Ph.D. Thesis

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

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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)

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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

INTRODUCTION

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. The incidence and prevalence of IBD are increasing worldwide. 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. 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 steroid-free remission. However, with the introduction of newer therapeutic options more rigorous endpoints have been identified. 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.

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. 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. In Hungary, starting from late 2012, a register of special drug reimbursement (hereinafter, Patient Registry) provides data on all administration of biological therapies. 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. The clinical definition of primary non-response (PNR) is lack of improvement of clinical signs and symptoms with induction therapy. LOR describes patients who respond to the therapy after an induction regimen, but subsequently lose response during maintenance treatment. 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. 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. 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. 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.

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. 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. Otherwise, TDM refers to the individualization of drug dosage by maintaining plasma or blood drug concentrations within a targeted therapeutic range or window. TDM and, in case of certain drugs, monitoring antidrug antibodies, have been shown to help therapy optimization, manage LOR and prolong maintenance treatment. 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. 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.

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

1.1. Data sources 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. Disease activity was assessed with the Crohn's Disease Activity Index (CDAI) in CD and the Mayo Scoring System in UC. On the basis of the activity scores, patients were divided into inactive, mildly, moderately and severely active groups. 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.

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. All patients suffering from UC were captured in the database based on the International Classification of Diseases (ICD-10) diagnosis code K51. 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 dispense of corticosteroids was captured using Anatomical Therapeutic Chemical Classification System codes (ATC).

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. 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. A patient was only considered to switch to another therapy 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. 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.

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. The patients underwent colonoscopy between January 2017 and March 2018. 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. Endoscopically active disease was defined as SES-CD≥3 in CD and

eMayo≥2 UC. 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 (InBody770 body composition analyzer) before colonoscopy. Each patient was informed about the study and provided written informed consent.

1.2. Description of methods

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.

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%.

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.

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).

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.

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% paraformaldehyde-phosphate buffer saline (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.

1.3. Statistical analysis

In **the first study**, the 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.

In **the second study**, 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.

In **the third study**, 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.

1.4. Ethical approval

Ethical approvals for the above mentioned studies were obtained from the Regional and Institutional Human Medical Biological Research Ethics Committee, University of Szeged (2640; 36/2016-SZTE; 3735) and from the Medical Research Council – Research and Ethics Committee (TUKEB), Hungary (Appr. no: 12288-3/2018/EKU).

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

Nine hundred and eleven 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 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.

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. 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. 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 ($p \leq 0.001$).

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. Colectomy was performed in 77 patients throughout the follow-up period.

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 at the first attendance (p≤0.001).

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

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). Smoking and history of appendectomy were significantly more common in CD vs. UC patients (p≤0.001 and p=0.003). The rate of surgery proved to be significantly higher in CD vs. UC (p≤0.001). 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, 568 UC patients were treated with anti-TNF-α agents. 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.

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. Despite the reimbursement rule that required treating physicians to stop biological treatment after one year, roughly 45% of patients continued the initial treatment.

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

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%). 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 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).

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 dispensings

Patients used significantly less corticosteroids after starting anti-TNF-α therapy than before ($p < 0.001$).

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 (29 CD, 21 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.

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). 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).

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

Examining 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). 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). 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). 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).

3.4. Correlation of different anti-TNF-α levels with endoscopic activity, body composition, inflammatory parameters and fecal calprotectin

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. 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. We found that body composition parameters had no significant impact on serum and tissue drug levels. 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 ($p<0.001$), but not with serum and tissue drug levels ($p=0.981$, tau=0.004; $p=0.06$, tau=0.232).

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. 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 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. 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. 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; 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. Immunomodulators were prescribed in 57% of CD and 19% of UC patients, steroids 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. 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. 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. 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. 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.

According to literature data, the incidence and prevalence rates of IBD are high in Hungary, which is consistent with our presented second study. 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. More patients started with IFX (70%) than with ADA (30%). 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. 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. 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.

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% and the annual risk of LOR varies from 13% for IFX to 20.3% for ADA. 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. 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. 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. 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. 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. 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. 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. 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. 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. 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. 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. 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. 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.

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.

(For references to the statements in the thesis booklet, see the dissertation.)

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