tumor necrosis factor (TNF)-α antagonist infliximab (IFX), while 7 years later it was introduced in the treatment of UC as well. Adalimumab (ADA) was registered in the treatment of CD in 2007 and it started to be used in UC 5 years later. The introduction of anti-TNF-α agents has dramatically changed the treatment of refractory IBD. Multiple randomized controlled trials (RCTs) have shown anti-TNF-α therapies to be efficacious for inducing and maintaining remission[13–20] and reducing the risks of hospitalizations and intestinal resections among patients with moderate-to-severe CD and UC[21–23]. However, long-term data on the real-life use of IFX and ADA in IBD and their impact on favorable health outcomes, such as reduced number of hospitalizations and surgeries are still lacking.

Data on the prevalence of anti-TNF-α therapy among IBD patients are available from the Inflammatory Bowel Disease Epidemiologic Database, University of Manitoba. According to the database, the cumulative prevalence of patients with current or prior anti-TNF-α exposure in 2014 was 20.4% in CD and 6.0% in UC. In 2014, the cumulative incidence of anti-TNF-α exposure within 5 years from the diagnosis was 23.4% in case of patients with CD and 7.8% amongst patients with UC[24]. A population-based cohort study from Denmark analyzed the data of 623 IBD patients receiving IFX throughout a 15-year period. They found IFX to be introduced at a younger age than the median age of the UC population. In UC patients, the median interval from the first prescription of IFX to therapy discontinuation increased significantly throughout the observational period. Median treatment length increased from 0.34 years (between 2005 and 2009) to 1.11 years (between 2010 and 2014)[25]. According to a review by Rencz et al., the estimated proportion of UC patients treated with biological therapy in Central and Eastern European countries varies between 0%-6.4%[26]. In Hungary, starting from late 2012, a register of special drug reimbursement (hereinafter, Patient Registry) provides data on all administration of biological therapies. Besides other data, the Patient Registry contains information on the dose of the drug, thus it is more detailed than data from earlier years.

Moreover, data can be captured on the need for dose escalation (DE) and drug change, since the most important limitation of long-term anti-TNF-α therapies is primary and secondary loss of response (LOR), which poses a significant challenge for clinicians[27]. The clinical definition of primary non-response (PNR) is lack of improvement of clinical signs and symptoms with induction therapy[28]. LOR describes patients who respond to the therapy after an induction regimen, but subsequently lose response during maintenance treatment. There is no consensus definition, but the majority of clinical trials and the European Crohn's and Colitis Organization (ECCO) workshop use clinical symptom indices to define response and remission. Patients who initially experience substantial increases in these scores but later suffer from clinical relapse during maintenance therapy are considered to experience a secondary LOR. Other definitions proposed for LOR, such as patients requiring dose intensification or discontinuing the drug after a period of use, do not capture all patients who experience LOR[29,30]. Ten to 40% of anti-TNF-α treated patients show PNR to anti-TNF-α treatment. According to literature data, the annual risk of LOR is 13-20.3% per patient year[31,32]. Several mechanisms have been examined with respect to LOR. Although the presence of antibodies against anti-TNF-α agents and low drug serum concentrations have been implicated as the most important predisposing factors for therapeutic failure, the exact mechanism of therapeutic failures remains debatable. Prospective studies have demonstrated that many patients exhibit clinical relapse despite adequate serum drug levels and no anti-drug antibodies; in contrast, many patients remain in clinical remission despite low serum levels[27,33]. Furthermore, there is an unmet need to establish biomarkers that predict therapeutic response to prevent the unnecessary exposure of non-responders to anti-TNF-α therapy, thus enhancing the safety and cost-effective use of this treatment. The severe consequences of chronic, untreated, even asymptomatic inflammatory processes lead to a change in disease behavior, not only in the treatment of the disease, but also in the follow-up of IBD patients. Thus, close follow-up of patients, monitoring of drug levels, and a treat-to-target approach are increasingly being reflected in the everyday practice of IBD therapy.

3. Therapeutic drug monitoring in IBD

Therapeutic drug monitoring (TDM) is generally defined as the clinical laboratory measurement of a chemical parameter, which, combined with appropriate medical interpretation, will directly influence drug prescribing procedures[34]. TDM has two types, reactive and proactive. Reactive TDM is performed when patients do not respond to treatment or have a flare up while on the treatment. It involves determining the next therapeutic step, i.e. whether DE or switching to an alternative therapy would be more beneficial. Proactive TDM

checks drug concentration and the presence of antidrug antibodies in patients in remission; it is performed to optimize dosing, avoid drug discontinuation and improve outcomes[31].

Otherwise, TDM refers to the individualization of drug dosage by maintaining plasma or blood drug concentrations within a targeted therapeutic range or window[35]. TDM and, in case of certain drugs, monitoring antidrug antibodies, have been shown to help therapy optimization, manage LOR and prolong maintenance treatment[36]. However, our previous measurements of serum TNF-α, IFX and antibody concentration were unable to predict the response in certain cases during anti-TNF-α therapy either, which was supposed to be due to the various mucosal concentrations of TNF-α blockers[37]. Moreover, it is also unclear what levels of biologics correlate best with mucosal healing. In the absence of routinely used biomarkers, the prediction of clinical responsiveness to anti-TNF-α therapies remains an important clinical problem. Simultaneous determination of anti-TNF-α level in the serum, intestinal mucosa and feces together with the assessment of the correlation of these levels with body composition could support therapeutic decisions and provide scientific information about drug bioavailability and distribution. Furthermore, there is an unmet need for establishing biomarkers that could predict therapeutic response to prevent the unnecessary exposure of non-responders to anti-TNF-α therapy, thus enhancing the safety and cost-effective use of this treatment.

AIMS

The aims of these comprehensive studies were:

1. To analyze the demographic and clinical characteristics, as well as the medical and surgical management of IBD patients treated at our tertiary IBD center in order to identify parameters associated with the need for surgery and/or biological therapy as surrogate markers of a worse disease course in IBD.
2. To evaluate the epidemiology and treatment characteristics of the anti-TNF-α treated Hungarian UC population by focusing on the analysis of treatment length, dose escalation, therapeutic switch and concomitant corticosteroid use in our country.
3. To broaden the potential methods of TDM via the determination of serum, tissue and fecal concentrations of anti-TNF-α agents as well as the mucosal expression of TNF-α, and to assess the relationship between drug levels in biological samples and clinical and endoscopic activity, as well as body composition in IBD patients receiving maintenance anti-TNF-α therapy.

PATIENTS AND METHODS

Study 1. To analyze the demographic and clinical characteristics, medical and surgical management of IBD patients treated at our tertiary IBD center in order to identify parameters associated with the need of surgery and/or biological therapy as surrogate markers of a worse disease course in IBD.

1.1. Data source and data collection

In the first study, IBD patients prospectively participated in a dedicated IBD registry launched at the First Department of Medicine, University of Szeged between January 2007 and March 2015. Age at inclusion, age at diagnosis, disease duration, smoking habits, history of appendectomy, the presence of familial IBD and extraintestinal manifestations, disease activity, number and type of surgeries, most important laboratory parameters and therapies used were registered at every appointment of each patient. The diagnosis of CD and UC was based on the Lennard-Jones and later the ECCO criteria[38,39]. Disease activity was assessed with the Crohn's Disease Activity Index (CDAI) and the Mayo Scoring System in UC[40,41]. On the basis of the activity scores, patients were divided into inactive, mildly, moderately and severely active groups. C-reactive protein (CRP) higher than 5 mg/l was considered to be abnormal suggesting active disease both in UC and CD. In the majority of patients, the first attendance at the First Department of Medicine, University of Szeged was the same as the time of the diagnosis. Worse disease course was characterized by active disease with or without extraintestinal complications or perianal manifestations, the need for biological therapy and/or major surgery. The registry included both incident and prevalent cases. Differences in demographic and clinical characteristics, presence of extraintestinal manifestations, number of surgeries, different types of treatment and predictors of outcomes in both diseases were analyzed statistically. Of therapies, corticosteroid, immunosuppressive and biological therapies were collected for statistical analysis.

1.2. Statistical analysis

Statistical analysis was carried out using STATA 9.0 software. The chi-square test, Pearson's phi-coefficient, Wilcoxon ranksum test and Kruskal-Wallis test were used. For examining predictive factors, a simple logistic regression model was performed. A probability level of $p<0.05$ was considered to be significant.

1.3. Ethical approval

This study was approved by the Clinical Research Coordination Center, Albert Szent-Györgyi Medical School, University of Szeged.
Number of ethical license: 2640.

Study 2. To evaluate the epidemiology and treatment characteristics of the anti-TNF-α treated Hungarian UC population by focusing on the analysis of treatment length, dose escalation, therapeutic switch and concomitant corticosteroid use in our country.

2.1. Data source

The second study was an observational, non-interventional, retrospective, epidemiological study using the National Health Insurance Fund (Hungarian acronym: NEAK) social security database, which includes data on in- and outpatient care, prescription medicine and special drug reimbursement. The database was analyzed between 2012 and 2016.

2.2. Data collection

All patients suffering from UC were captured in the database based on the International Classification of Diseases (ICD-10) diagnosis code K51. Those patients were included in the analysis who started biological therapy after September 2012, as financial reimbursement data and adequate Patient Registry have been available since then. Biological therapy could be captured based on prescription data before 2012 September and patients receiving any during this time period were excluded from the analysis. Therefore, all patients have a record in the Patient Registry corresponding to each of their biological therapy administration. The date of the UC diagnosis was defined as the date when the first UC diagnosis code appeared in the in- and outpatient care or medication database. The start of biological therapy was defined as the date of the first Patient Registry sheet, since first appearance corresponds with the first occasion of biological agent received. To define the time interval elapsed from the diagnosis to the start of biological therapy, the difference of these dates was calculated and recorded. The active substance of all biological therapy administrations was determined based on the procedure code (ICHI – International Classification of Health Interventions). The dispense of corticosteroids was captured using Anatomical Therapeutic Chemical Classification System (ATC codes). The drug named Remicade (IFX; later its biosimilar also appeared under the name of Inflectra) was approved in Hungary in 2006, while Humira (ADA) was approved in the second half of 2012. Starting from late 2012, these drugs have been available through itemized reimbursement, where detailed documentation is required to support the responsible use of these agents. The

biological treatment of the patients was compiled into treatment episodes. A treatment episode was defined as a series of treatment using the same substance (regardless of the number of treatments), where the time between two consecutive treatments is no longer than 180 days. Every treatment episode started with the induction period, which was defined differently for ADA and IFX based on the medical and reimbursement protocols. All treatments were called maintenance therapy after the induction period. An episode could end due to three different reasons. Firstly, when the patient received no more biological treatment in the study period. Secondly, when the patient stopped biological treatment and the treatment was restarted with the same drug after more than 180 days. Thirdly, when the patient started to use a different active substance. The length of the treatment period was defined as the time from the starting date of the induction to the date of the last registry sheet in that treatment episode. As a result of this definition, treatment length could not be calculated for episodes which consisted of only one treatment. Furthermore, as the effect of the treatment lasts longer than the date of the last registry sheet, treatment length was slightly underestimated. Due to the low number of patients who received more than one episode of treatment (defined by the above mentioned criteria), only the first treatment episode could be analyzed in this study. All treatments during maintenance therapy were categorized as dose-escalated (DE) or non-dose-escalated (non-DE) treatments. A treatment was considered DE if the dose was greater than 1.5 times the median dose of the compound across all patients. All other treatments were considered to be non-DE. The DE period incorporates the time interval of all dose-escalated treatments of a patient, while the time of DE was the date of the first DE treatment. There was a possibility for patients to change their medication in case of ineffectiveness as there were two different active substances available (IFX and ADA). A patient was only considered a switch when they were put on the other agent within less than 180 days from stopping the treatment with the previous one. A patient was considered to have a PNR if their first episode of biological treatment consisted of an induction period only. A therapeutic episode could end due to treatment stopping or switching. A patient was considered to have LOR if the therapy was stopped, the dose was escalated or the drug was switched after the induction period of the biological therapy but before 1 year of continuous biological therapy. To check whether the therapy was stopped or switched within 1 year from the start of the therapy, the end date of the last administration of the biological agent had to be estimated. In case of ADA, this was calculated by adding 30 days to the date of the last registry sheet of ADA treatment. In case of IFX, 60 days were added. In both cases, treatment stop could only be ascertained if there was an at least 180-day-long follow-up period after the last registry sheet of the current patient with the corresponding treatment.

Therefore, there were some patients in whom PNR or LOR status could not be determined, so these patients were censored in these analyses. It was assumed that this censoring is independent of the fact whether or not the patient experiences PNR/ LOR in real life, so that bias is negligible. An analysis of concomitant corticosteroid use was performed in the following subgroup of patients: the first biological treatment episode of the analyzed patients had to be at least 6 months long (adequate length of biological therapy) and they had to have at least 2 years of follow-up after the initiation of biological therapy. The number of corticosteroid dispensing was counted in the 2-year-period preceding and following the start of biological therapy.

2.3. Statistical analysis

The number of patients on biological therapy was described using patient counts. Demographic data were characterized using histograms and median age. Since all patients in the study started biological therapy, there was no censoring in the time to biologic initiation data, thus it was characterized using a histogram. Survival analysis was performed to study length of treatment, time to DE and time on escalated dose, Kaplan-Meier estimators were used to characterize survival function. When analyzing corticosteroid use, the number of corticosteroid prescriptions was not used as a continuous variable; an ordinal scale was assumed instead. A nonparametric Mann-Whitney test was used to compare corticosteroid use before and after the start of biological therapy.

2.4. Ethical approval

This study has been approved by the Medical Research Council – Research and Ethics Committee (TUKEB), Hungary (Appr. no: 12288-3/2018/EKU).

Study 3. To broaden the potential methods of TDM via the determination of serum, tissue and fecal concentrations of anti-TNF-α agents, as well as the mucosal expression of TNF-α, and to assess the relationship between drug levels in biological samples and clinical and endoscopic activity, as well as body composition in IBD patients receiving maintenance anti-TNF-α therapy.

3.1. Patient population and study design

The third study enrolled consecutive patients with luminal CD and UC receiving maintenance IFX or ADA therapy at the First Department of Medicine, University of Szeged for refractory disease defined by the ECCO Consensus Report[42,43]. The patients underwent colonoscopy

between January 2017 and March 2018. In CD patients, the clinical disease activity was determined using the CDAI, in patients with UC, clinical disease activity was measured using the Mayo score[40,41].

Clinically active disease was defined as CDAI>150 in CD and as partial Mayo score >2 in UC. Endoscopic activity was determined with disease-specific endoscopic scores[40,44]. Endoscopically active disease was defined as SES-CD≥3 in CD and eMayo≥2 UC[45–47]. Assessment of clinical disease activity, collection of blood samples and fecal specimens and colonoscopy with biopsy samples were performed within the same 7-day period in all patients. In case of IFX therapy, samples were taken within 2 weeks before the subsequent infusion. Serum samples were obtained for the determination of routine inflammatory parameters, anti-TNF-α and anti-drug antibody levels. Stool samples were obtained to determine the fecal calprotectin and drug concentration. During endoscopy, biopsy samples were taken exclusively from the colon. For the determination of tissue drug levels, biopsy samples were obtained from the inflamed (from the edge of the ulcer or in the absence of an ulcer, from the most inflamed region) and uninflamed parts of the colon. If there was no endoscopic activity, tissue samples were obtained from the uninflamed tissue that was previously involved. Each patient underwent body composition analysis before colonoscopy. Each patient was informed about the study and provided written informed consent.

3.2. Measurement of serum anti-TNF-α concentration

Serum IFX (#ref: TR-Q-INFLIXIv2) and ADA (#ref: TR-ADAv1) concentrations were determined using ELISA as per the manufacturer's protocol (Matriks Biotek Laboratories, Ankara, Turkey). The sensitivity of IFX and ADA assays was 30 ng/mL and 10 ng/ml, respectively. The intra- and inter-assay coefficients of variation for both assays were < 20%. Subtherapeutic concentration was defined as serum IFX concentration below 3 μg/ml and serum ADA concentration below 5 μg/ml[48,49].

3.3. Measurement of serum anti-drug antibody concentration

The level of antibodies for IFX (#ref: TR-ATIv5) and ADA (#ref: TR-AADAv2) in serum was determined using ELISA assay, as per the manufacturer's protocol (Matriks Biotek Laboratories, Ankara, Turkey). The sensitivity of the anti-IFX and anti-ADA kits was 5 ng/ml and < 30 ng/ml, respectively. The intra- and inter-assay coefficients of variation of both assays were < 15%.

3.4. Measurement of tissue anti-TNF-α concentration

All tissue samples were obtained with biopsy forceps and placed in ice-cold Tris-Triton buffer. After homogenization and sonication, the protein supernatant was kept at a temperature of −80°C. Mucosal drug levels were determined using an ELISA kit (Matriks Biotek Laboratories, Ankara, Turkey) and expressed as μg/mg protein. The total protein level of the tissue samples was measured using the Bradford protein assay method[50].

3.5. Measurement of fecal anti-TNF-α drug concentration

Fecal samples were diluted at a ratio of 1:1 in Tris/triton-X100 lysis buffer (contains in mM: 10 Tris pH: 7.4, 100 NaCl, 1 EDTA, 1 EGTA, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate). Samples were then homogenized by vortexing for 1 min, and then centrifuging at 3000 g for 10 min. The supernatants were collected and centrifuged again at 10000 g for 10 min. The final supernatants were collected and stored at −20°C until analysis. The IFX and ADA concentrations were measured using ELISA kits (Matriks Biotek Laboratories, Ankara, Turkey).

3.6. Validation assay for the measurement of tissue and fecal anti-TNF-α concentrations

Our group was the first to use the commercial ELISA kits (Matriks Biotek Laboratories, Ankara, Turkey) for the measurement of anti-TNF-α levels in tissue and feces. Therefore, we performed validation assays to test whether the tissue and fecal matrix themselves have an influence on anti-TNF-α determination and whether the kits from Matriks Biotek Laboratories can determine anti-TNF-α from human tissue and fecal extracts. Control tissue and fecal samples (potentially free of TNF-α) were prepared as described previously. Active human IFX protein was added to the pooled extracts to get the final IFX concentration of 2.0 μg/ml (calculated). The stock extract containing the added anti-TNF-α (100%) was serially diluted to get 1.0, 0.5, 0.25. 0.125, 0.0626, 0.0312 and 0 μg/ml (control pooled extracts without added TNF-α) of the original anti-TNF-α concentration with the same pooled control tissue or feces extracts containing no added anti-TNF-α. Then all samples, including negative controls (prepared from apparently healthy or treatment naive patients) were subjected to anti-TNF-α measurement with the above-mentioned ELISA kits for human IFX.

3.7. Immunofluorescent labelling and confocal microscopy for the detection of mucosal TNF-α expression

Biopsy samples for immunofluorescent labelling and tissue samples for TNF-α and anti-TNF-α measurements were collected at the same time. Samples were frozen in Shandon Cryomatrix (ThermoFisher Scientific, Cat. No.: 6769006) and stored at −20°C until sectioning; 7-µm thick sections were cut with a cryostat (Leica CM 1860 UV) at −20°C. Sections were fixed in 4% PFA-PBS for 15 min; thereafter, they were washed in 1x Tris buffered saline (TBS) for 3×5 min. Antigen retrieval was performed and the sections were blocked with 0.1% goat serum and 10% BSA in PBS for 2 h. Sections were incubated overnight at 4°C with primary anti-TNF-α antibody (Abcam, Cat No.: ab6671), which recognizes membrane bound and soluble TNF-α as well. For secondary antibody labelling, samples were incubated with Alexa Fluor-488 conjugated Goat anti-Rabbit secondary antibody (ThermoFisher Scientific; Cat. No.: A11034). Nuclear staining was performed with 1-µg/mL Hoechst33342 (ThermoFisher Scientific; Cat. No.: 62249) for 15 min and sections were placed in Fluoromount mounting medium (Sigma-Aldrich; Cat. No.: F4680); they were then left to dry. For isotype control, rabbit polyclonal IgG was used (ab37415) under the same conditions as the primary antibody. Images were captured with a Zeiss LSM880 confocal microscope using a 40x oil immersion objective (Zeiss, NA: 1.4). Four randomly selected areas were imaged per sample with constant imaging parameters (zoom: 1X, laser power, and detector gain) and the total and TNF-α positive cell numbers were determined in the whole frame via manual counting by two independent investigators in a blinded manner. Mucosal TNF-α positive cell numbers were determined by enhancing the signal-to-noise ratio with the selection of proper and constant thresholds. Results are presented as TNF-α positive cell number/total cell number to normalize the data.

3.8. Investigation of the association between clinical and endoscopic activities; inflammatory laboratory and fecal parameters; as well as the serum, mucosal and fecal anti-TNF-α levels with the determination of body composition

Serum samples for the determination of inflammatory laboratory parameters (CRP, iron level, albumin level, leukocyte number, hematocrit and platelet number) and for measuring the serum anti-TNF-α and anti-drug antibody levels were collected at the same time. Stool samples were obtained to determine fecal calprotectin and drug concentrations. Body composition was determined with an InBody770 body composition analyzer.

3.9. Statistical analysis

Continuous variables are presented as mean (minimum–maximum) values, while categorical variables are presented as counts (percentages). Continuous variables of paired groups were

compared with the exact Wilcoxon signed rank test, while the correlations between continuous variables were investigated with Kendall rank correlation.

3.10. Ethical approval

Ethical approval for the study was obtained from the Regional and Institutional Human Medical Biological Research Ethics Committee, University of Szeged (36/2016-SZTE; 3735).

RESULTS

Study 1. To analyze the demographic and clinical characteristics, medical and surgical management of IBD patients treated at our tertiary IBD center in order to identify parameters associated with the need of surgery and/or biological therapy as surrogate markers of a worse disease course in IBD.

1.1. Demographic data and clinical characteristics of CD and UC patient populations

In the first study, 911 IBD (CD: 428, UC: 483) patients were enrolled in our prospective database between January 2007 and April 2015. The male/female ratio was 422/489. The mean age at the onset of symptoms and at diagnosis was 26.6±11.3 and 27.9±11.4 years among CD patients, while among UC patients the average age at the onset of symptoms and at diagnosis was 30.3±12.4 and 30.9±12.5 years. At the diagnosis of CD, ileocolonic and colonic locations and non-stricturing, non-penetrating behavior were the most common (35.3%, 34.6% and 44.4%) disease phenotypes. Proctitis and left-sided colitis were the most frequent disease extents (34.2% and 34.6%) at the diagnosis of UC. At the time of the first appointment, registered disease activity was mild in 41.6%, moderate in 41.6%, and severe in 12.2% of UC patients. The remaining 4.6% of the patients was documented to have inactive disease at the time of the first appointment. Positive family history of IBD was found in 8.8% of CD patients and 11.3% of UC patients. Considering extraintestinal manifestations, arthralgia and arthritis occurred in 54% of CD patients and 57.8% of UC patients. Table 1. presents the demographic and clinical characteristics of the enrolled patients.

	CD patients (n=428)	**UC patients (n=483)**
Mean age at present, yr	41.2±13.8 (SD:0.67)	47.4±15.2 (SD:0.69)
Mean age at diagnosis, yr	27.9±11.4	30.9±12.5
Mean age at onset of symptoms, yr	26.6±11.3 (SD: 0.56)	30.3±12.4 (SD:0.58)
Male/female ratio	194/234	228/255

Smoking habits, n (%)		
- **Ex-smoker**	109/385 (28.3)	148/448 (33)
- **Current smoker**	107/385 (27.8)	37/448 (8.2)
- **Never smoked**	169/385 (43.9)	263/448 (58.7)
Appendectomy, n (%)	76/378 (20.1)	26/448 (5.8)
Use of oral contraceptives, n (%)	119/372 (32)	146/437 (33.4)
Location/extent, n (%)		
- **Ileal**	110 (25.7)	NA
- **Colonic**	156 (34.6)	NA
- **Ileocolonic**	151 (35.3)	NA
- **Isolated upper GI**	11 (2.6)	NA
- **Proctitis**	NA	165 (34.2)
- **Left-sided colitis**	NA	167 (34.6)
- **Extensive colitis**	NA	151 (31.3)
Behavior, n (%)		
-Non-stricturing, non-penetrating	190 (44.4)	NA
-Stricturing	101 (23.6)	NA
-Penetrating	136 (31.8)	NA
-Perianal manifestation	63 (14.7)	NA
Extraintestinal manifestations, n (%)		
- **Eye**	27 (6.3)	18 (3.7)
- **Skin**	55 (12.9)	69 (14.3)
- **Joint**	231 (54)	279 (57.8)

Table 1. Demographic and clinical characteristics of the enrolled patients.

1.2. Medical therapy and surgery rates in CD

Sixty point five% of CD patients received thiopurine, 23.6% methylprednisolone, 45.6% biological therapy and 28% combined thiopurine and biological therapy. In CD, the use of thiopurine was more common in patients with colonic and ileocolonic locations vs. ileal location (34.3% and 37.7% vs. 27.9%, p=0.03). The need for biological therapy was not associated with any location, disease behavior or the presence of extraintestinal manifestations. Surgery was needed in 228 CD patients (53.27%); the number of surgical interventions (both abdominal and perianal) was 440. Bowel resection and perianal surgery was performed in 66% and 34% of the cases. Surgery proved to be more common in patients with ileal location compared to colonic and ileocolonic locations (p=0.06) and with penetrating behavior compared to non-stricturing, non-penetrating and stricturing behavior ($p \leq 0.001$). A higher proportion (58.2%) of surgery was shown in patients above 40 years (p=0.051). In CD, neither baseline CDAI or CRP were associated with the need for biological therapy and/or surgery.

1.3. Medical therapy and surgery rates in UC

Forty-three point five% of UC patients were on thiopurine, 34.2% on methylprednisolone, 26.3% on biological therapy and 14.8% on combined thiopurine and biological therapy during the whole period. The use of thiopurines and biologicals did not show any association with disease extent. Colectomy was performed in 77 patients throughout the follow-up period. Fifty-one% of the patients underwent laparoscopic surgery. One-, two, -and three-stage surgeries were performed in 13.3%, 40% and 46.7% of the operated patients. A significantly higher proportion (75.3%) of surgery was performed in patients above 40 years (p=0.035). Disease extent did not show any association with the need for surgery. The rate of surgery did not differ significantly in patients receiving biologicals; however, it was lower in patients treated with thiopurines (p=0.045). The need for biological therapy and need for biological therapy and surgery together were more common in patients with more severe disease activity determined with the pMayo score and CRP levels at the first attendance ($p \leq 0.001$).

1.4. Comparison of the demographic and clinical parameters of CD and UC patients

The median follow-up time was 3.6 years. The ratio of males/females did not differ significantly between the two disease groups (p=0.435). The median lag time between the onset of symptoms and diagnosis proved to be significantly longer in UC than in CD (4.6 years vs. 2.1 years, p=0.01; Table 2). However, after excluding cases where we were not able to perform a complete

follow-up of the medical records, no significant difference remained regarding the median lag time between the onset of symptoms and diagnosis.

		Follow-up duration (year)			
Group	No. of patients	Median	P25%	P75%	P-value
Total	911	3.6	0.0	9.6	<0.01
UC	483	4.6	0.0	10.3	
CD	428	2.1	0.0	8.6	
	Non-zero follow-up duration (year)				
Total	589	7.9	3.9	12.2	0.86
UC	349	8.0	3.7	12.7	
CD	240	7.7	4.2	11.9	

Table 2. Median lag time between onset of symptoms and diagnosis in CD and UC.

Smoking and history of appendectomy were significantly more common in CD vs. UC patients ($p \leq 0.001$ and $p=0.003$). No difference was shown in the use of oral contraceptives between the two patient groups. No association was found between either positive family history of IBD, or any of the accompanying extraintestinal manifestations and any of the disease locations/extents or complicated disease behaviors in CD. The ratio of males/females did not differ regarding the use of thiopurines and/or biological therapy. The rate of surgery proved to be significantly higher in CD vs. UC ($p \leq 0.001$). No relationship was found between smoking, appendectomy, positive family history of IBD and the presence of extraintestinal manifestations and the need for surgery or biological therapy. The use of oral contraceptives was protective regarding the need for surgery and biologicals in both diseases. The rates of surgery and biological therapy together were significantly higher in patients diagnosed more than 1 year after the onset of symptoms in CD and UC as well ($p=0.012$ and $p=0.002$).

Study 2. To evaluate the epidemiology and treatment characteristics of the anti-TNF-α treated Hungarian UC population by focusing on the analysis of treatment length, dose escalation, therapeutic switch and concomitant corticosteroid use in our country.

2.1. Demographic results

During the observational period between 2012-2016, the number of UC patients increased from 21 809 to 23 280. In total, 2.44% of these patients (n=568) treated with anti-TNF-α agents were identified during this period. Out of these patients, 172 (30%) started with ADA, while 396 (70%) started with IFX as the first biological therapy. The usual onset of anti-TNF-α therapy was between 30 and 39 years with a median age of 39 years. Furthermore, there was a slightly higher proportion of males (54%) in the biologically treated population. The demographic data of the enrolled patients are shown in Table 3.

	Total (n=568)
Demographics	
Male, n (%)	301 (54)
Age (%)	
<30	114 (20.1)
30-39	176 (31.0)
40-49	120 (21.1)
50-59	95 (16.7)
60+	63 (11.1)
Biological therapy, n (%)	
ADA	172 (30)
IFX	396 (70)

Table 3. Demographic data of the enrolled patients.

2.2. Length of biological therapy episodes

During the first therapeutic episode, approximately 70-80% of the patients reached maintenance therapy. A distinct drop in therapy length can be observed between 10-12 months, which is partly attributed to the mandatory stop rule after one year of therapy present in the NEAK reimbursement policy (Figure 1). Despite the reimbursement rule that required treating physicians to stop biological treatment after one year, roughly 45% of patients continued the initial treatment. Regulations regarding the forced stop of biological therapy in CD and UC were abolished in February 2013 and January 2022, respectively.

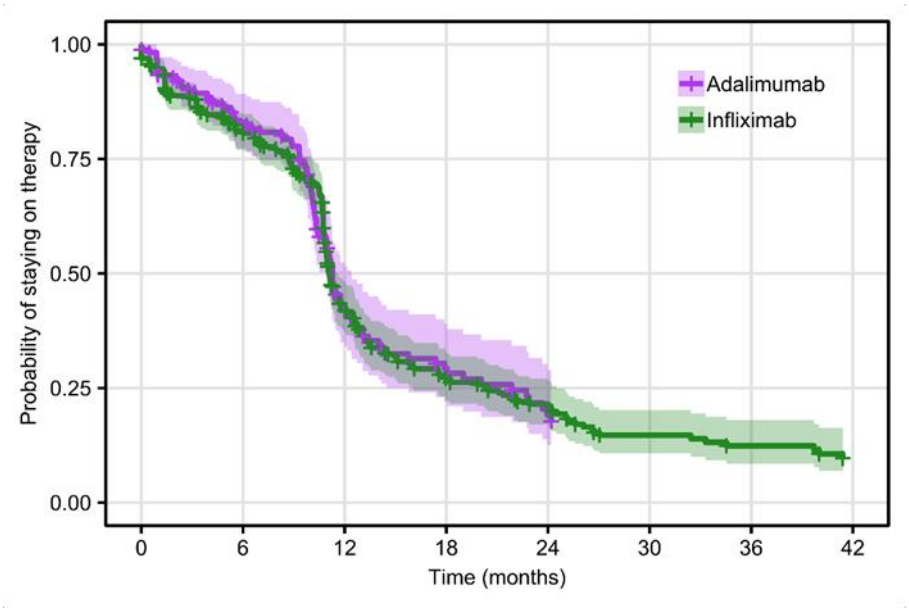

Figure 1. Kaplan-Meier estimation of the length of the first episode of biological treatment with 95% confidence interval. Start: start of biological therapy, event: end of first episode, censoring: death, end of follow-up.

2.3. Time from UC diagnosis to the initiation of biologics

Thirty-five% of anti-TNF-α treated patients started their first anti-TNF-α therapy within 3 years from diagnosis. A third of these patients began anti-TNF-α therapy within the first year. On the other hand, 33.3% of the patients started anti-TNF-α therapy more than 10 years after the diagnosis of UC.

2.4. DE and medication switch

DE is a potential therapeutic event only in patients who reach maintenance therapy in a given treatment episode. Due to the low patient numbers, DE analysis could only be performed in the first treatment episode for all patients. A total of 13.6% (n=77) of the patients were DE. A higher proportion of ADA-treated patients (19.8%) underwent DE compared to IFX-treated patients (10.9%) (Figure 2). While the long-term likelihood of being DE was similar in both treatment arms (about 30% after 18 months), the time passed until DE differed remarkably in the two agent groups. The majority of DEs of ADA patients occurred within the first 2 months of the therapy. On the other hand, IFX patients were mainly escalated after 1 year. The median time on escalated dose was 3.3 months (95% CI: 1.9-4.8 months) with no significant difference between the arms (Figure 3). The frequency of switch was 15.7% with 89 patients switching

medication. Switching was more common in previously dose escalated patients (19.5% of them switched medication).

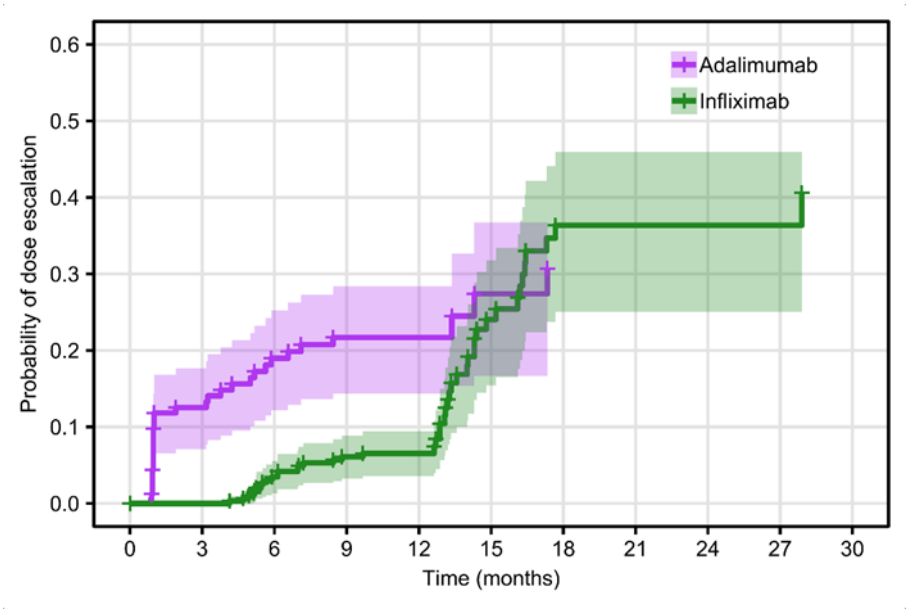

Figure 2. Cumulative probability function of dose escalation over time within the first episode of biological therapy with 95% confidence interval. Start: start of maintenance therapy, event: dose escalation, censor: end of therapy, death, end of follow-up.

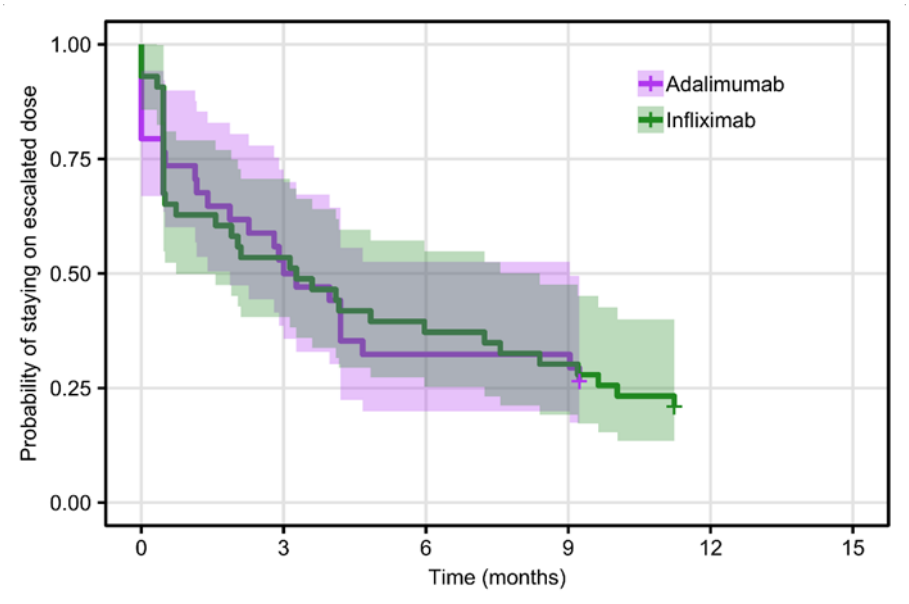

Figure 3. Kaplan-Meier estimation of time on escalated dose with 95% confidence interval. Start: dose escalation, event: de-escalation or end of treatment, censor: death or end of follow-up while on escalated dose.

2.5. LOR

In total, 112 patients had no observable maintenance treatment on their first biological drug due to treatment stopping, switching or insufficient follow-up. Out of these patients, treatment stop could not be ascertained in 52 patients. Therefore, 60 patients out of 516 were determined to experience PNR (11.6%). All other patients except the aforementioned 112 were at risk of experiencing LOR (456 patients in total). Out of these patients, the LOR status could not be determined due to insufficient follow-up in 53 patients. Among the remaining patients, 147 experienced LOR and 256 did not. Therefore, there were 147 out of the 403 possible patients who experienced LOR (36.5%).

2.6. Effect of anti-TNF-α therapy on corticosteroid dispensing

Patients used significantly less corticosteroids after starting anti-TNF-α therapy than before ($p<0.001$). Figure 4 shows the distribution of corticosteroid prescriptions in a 4-year period (2 years prior to and 2 years after the start of biological therapy).

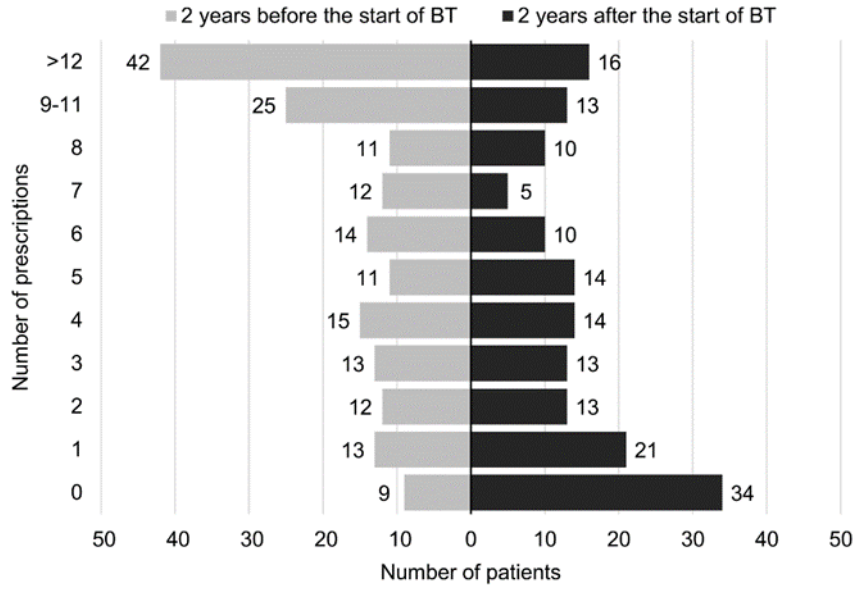

Figure 4. Distribution of patients based on the number of corticosteroid prescriptions dispensed in a 4-year period (2 years prior to and 2 years after the start of biological therapy). BT – biological therapy.

Study 3. To broaden the potential methods of TDM via the determination of serum, tissue and fecal concentrations of anti-TNF-α agents, as well as the mucosal expression of TNF-α, and to assess the relationship between drug levels in biological samples and clinical and

endoscopic activity, as well as body composition in IBD patients receiving maintenance anti-TNF-α therapy.

3.1. Patient population

Fifty consecutive patients were analyzed in the study from whom matched serum, tissue and fecal samples were obtained. Twenty-nine patients had CD, and 21 had UC. Twenty-six patients were receiving maintenance ADA (52%), 24 were receiving maintenance IFX therapy (48%). The mean CDAI was 126.5, and the mean pMayo score was 2.5 at baseline. Twenty-one patients presented with clinically active disease and 38 patients showed endoscopic activity. The baseline characteristics of the patients are shown in Table 4. For each patient, we analyzed one serum sample, one fecal sample and 3-3 tissue samples from two distinct sites. In 12 patients, only 3 tissue samples were obtained from the uninflamed tissue that was previously involved because of the lack of endoscopic activity. Conversely, in 5 patients no part of the colon was uninflamed.

	Patients (n=50)
Mean age at present, yr (min-max)	40.5 (20-66)
Mean age at diagnosis, yr (min-max)	27.5 (12-56)
Mean disease duration, yr (min-max)	12.9 (1-34)
Type of IBD (CD/UC), n (%)	29(58)/21(42)
Gender (female/male), n (%)	23(46)/27(54)
Number of patients with clinically active disease, n (%) - **Mean CDAI (min-max)** - **Mean pMayo (min-max)**	21 (42) 126.5 (22-272) 2.5 (0-9)
Number of patients with endoscopically active disease, n (%)	38 (76)
Type of anti-TNF-α agent: IFX/ADA, n (%)	26 (52)/24(48)

Mean duration of anti-TNF-α therapy, months (min-max)	27.4 (6-84)
Previous anti-TNF-α therapy, n (%)	23 (46)
Number of patients receiving systemic corticosteroids, n (%)	7 (14)
Number of patients receiving combination therapy with immunomodulators, n (%)	21 (42)
Location/extent, n (%)	
- **Ileal**	1 (3.4)
- **Colonic**	9 (31)
- **Ileocolonic**	19 (65.5)
- **+Upper GI tract**	1 (3.4)
- **Extensive colitis**	10 (47.6)
- **Left-sided colitis**	11 (52.3)
- **Proctitis**	0 (0)

Table 4. Baseline characteristics of patient population enrolled in the study.

3.2. Mucosal TNF-α and anti-TNF-α drug levels in the inflamed and uninflamed parts of the colon

The ratio of TNF-α positive/total cells was significantly higher in the inflamed vs. uninflamed parts of the colon (p=0.01) (Figure 5). Based on this, we hypothesized that tissue drug levels may differ in these different regions of the colon. However, tissue drug levels obtained from the inflamed parts of the colon did not differ to a statistically significant degree compared to the samples obtained from the uninflamed colonic segments (0.08 vs. 0.007 μg/mg, p=0.106) (Figure 5).

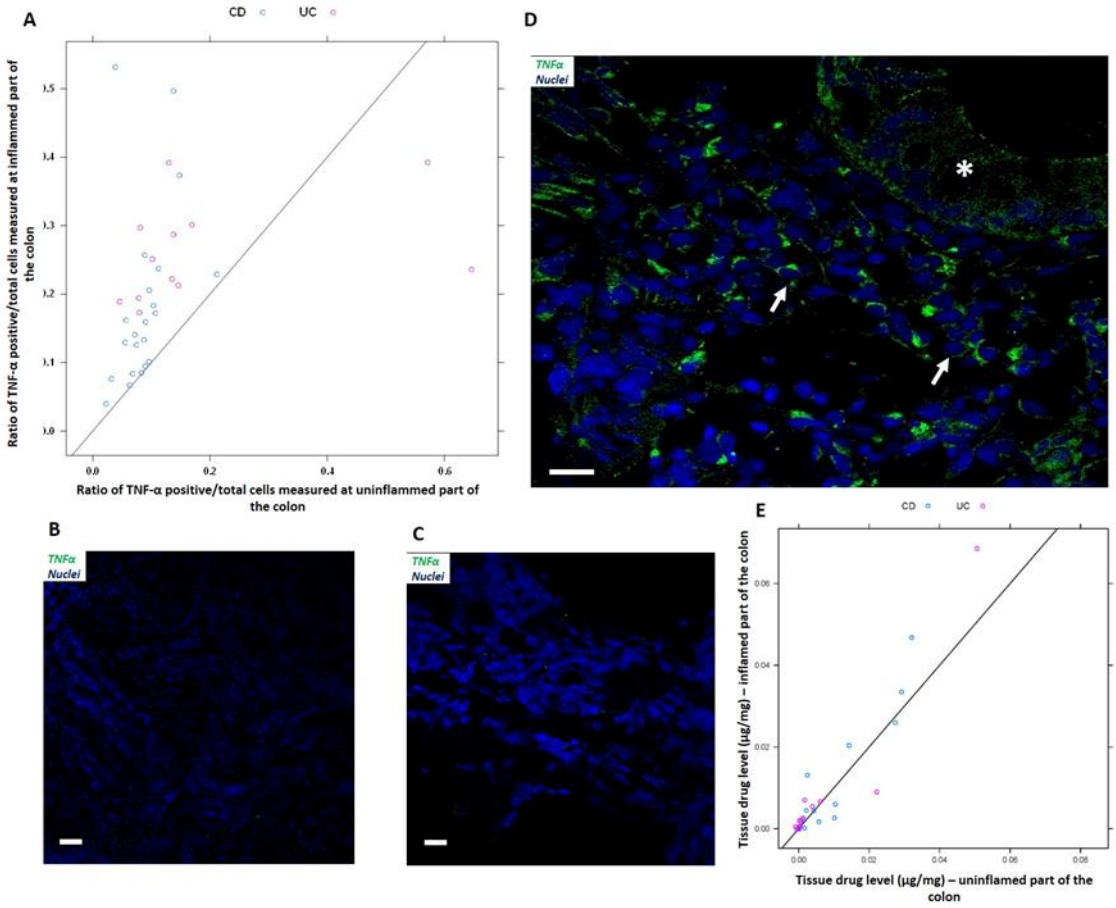

Figure 5. A: The ratio of TNF-α positive/total cells was significantly higher in the inflamed vs. uninflamed part of the colon. B: Confocal microscopic picture of no primary staining. C: Confocal microscopic picture of an uninflamed area of the colon. D: Confocal microscopic picture of an inflamed area of the colon. Arrows: TNF-α positive cells; asterix: colonic crypt. Scale bar: 10µm. E: Tissue drug levels obtained from the inflamed part of the colon did not differ significantly from the samples obtained from the uninflamed segment (p=0.106).

3.3. Measurements of anti-TNF-α concentrations in the serum, tissue and feces

We aimed to determine whether there was a correlation between drug levels in the serum, feces and tissue of the inflamed and uninflamed segments. Generally, an inverse correlation was found between the serum and tissue drug levels. When we examined the correlation as per the inflammatory state of the tissue, neither the drug level of the inflamed tissue (p=0.988, tau=0.0036), nor that of the uninflamed tissue correlated significantly with the serum drug level (p=0.155, tau=0.156). Anti-TNF-α antibody positivity was detected in 20 patients. Lower serum drug levels were shown in patients with antibody positivity; however, this was statistically significant only in IFX-treated patients (serum IFX levels 14.02 vs. 0.62 µg/ml, p=0.002 vs. serum ADA levels 15.06 vs. 8.6 µg/ml, p=0.07). Tissue drug levels did not show any association with the presence of anti-drug antibodies (data not shown).

Fecal anti-TNF-α concentration was higher than 0 μg/ml in 10 patients (7 CD, 3 UC patients, 5 patients on ADA, 5 patients on IFX treatment). Due to non-normally distributed data, we calculated median values with using ½ of LOD. Median fecal ADA concentration was 0.015 μg/ml, while median fecal IFX concentration was 0.05 μg/ml. Because of the low number of patients with fecal anti-TNF-α positivity, we dichotomized these quantitative variables and compared tissue drug concentrations in patients with and without detectable fecal anti-TNF-α. We found that patients with detectable fecal anti-TNF-α had substantially lower tissue drug levels; however, the difference was not significant (p=0.124) (Figure 6). When we examined the samples stratified by the type of anti-TNF-α, we found that significant difference was present only in IFX-treated patients; mucosal IFX concentration was lower in patients with detectable fecal IFX (0.002 μg/ml vs. 0.02 μg/ml, p=0.001). Serum and fecal drug levels did not show any association (data not shown).

Detectable fecal anti-TNF-α was present in 9.5% of the patients who did not develop anti-drug-antibody compared to 11.9% in those with anti-drug antibody positivity. The difference was non-significant (p=0.462). On the basis of these data, we hypothesized that fecal loss of anti-TNF-α (especially IFX) might be associated with decreased mucosal accumulation of the drug and not influenced by antibody positivity.

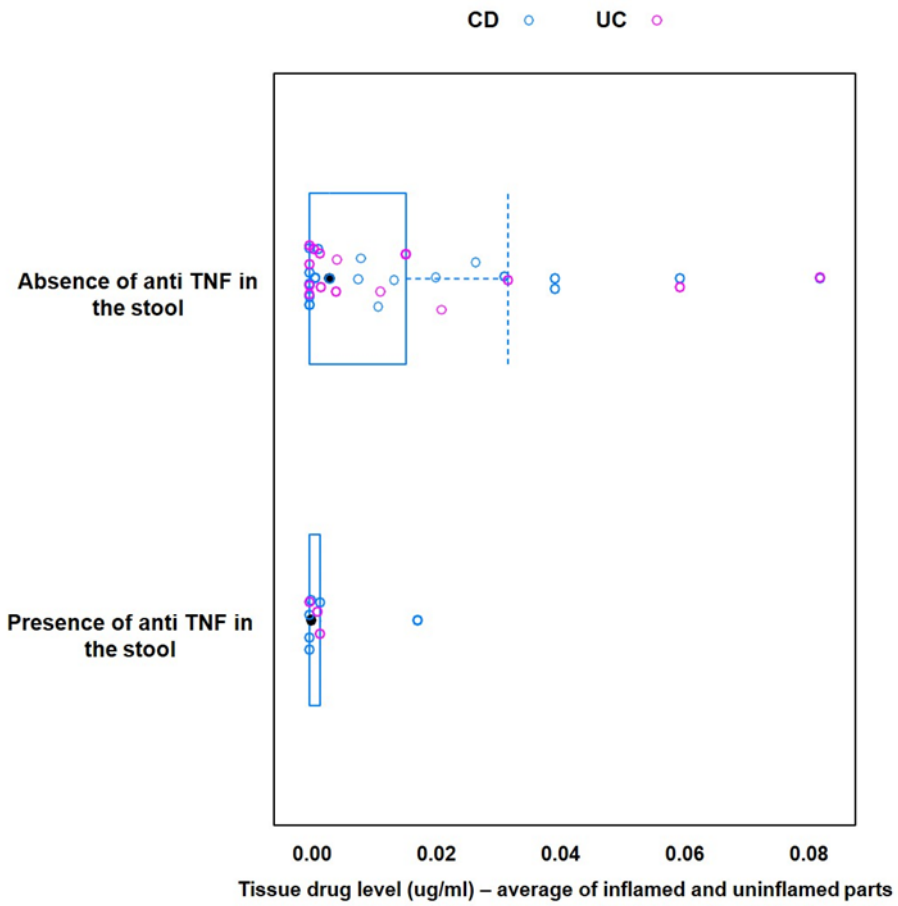

Figure 6. Patients with detectable fecal anti-TNF-α had substantially but not significantly lower tissue drug levels (p=0.124).

3.4. Correlation of different anti-TNF-α levels with endoscopic activity

In the next stage of the study, we aimed to determine which drug concentration of the different biological samples correlated most accurately with the endoscopic and clinical activity in order to predict response to anti-TNF-α therapy. This was a crucial point because in our cohort, only 12 patients had sub-therapeutic serum levels despite 38 patients having endoscopically active disease. As a confirmation of this discordance between serum drug levels and therapeutic response, no significant correlation was observed between endoscopic activity and serum drug concentrations (p=0.993). However, the tissue drug levels of samples obtained from the uninflamed part of the colon proved to be significantly different according to activity (p=0.035), with higher levels observed in those in remission. Figure 7 shows the correlation between serum and tissue drug levels and clinical and endoscopic disease activity. There was no difference in the samples obtained from the inflamed part of the colon as per the activity (p=0.217). It was noteworthy that the presence of the drug in the feces was significantly different as per the

activity (p=0.002); every patient with detectable fecal anti-TNF showed endoscopic activity and none of the patients in remission had detectable drug levels in their feces.

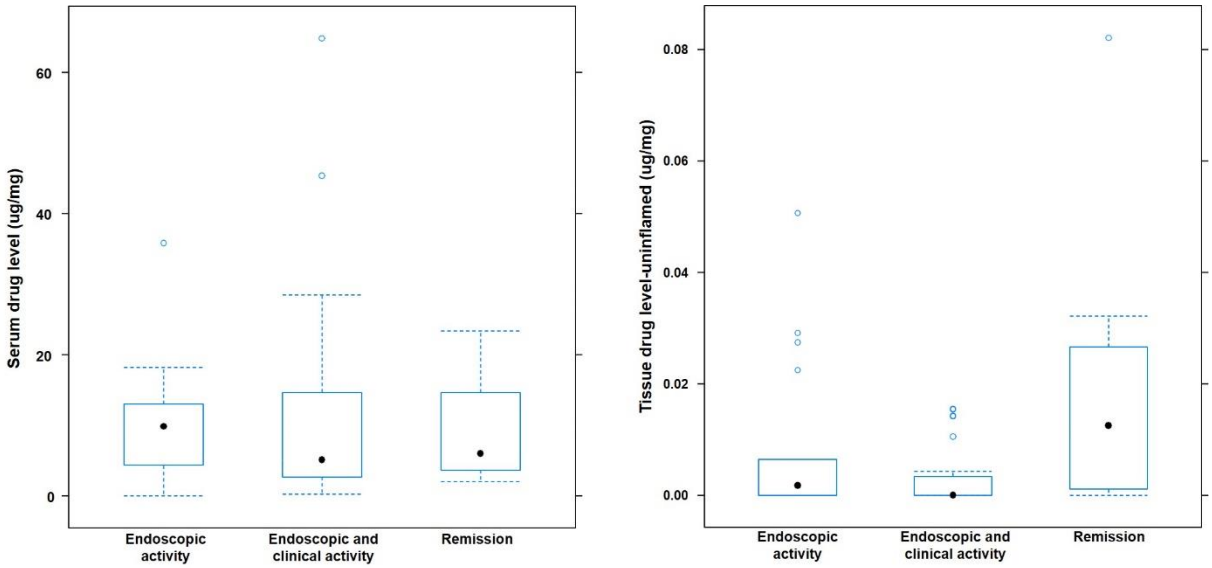

Figure 7. Correlation between serum and tissue (uninflamed) drug levels and clinical and endoscopic disease activity.

3.5. Correlation of different anti-TNF-α levels, body composition, inflammatory parameters and fecal calprotectin

We hypothesized that body mass and composition may influence anti-TNF-α levels and thus the therapeutic response; therefore, we performed multivariate regression analysis with serum drug level and tissue drug level as response variables. However, we found that body composition parameters, including body mass index, total body water, as well as minerals and skeletal muscle mass had no significant impact on serum and tissue drug levels (data not shown). Investigation of the correlation of inflammatory laboratory markers and endoscopic activity showed that only CRP was significantly associated with mucosal activity ($p<0.001$). Fecal calprotectin showed significant correlation with the presence of fecal anti-TNF-α ($p=0.016$) and clinical and endoscopic activity ($p<0.001$) (Figure 8), but not with serum and tissue drug levels ($p=0.981$, tau=0.004; $p=0.06$, tau=0.232).

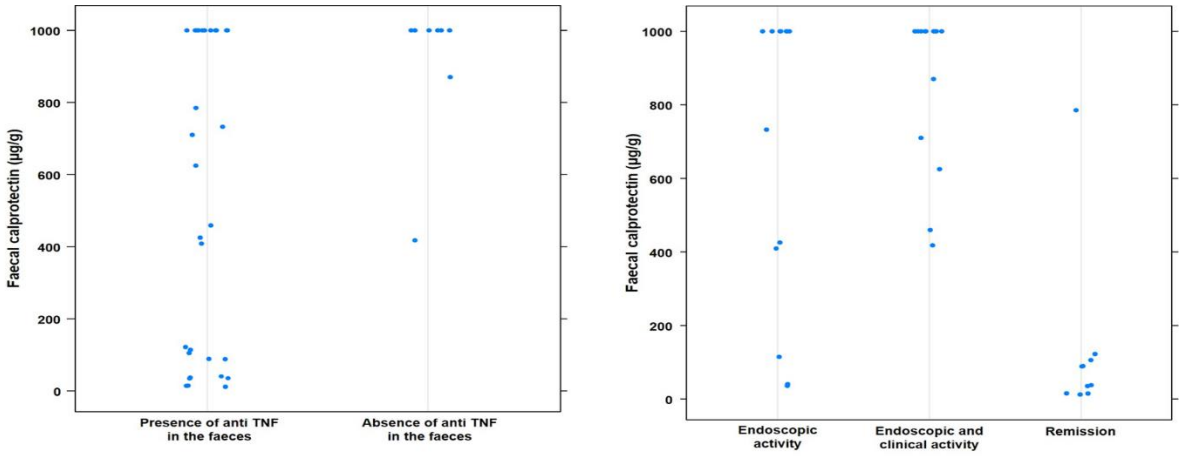

Figure 8. Fecal calprotectin showed significant correlation with the presence of fecal anti-TNF-α and with clinical and endoscopic activity.

DISCUSSION

IBD is a chronic, progressive and disabling condition that has an increasingly serious impact on healthcare and financial resources worldwide. Since biological agents were introduced to the market, the treatment approach and goals in IBD have changed significantly. However, primary and secondary LOR affecting 30-40% of patients is still a matter of debate, posing a significant challenge to physicians. Even more importantly, no reliable approach is available to predict the resistance or non-responsiveness of patients to different treatments. Consequently, there is an unmet need to develop approaches that would optimize and personalize the selection of treatment for IBD patients. In this thesis, we have analyzed the demographic and clinical characteristics of IBD patients at a national level and in our tertiary IBD center. Furthermore, we have evaluated the results of TDM in the serum, tissue and feces of IBD patients receiving anti-TNF-α therapy.

The first study I presented was based on our prospective IBD database and it analyzed 428 CD and 483 UC patients who were followed up for a median of 3.6 years. The median lag time between the onset of symptoms and diagnosis proved to be significantly longer in UC than in CD. Thirty-three point five% of the patients underwent surgery, the majority of them for CD. Biological therapy was given to 40%, immunomodulators to 53% of the patients. More patients needed surgery or biological therapy if they were diagnosed more than 1 year after the onset of symptoms. Surgery was more common in CD patients with ileal location and penetrating behavior but it did not show any relationship with the extent of UC. The rate of surgery was lower in patients treated with thiopurines in UC. A higher proportion of surgery was found in patients above 40 years in both CD and UC. The use of oral contraceptives was protective regarding surgery and the need for biological therapy, and age above 40 years also proved to be protective regarding the need for biologicals in both diseases.

The overall prevalence and incidence of both CD and UC are increasing worldwide. Previous data suggested that approximately 2-14% of patients with CD and UC have a family history of IBD[51]. Our data revealed a bit higher proportion of IBD patients with a positive family history of the disease. Smoking is one of the most consistently examined risk factors in IBD being associated with a more aggressive disease course in CD[52–54]. In our cohort, smoking did not prove to be a predictor of unfavorable disease outcome although the proportion of current smokers was significantly higher in CD vs. UC. Appendectomy also shows different consequences in CD and UC[55–57]; however, our results could not confirm that appendectomy

was associated with the need for biological therapy and/or surgery in CD and we could not reveal any protective effect in UC.

An Australian study of Niewiadomski et al. established a population-based registry to assess disease course in IBD[58]. Immunomodulators were prescribed in 57% of CD and 19% of UC patients, corticosteroids were used in 74% of CD and 63% of UC patients. Only 13% of CD patients were started on biological therapy. In our study, more than half of the patients received immunomodulators and more than one third of the patients received biological therapy during the whole follow-up period. The use of thiopurine was more common in CD patients with colonic and ileocolonic locations. The use of methylprednisolone seems to be lower in our cohort, although it also varies in the IBSEN studies with 43% of the UC patients and 72% of CD patients taking systemic glucocorticoids during the 5-year follow-up[59,60]. In the Australian study, age < 25 years at diagnosis, as well as ileocolonic and perianal disease were risk factors for biological therapy among CD patients[58]. In our cohort, no relationship was found between the location and behavior of CD and disease extent of UC and the use of biological therapy; however, the need for biologicals was higher in patients below the age of 40 and in UC patients with a more severe initial disease activity. In our cohort, we found a lower rate of surgery in patients treated with thiopurines. In CD, ileal location and penetrating behavior, in UC, more severe initial disease activity (assessed with CRP and the pMayo score), and in both diseases age above 40 years at diagnosis correlated with surgery[42].

The systematic review and meta-analysis of Dias et al. highlights that a delay in diagnosis is a crucial factor for unfavorable disease outcome in IBD, since due to delays in diagnosis, disease activity and bowel damage start to worsen if appropriate therapy is lacking[61]. A delay in confirming the diagnosis of IBD is associated with an increased need for surgery, poorer treatment outcomes, impaired quality of life, and more extended disease[62,63]. A French study revealed that a diagnostic delay of more than 12 months is associated with the presence of disease complications, suggesting that a delay in diagnosis beyond 12 months may result in missing the therapeutic window to intervene before disease complications occur [64]. Our results also suggest that more than 1 year of diagnostic delay may be associated with a higher risk of surgery and need for biological therapy in CD and UC.

Real-life data on anti-TNF-α use and treatment characteristics are limited worldwide. We conducted a population-based, retrospective study based on the database of the National Health Insurance Fund to fill in the gaps in our knowledge about the treatment patterns of Hungarian IBD patients. According to literature data, the incidence and prevalence rates of IBD are high in Hungary, which is consistent with our study[2]. In our cohort, 568 UC patients started anti-

TNF-α therapy between late 2012 and 2016, which is only about 2.5% of the total Hungarian UC population. Although the use of biologics is much more common in CD than in UC all over the world, exposure to anti-TNF-α agents among the Hungarian UC population is lower than expected – based on the prevalence values of 6.0% in Canada and 0%-6.4% in Central and Eastern European countries[24,26]. More patients started with IFX (70%) than with ADA (30%). The usual onset of anti-TNF-α therapy is between 30 and 39 years with a median age of 39 years. Furthermore, a slight overrepresentation of males (54%) was found in the biologically treated population. Thirty-five% of our anti-TNF-α treated patients started their first anti-TNF-α therapy within 3 years from diagnosis, one third of them began it within the first year. Due to the reimbursement policy, there was a distinct drop in therapy length at around 1 year as 55% of patients have therapy length of less than a year. The remaining patients were kept on the therapy for a longer period of time based on the decision of the treating physician due to the persisting clinical symptoms and/or incomplete mucosal healing. Two Hungarian prospective studies assessed the disease course and frequency of relapse in UC and CD following discontinuation of IFX therapy after 1 year in patients with remission. According to these studies, anti-TNF-α therapy was restarted at a median of 4 months after discontinuation in 35% of UC patients and it was restarted at a median of 6 months after discontinuation in 45% of CD patients[65,66]. Immunosuppressive and biological treatments have a corticosteroid sparing effect and this was observed in a vast majority of studies; however real-world data are still lacking. A significant decrease in corticosteroid use could be observed in our patients with adequately long (at least 6 months) biological therapy. A retrospective analysis demonstrated that while both azathioprine and anti-TNF-α therapy reduce the number of corticosteroid prescriptions, patients on anti-TNF-α agents were more likely to be in corticosteroid-free remission through 24 months[67].

In the second study presented in this thesis, the rate of PNR was 11.6% and the rate of LOR was 36.5%. According to the literature on anti-TNF-α therapy, PNR rates vary from 10 to 30% in clinical trials and clinical practice and the annual risk of LOR varies from 13% for IFX to 20.3% for ADA[68]. There are limited data on LOR in UC patients; the ACT-1 and 2 trials evaluated LOR in UC patients. Clinical non-remission was 66% at week 54 in ACT-1 and 74.4% at week 30 in ACT-2[14]. Among our patients, PNR rates were consistent with literature data. In case of LOR, the mandatory stop rule made a reliable estimation difficult, and our results may be underestimated due to the statistical method used. DE and switching are performed in patients who cannot maintain remission or lose response to the anti-TNF-α agent. The long term (after one year) DE numbers are similar for the two agents with a total of 30%

requiring DE. However, most of the DEs were performed relatively soon (after 1-2 months) in patients on ADA, while it occurred later (after 1 year) in patients on IFX. As the number of patients on therapy after one year of treatment is much lower than in the second month, the total number of dose escalated patients on ADA is higher (19.8%) than on IFX (10.9%). A wide range of DE rates have been reported in the literature. In case of ADA, a study reported that approximately 8% of patients were dose escalated up until 1 year of treatment, while other studies reported that a higher proportion of patients, around 20-40%, required DE of ADA during the first year of treatment[69–71]. In case of IFX therapy, the DE rate was reported to be 37% among CD patients and 42-58% among UC patients[72,73]. According to a meta-analysis, the random-effects pooled incidence of DE was 38% (95% CI 28-50) for IFX and 36% (95% CI 30-43) for ADA among CD patients[74]. Our research group also conducted a retrospective study evaluating the clinical and treatment characteristics of anti-TNF-α treated IBD patients at the First Department of Medicine, University of Szeged. Among our patients, 22% of the IFX-treated patients and 35% of the ADA-treated patients required DE during the first cycle of anti-TNF-α therapy[75]. Switching therapy was less common than DE; 15.7% of all patients required switching, while the frequency of switch in the dose-escalated population was 19.5%.

The presence of antibodies against anti-TNF-α agents and low drug serum concentrations have been implicated as the most important predisposing factors for therapeutic failure, the exact mechanism of which remains debatable. To gain deeper understanding about drug distribution and clearance of anti-TNF-α agents, the third study in this thesis examined the relationship between anti-TNF-α levels in different biological samples (blood, tissue and feces) and endoscopic and clinical activity in patients receiving maintenance anti-TNF-α therapy. We aimed to achieve novel insights into the mechanism of LOR, which could help to assess therapeutic response and outcome. A study by Atreya et al. detected intestinal membrane-bound TNF positive immune cells following topical antibody administration during confocal laser endomicroscopy in patients with CD. Patients with high numbers of cells expressing membrane-bound TNF-α had significantly higher short-term response rates at week 12 compared to patients with low numbers of membrane-bound TNF cells. Furthermore, they demonstrated a correlation between response to anti-TNF-α and the number of TNF-expressing cells in the intestinal mucosa[76]. In the study by Olsen et al., normalized expression levels of TNF-α quantified using real-time PCR in mucosal biopsies predicted long-term remission after IFX discontinuation, suggesting that this would be an important criterion for deciding when to discontinue treatment with IFX in UC patients[77].

First, we found that the number of TNF-α positive cells was significantly higher in the inflamed part than in the uninflamed part of the colon. This finding would be consistent with the suggestion of Yarur et al. namely that local inflammation with high TNF-α levels may serve as a sink for anti-TNF-α; however, this was not confirmed by tissue drug levels as they did not differ significantly in these regions of the colon. Therefore, for further analysis, we used the average value of tissue drug concentrations. Tissue and fecal drug levels did not show any association with serum drug levels, moreover serum anti-TNF-α concentration did not correlate with endoscopic activity. This can be an aberration, since most of the studies with a high number of enrolled patients confirmed that anti-TNF-α trough levels are associated with sustained clinical response and mucosal healing. However, this correlation largely depends on the determination of cut-off values regarding the drug. For example, in the study by Ungar et al. favorable clinical outcomes were associated with elevated IFX trough levels, which were higher than 10mg/mL[78]. On the other hand, Chaparro et al. defined the best cut-off value for IFX to be 3.4 μg/mL. Although in this study, the authors could confirm an association between anti-TNF-α trough levels and mucosal healing, the accuracy of the IFX and ADA concentrations being able to predict mucosal healing was poor, suggesting that a relevant proportion of patients would be misclassified[79]. Differences between the definition of mucosal healing in the different studies may also be a reason for the various relationships between disease outcomes and trough levels. Altogether, the lack of association between anti-TNF-α concentrations and clinical and endoscopic outcomes can be influenced by the relatively small number of samples analyzed in this study. Moreover, we presented only a transversal analysis of these patients. However, real-life experience does not always corroborate the findings of larger studies. Our results showed that mucosal anti-TNF-α levels are higher in patients in remission, and patients with detectable fecal anti-TNF-α generally had lower tissue drug levels. However, this difference was significant only in the case of IFX-treated subjects. Therefore, we hypothesized that fecal loss of IFX might be associated with decreased mucosal accumulation of the drug. This hypothesis was also confirmed by our results regarding the correlation between fecal drug concentration and endoscopic activity suggesting a link between LOR and increased loss of anti-TNF-α in the feces. Moreover, anti-drug antibody positivity did not influence tissue or fecal drug levels. Based on these data, we can suggest that fecal drug concentration would be a better predictor of endoscopic activity and LOR, and fecal drug monitoring may improve the estimation accuracy of tissue drug levels. Moreover, a correlation was also detected between fecal drug levels and fecal concentration of the inflammatory biomarker, calprotectin. Yarur et al. revealed that tissue anti-TNF-α concentration correlated with the degree of endoscopic inflammation[80].

In keeping with their findings, our results also showed that high serum drug levels may not always translate into high tissue levels; low tissue levels may be attributable to other factors, such as the rapid clearance of anti-TNF-α from the inflamed tissue. Fecal loss of IFX in the background of therapeutic failure was shown by Brandse et al. for the first-time using ELISA in UC patients[81]. However, fecal drug concentration is currently not routinely measured as per TDM strategies. LOR is a major problem in the presently available, most effective biological therapies used for refractory or severe IBD cases. The establishment of biomarkers that predict therapeutic response and help prevent exposure of non-responders to anti-TNF-α therapy to enhance the safety and ensure cost-effective use of this treatment is a very important goal in the management of the disease.

Our studies have some strengths and limitations that should be mentioned. The major strength of the first study is the prospective inclusion and follow-up of incident IBD patients diagnosed in one of the largest IBD centers of Hungary. The relatively high number of patients represents the Hungarian IBD population correctly. The patients are unselected and represent the whole spectrum of disease severity. Additionally, this study is characterized by a long follow-up period of both UC and CD patients. There were cases where we could not perform a complete follow-up of the medical records. After excluding these data from the statistical analysis, no significant difference was found regarding the median lag time between the onset of symptoms and diagnosis. In the second study, a nationwide claims and insurance database was used, which is based on the sole insurance fund in Hungary with almost complete population coverage. All patients receiving biologics in Hungary in the given timeframe could be captured. A major limitation is the retrospective nature of the study, as the primary aim of data collection was not the clinical evaluation of patients, but to serve financial and reimbursement purposes. No data were available on clinical outcomes, such as laboratory values, disease severity indices or patient reported outcomes. Dosing information on corticosteroid dispensing is limited. Due to the low number of deaths in the study population, mortality could not be analyzed. Due to the high cost of biological therapy, yearly limits exist on the amounts that can be reimbursed in the Hungarian system. Therefore, biologic treatment is only available for patients with the most aggressive IBD phenotype. This may be the reason why the clinical outcomes of Hungarian patients are worse than those observed in other western countries. It should be noted that the amount available for reimbursement continuously increased during the years studied. Limitations of the third study are as follows: primarily, the study was performed in a single center and included a relatively limited number of patients, therefore the influence of confounding factors associated with the pharmacokinetics of anti-TNF-α agents cannot be

excluded. Secondly, we pooled data of patients on IFX and ADA therapy that might introduce potential bias into the results; however, we performed subanalysis on the type of anti-TNF-α drugs where it was possible. We know we should be careful about over-interpreting the results as our sample size is small, both inflammatory bowel diseases were examined, and we used two types of anti-TNF-α drugs, the pharmacokinetic profiles of which are known to be vastly different. Furthermore, the patients represented had clinically diverse active disease, as well as varying endoscopic activity. It can occur that a single valuation per se cannot allow for the conclusion that serum levels do not correlate with endoscopic activity. However, we performed all analyses at a certain time-point during regular monitoring representing the real-life clinical situation and treat-to target approach. At this time, serum levels did not show any correlation with endoscopic activity, although tissue and fecal drug levels proved to be different according to activity. We cannot conclude that serum levels will not correlate with endoscopic activity later; however, the median duration of anti-TNF-α therapy was 27.4 months with a minimum duration of 6 months, thus we do think that enough time elapsed to assess treatment efficacy. One of the strengths of this prospective study is that it is unique work on the combined measurement of serum, tissue and fecal anti-TNF-α levels. We successfully validated anti-TNF-α measurement from human tissue and fecal extracts. We correlated fecal and tissue anti-TNF-α levels with disease activity, fecal calprotectin and body composition for the first time. Antibodies against anti-TNF-α agents were measured, hence the influence of immunogenicity was evaluated. Although the rate of immunogenicity is rather high, it might have impacted the serum pharmacokinetic profile of anti-TNF-α, but it still matches the published literature data. Furthermore, we should also consider immunogenicity during data assessment.

CONCLUSIONS

In the first study, we used objective measures, surgery and the need for biological therapy as surrogate markers of worse disease course. Our follow-up study on almost 1000 IBD patients revealed that at the diagnosis of IBD in a referral center, one factor predictive of surgery was age above 40. However, age above 40 years and the use of oral contraceptives proved to be protective regarding the need for biologicals in both diseases. At the same time, more than 1 year of diagnostic delay, disease activity at diagnosis in UC, CD, ileal location and penetrating behavior also seem to be important factors that may influence disease outcome. The use of thiopurines seemed to be protective in UC. In the second, retrospective real-world data study, the treatment patterns of 568 UC patients treated with biological agents between late 2012 and 2016 were analyzed. In most cases, the treatment lasted for nearly a year, when the majority of

patients stopped therapy, which reflects the reimbursement and medical protocols that require them to do so. In a large proportion of UC patients – based on the decision of the treating physician and the medical need – the treatment was continued past this time point, which might suggest that the firm one year stop policy was not optimal for UC patients requiring biological therapy. The concomitant corticosteroid use of the patients was also analyzed. It was found that in patients with adequately long biological therapy the corticosteroid use was significantly reduced after the initiation of biological therapy. The long-term likelihood of being DE was similar in both ADA-, and IFX-treated patients; however, the majority of DE occurred within the first two months of treatment in case of ADA, while IFX-treated patients were mainly escalated after one year of treatment. Therapy switch was more common in previously DE patients. Based on the third study, we suggested that the measurement of fecal anti-TNF-α concentration would increase the benefit of TDM, while the simultaneous determination of fecal drug concentration and fecal calprotectin may be more accurate for evaluating therapeutic response in the future. Moreover, because of its complexity, information gained with a fecal drug test may also be valuable in combination with calprotectin, since by incorporating the measurement of fecal anti-TNF-α concentration in TDM, we can also get information about the clearance of the drug. However, further studies are needed to confirm our observations.

Improving treatment strategies to minimize structural bowel damage and complications has an increasing role in IBD. Hopefully the new trends in IBD management and the optimization of therapeutic options combined with new drugs can change the course of disease and provide better therapy and quality of life for patients suffering from IBD.

SUMMARY OF NEW FINDINGS

- We identified that more than 1 year of diagnostic delay, more severe disease activity at diagnosis in UC, CD itself, ileal location and penetrating behavior of CD are the most important factors that may negatively influence disease outcome in IBD,
- we showed that age above 40 years is predictive of surgery in both CD and UC,
- according to our results, the use of thiopurines is protective regarding surgery in UC,
- we found that exposure to anti-TNF-α agents is low in the Hungarian UC population,
- our results showed that anti-TNF-α therapy was introduced within 1-year of disease duration in only 1/3 of the patients – this is the period of the window of opportunity,
- we confirmed that the rate of PNR and LOR in Hungary are consistent with the literature data,

- we verified that the need for corticosteroid use significantly decreased after the initiation of anti-TNF-α therapy,
- our results showed that serum anti-TNF-α concentration did not correlate with endoscopic activity, but mucosal anti-TNF-α levels are higher in patients in remission,
- we confirmed that patients with detectable fecal anti-TNF-α generally had lower tissue drug levels, which was significant in IFX-treated patients,
- we suggest that fecal drug concentration would be a better predictor of endoscopic activity and LOR.

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to my supervisor Dr. Klaudia Farkas for her support, encouraging, personal guidance and useful critiques of this research work. In addition, I would like to thank for the opportunities and support to Prof. Dr. Tamás Molnár. Special thanks should be given to the Colorectal research group, Prof. Dr. Ferenc Nagy, Dr. Zoltán Szepes, Dr. Renáta Bor, Dr. Anna Fábián, Dr. Anita Bálint, Dr. Ágnes Milassin, Dr. Mariann Rutka, Dr. Tamás Resál, Dr. Péter Bacsur, Dr. Tibor Tóth, Csilla Tóth-Káli and Gabriella Pócsik, and to all of our collaborative partners (MTA-SZTE Momentum Epithelial Cell Signalling and Secretion Research Group, Dr. Tibor Nyári, Dr. Imre Földesi, Dr. Diána Kata) - without them this work could not be done. I am grateful to Prof. Dr. György Ábrahám and Prof. Dr. Csaba Lengyel, former and present head of the Department of Medicine, who gave me the opportunity to work at the Department.

Lastly, I would like to thank my family and friends for all their love, never-ending support, endless patience and encouragement. To them I dedicate this thesis.

REFERENCES

1. Kucharzik T, Maaser C, Lügering A, et al. Recent understanding of IBD pathogenesis: implications for future therapies. Inflamm Bowel Dis 2006;12(11):1068–83.

2. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 2017;390(10114):2769–78.

3. Burisch J, Jess T, Martinato M, Lakatos PL, ECCO -EpiCom. The burden of inflammatory bowel disease in Europe. J Crohns Colitis 2013;7(4):322–37.

4. Burisch J, Pedersen N, Čuković-Čavka S, et al. East-West gradient in the incidence of inflammatory bowel disease in Europe: the ECCO-EpiCom inception cohort. Gut 2014;63(4):588–97.

5. Shivananda S, Lennard-Jones J, Logan R, et al. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). Gut 1996;39(5):690–7.

6. Jacobsen BA, Fallingborg J, Rasmussen HH, et al. Increase in incidence and prevalence of inflammatory bowel disease in northern Denmark: a population-based study, 1978-2002. Eur J Gastroenterol Hepatol 2006;18(6):601–6.

7. Gheorghe C, Pascu O, Gheorghe L, et al. Epidemiology of inflammatory bowel disease in adults who refer to gastroenterology care in Romania: a multicentre study. Eur J Gastroenterol Hepatol 2004;16(11):1153–9.

8. Lapidus A. Crohn's disease in Stockholm County during 1990-2001: an epidemiological update. World J Gastroenterol 2006;12(1):75–81.

9. D'Haens GR, Sartor RB, Silverberg MS, Petersson J, Rutgeerts P. Future directions in inflammatory bowel disease management☆. Journal of Crohn's and Colitis 2014;8(8):726–34.

10. Billiet T, Ferrante M, Van Assche G. The use of prognostic factors in inflammatory bowel diseases. Curr Gastroenterol Rep 2014;16(11):416.

11. Monstad I, Hovde Ø, Solberg IC, A. Moum B. Clinical course and prognosis in ulcerative colitis: results from population-based and observational studies. Ann Gastroenterol 2014;27(2):95–104.

12. da Silva BC, Lyra AC, Rocha R, Santana GO. Epidemiology, demographic characteristics and prognostic predictors of ulcerative colitis. World J Gastroenterol 2014;20(28):9458–67.

13. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 2002;359(9317):1541–9.

14. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353(23):2462–76.

15. Sands BE, Anderson FH, Bernstein CN, et al. Infliximab Maintenance Therapy for Fistulizing Crohn's Disease. New England Journal of Medicine 2004;350(9):876–85.

16. Sandborn WJ, van Assche G, Reinisch W, et al. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology 2012;142(2):257-265.e1-3.

17. Sandborn WJ, Feagan BG, Marano C, et al. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology 2014;146(1):85–95; quiz e14-15.

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

19. Colombel J-F, Sandborn WJ, Rutgeerts P, 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.

20. Targan SR, Hanauer SB, van Deventer SJ, 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.

21. 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.

22. Sandborn WJ, Rutgeerts P, Feagan BG, et al. Colectomy rate comparison after treatment of ulcerative colitis with placebo or infliximab. Gastroenterology 2009;137(4):1250–60; quiz 1520.

23. Feagan BG, Panaccione R, Sandborn WJ, 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.

24. Targownik LE, Tennakoon A, Leung S, Lix LM, Singh H, Bernstein CN. Temporal Trends in Initiation of Therapy With Tumor Necrosis Factor Antagonists for Patients With Inflammatory Bowel Disease: A Population-based Analysis. Clin Gastroenterol Hepatol 2017;15(7):1061-1070.e1.

25. Larsen L, Drewes AM, Broberg MCH, et al. Changing Infliximab Prescription Patterns in Inflammatory Bowel Disease: A Population-Based Cohort Study, 1999-2014. Inflamm Bowel Dis 2018;24(2):433–9.

26. Rencz F, Péntek M, Bortlik M, et al. Biological therapy in inflammatory bowel diseases: Access in Central and Eastern Europe. World J Gastroenterol 2015;21(6):1728–37.

27. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OØ, 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. Am J Gastroenterol 2014;109(7):1055–64.

28. Sprakes MB, Ford AC, Warren L, Greer D, Hamlin J. Efficacy, tolerability, and predictors of response to infliximab therapy for Crohn's disease: a large single centre experience. J Crohns Colitis 2012;6(2):143–53.

29. Allez M, Karmiris K, Louis E, et al. Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: definitions, frequency and pharmacological aspects. J Crohns Colitis 2010;4(4):355–66.

30. Roda G, Jharap B, Neeraj N, Colombel J-F. Loss of Response to Anti-TNFs: Definition, Epidemiology, and Management. Clin Transl Gastroenterol 2016;7(1):e135.

31. Fine S, Papamichael K, Cheifetz AS. Etiology and Management of Lack or Loss of Response to Anti-Tumor Necrosis Factor Therapy in Patients With Inflammatory Bowel Disease. Gastroenterol Hepatol (N Y) 2019;15(12):656–65.

32. Papamichael K, Gils A, Rutgeerts P, et al. Role for therapeutic drug monitoring during induction therapy with TNF antagonists in IBD: evolution in the definition and management of primary nonresponse. Inflamm Bowel Dis 2015;21(1):182–97.

33. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology 2015;148(7):1320-1329.e3.

34. Touw DJ, Neef C, Thomson AH, Vinks AA, Cost-Effectiveness of Therapeutic Drug Monitoring Committee of the International Association for Therapeutic Drug Monitoring and Clinical Toxicology. Cost-effectiveness of therapeutic drug monitoring: a systematic review. Ther Drug Monit 2005;27(1):10–7.

35. Birkett D. Pharmacokinetics made easy: Therapeutic drug monitoring. 1997;

36. Papamichael K, Cheifetz AS, Melmed GY, et al. Appropriate Therapeutic Drug Monitoring of Biologic Agents for Patients With Inflammatory Bowel Diseases. Clin Gastroenterol Hepatol 2019;17(9):1655-1668.e3.

37. Pallagi-Kunstár É, Farkas K, Szepes Z, et al. Utility of serum TNF-α, infliximab trough level, and antibody titers in inflammatory bowel disease. World J Gastroenterol 2014;20(17):5031–5.

38. Lennard-Jones JE. Classification of inflammatory bowel disease. Scand J Gastroenterol Suppl 1989;170:2–6; discussion 16-19.

39. Satsangi J, Silverberg MS, Vermeire S, Colombel J-F. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut 2006;55(6):749–53.

40. Best WR, Becktel JM, Singleton JW, Kern F. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology 1976;70(3):439–44.

41. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med 1987;317(26):1625–9.

42. Gomollón F, Dignass A, Annese V, et al. 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part 1: Diagnosis and Medical Management. J Crohns Colitis 2017;11(1):3–25.

43. Magro F, Gionchetti P, Eliakim R, 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. J Crohns Colitis 2017;11(6):649–70.

44. Daperno M, D'Haens G, Van Assche G, et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointest Endosc 2004;60(4):505–12.

45. Sipponen T, Nuutinen H, Turunen U, Färkkilä M. Endoscopic evaluation of Crohn's disease activity: comparison of the CDEIS and the SES-CD. Inflamm Bowel Dis 2010;16(12):2131–6.

46. Molander P, Sipponen T, Kemppainen H, et al. Achievement of deep remission during scheduled maintenance therapy with TNFα-blocking agents in IBD. J Crohns Colitis 2013;7(9):730–5.

47. Klenske E, Bojarski C, Waldner M, Rath T, Neurath MF, Atreya R. Targeting mucosal healing in Crohn's disease: what the clinician needs to know. Therap Adv Gastroenterol 2019;12:1756284819856865.

48. Papamichael K, Cheifetz AS. Use of anti-TNF drug levels to optimise patient management. Frontline Gastroenterol 2016;7(4):289–300.

49. Mazor Y, Almog R, Kopylov U, et al. Adalimumab drug and antibody levels as predictors of clinical and laboratory response in patients with Crohn's disease. Aliment Pharmacol Ther 2014;40(6):620–8.

50. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 1976;72(1):248–54.

51. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature 2011;474(7351):307–17.

52. Cosnes J, Carbonnel F, Carrat F, Beaugerie L, Cattan S, Gendre J. Effects of current and former cigarette smoking on the clinical course of Crohn's disease. Aliment Pharmacol Ther 1999;13(11):1403–11.

53. Cosnes J. Tobacco and IBD: relevance in the understanding of disease mechanisms and clinical practice. Best Pract Res Clin Gastroenterol 2004;18(3):481–96.

54. Lakatos PL, Szamosi T, Lakatos L. Smoking in inflammatory bowel diseases: good, bad or ugly? World J Gastroenterol 2007;13(46):6134–9.

55. Andersson RE, Olaison G, Tysk C, Ekbom A. Appendectomy is followed by increased risk of Crohn's disease. Gastroenterology 2003;124(1):40–6.

56. Gardenbroek TJ, Eshuis EJ, Ponsioen CIJ, Ubbink DT, D'Haens GR a. M, Bemelman WA. The effect of appendectomy on the course of ulcerative colitis: a systematic review. Colorectal Dis 2012;14(5):545–53.

57. Radford-Smith GL, Edwards JE, Purdie DM, et al. Protective role of appendicectomy on onset and severity of ulcerative colitis and Crohn's disease. Gut 2002;51(6):808–13.

58. Niewiadomski O, Studd C, Hair C, et al. Prospective population-based cohort of inflammatory bowel disease in the biologics era: Disease course and predictors of severity. J Gastroenterol Hepatol 2015;30(9):1346–53.

59. Henriksen M, Jahnsen J, Lygren I, et al. Clinical course in Crohn's disease: results of a five-year population-based follow-up study (the IBSEN study). Scand J Gastroenterol 2007;42(5):602–10.

60. Henriksen M, Jahnsen J, Lygren I, et al. Ulcerative colitis and clinical course: results of a 5-year population-based follow-up study (the IBSEN study). Inflamm Bowel Dis 2006;12(7):543–50.

61. Dias CC, Rodrigues PP, da Costa-Pereira A, Magro F. Clinical prognostic factors for disabling Crohn's disease: a systematic review and meta-analysis. World J Gastroenterol 2013;19(24):3866–71.

62. Schoepfer AM, Dehlavi M-A, Fournier N, et al. Diagnostic delay in Crohn's disease is associated with a complicated disease course and increased operation rate. Am J Gastroenterol 2013;108(11):1744–53; quiz 1754.

63. Gower-Rousseau C, Dauchet L, Vernier-Massouille G, et al. The natural history of pediatric ulcerative colitis: a population-based cohort study. Am J Gastroenterol 2009;104(8):2080–8.

64. Nahon S, Lahmek P, Lesgourgues B, et al. Diagnostic delay in a French cohort of Crohn's disease patients. J Crohns Colitis 2014;8(9):964–9.

65. Farkas K, Lakatos PL, Nagy F, et al. Predictors of relapse in patients with ulcerative colitis in remission after one-year of infliximab therapy. Scand J Gastroenterol 2013;48(12):1394–8.

66. Molnár T, Lakatos PL, Farkas K, et al. Predictors of relapse in patients with Crohn's disease in remission after 1 year of biological therapy. Aliment Pharmacol Ther 2013;37(2):225–33.

67. Kane SV, Jaganathan S, Bedenbaugh AV, Palmer L, Schwartz DA. Anti-tumor necrosis factor agents reduce corticosteroid use compared with azathioprine in patients with Crohn's disease. Curr Med Res Opin 2014;30(9):1821–6.

68. Flamant M, Roblin X. Inflammatory bowel disease: towards a personalized medicine. Therap Adv Gastroenterol 2018;11:1756283X17745029.

69. Taxonera C, Iglesias E, Muñoz F, et al. Adalimumab Maintenance Treatment in Ulcerative Colitis: Outcomes by Prior Anti-TNF Use and Efficacy of Dose Escalation. Dig Dis Sci 2017;62(2):481–90.

70. Black CM, Yu E, McCann E, Kachroo S. Dose Escalation and Healthcare Resource Use among Ulcerative Colitis Patients Treated with Adalimumab in English Hospitals: An Analysis of Real-World Data. PLoS One 2016;11(2):e0149692.

71. Sandborn WJ, Sakuraba A, Wang A, et al. Comparison of real-world outcomes of adalimumab and infliximab for patients with ulcerative colitis in the United States. Curr Med Res Opin 2016;32(7):1233–41.

72. Gisbert JP, Panés J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. Am J Gastroenterol 2009;104(3):760–7.

73. Taxonera C, Olivares D, Mendoza JL, Díaz-Rubio M, Rey E. Need for infliximab dose intensification in Crohn's disease and ulcerative colitis. World J Gastroenterol 2014;20(27):9170–7.

74. Qiu Y, Chen B-L, Mao R, et al. Systematic review with meta-analysis: loss of response and requirement of anti-TNFα dose intensification in Crohn's disease. J Gastroenterol 2017;52(5):535–54.

75. Bacsur P, Skribanek S, Milassin Á, et al. Anti-TNF-alfa terápiában részesülő gyulladásos bélbetegek hosszútávú utánkövetése [Long-term follow-up of inflammatory bowel diseases patients receiving anti-tumor necrosis factor alpha therapy]. Orvosi Hetilap 2020;161(47):1989–94.

76. Atreya R, Neumann H, Neufert C, et al. In vivo imaging using fluorescent antibodies to tumor necrosis factor predicts therapeutic response in Crohn's disease. Nat Med 2014;20(3):313–8.

77. Olsen T, Rismo R, Gundersen MD, et al. Normalization of mucosal tumor necrosis factor-α: A new criterion for discontinuing infliximab therapy in ulcerative colitis. Cytokine 2016;79:90–5.

78. Ungar B, Levy I, Yavne Y, et al. Optimizing Anti-TNF-α Therapy: Serum Levels of Infliximab and Adalimumab Are Associated With Mucosal Healing in Patients With Inflammatory Bowel Diseases. Clin Gastroenterol Hepatol 2016;14(4):550-557.e2.

79. Chaparro M, Barreiro-de Acosta M, Echarri A, et al. Correlation Between Anti-TNF Serum Levels and Endoscopic Inflammation in Inflammatory Bowel Disease Patients. Dig Dis Sci 2019;64(3):846–54.

80. Yarur AJ, Jain A, Sussman DA, et al. The association of tissue anti-TNF drug levels with serological and endoscopic disease activity in inflammatory bowel disease: the ATLAS study. Gut 2016;65(2):249–55.

81. 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(2):350-355.e2.