Association between serum, mucosal and fecal infliximab levels and response to anti TNF-α therapy in patients with inflammatory bowel disease – new insights into the mechanism of loss of response to biological therapy (K119809)

The main focus of the research was a comprehensive study that aimed to analyse the serum, tissue and faecal concentrations of anti-TNF agents as well as the mucosal expression of TNF-α and to assess the relationship of the drug levels in biological samples with the endoscopic activity, clinical activity and body composition in inflammatory bowel disease (IBD) patients receiving maintenance anti-TNF therapy.

Fifty consecutive patients with luminal Crohn's disease (CD) and (UC) receiving maintenance infliximab (IFX) or adalimumab (ADA) therapy at the First Department of Medicine, University of Szeged who underwent colonoscopy were finally enrolled in the study. Serum samples were obtained for determining the routine inflammatory parameters and for the determination of anti-TNF-α and anti-drug antibody levels. Stool samples were obtained to determine the faecal calprotectin and drug concentration. During endoscopy, biopsy samples were taken only from the colon. For the determination of tissue drug levels, samples were obtained from the inflamed (the mostly affected region with avoiding samples from ulcers) and un-inflamed parts of the colon. In the lack of endoscopic activity, tissue samples were obtained from the un-inflamed tissue that was previously involved. Each patient was informed about the study and provided written informed consent for study participation. The serum IFX and ADA concentrations, the levels of antibodies for IFX and ADA and mucosal and fecal drug levels were determined using the ELISA assay. Mucosal TNF-α expression was detected with the use of confocal microscopy after immunofluorescent labelling of the biopsy samples and the results were presented as TNF-α positive cell number/total cell number to normalize the data. Body composition was determined with an InBody770 body composition analyser.

Of the enrolled patients, 21 patients represented with clinically active disease and 38 patients showed endoscopic activity. The ratio of TNF-α positive/total cells was significantly higher in the inflamed vs. un-inflamed part of the colon (Figure 1). However, the difference in the tissue drug levels obtained from the inflamed part of the colon did not differ significantly compared to the un-inflamed samples ($p = 0.1065$) and did not show any association with the presence of anti-drug antibodies.

During the study we aimed to determine whether there was a correlation between the serum and faecal drug level and the tissue drug levels of the inflamed and un-inflamed segments. Neither the drug level of the inflamed tissue ($p = 0.988$), nor that of the un-inflamed tissue

correlated significantly with the serum drug level ($p = 0.155$). Lower serum drug levels were shown in patients with antibody positivity. Patients with detectable faecal anti-TNF had substantially lower tissue drug levels; however, the difference was not significant ($p = 0.124$). Thus, we hypothesized that faecal loss of anti-TNF might be associated with decreased mucosal accumulation of the drug.

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 for the prediction of responders to anti-TNF therapy. As a confirmation of this discordance between the serum drug levels and therapeutic response, no significant correlation was observed between endoscopic activity and serum drug concentrations ($p = 0.9925$). However, the tissue drug levels of samples obtained from the un-inflamed part of the colon proved to be significantly different according to activity ($p = 0.03467$), with higher levels observed in those in remission. The presence of the drug in the faeces was significantly different as per the activity ($p = 0.0022$); almost no subject in remission or with only endoscopic activity had detectable drug levels; however, 50% of the clinically active patients had non-zero faecal drug concentration. We also aimed to model which simple parameters may predict endoscopic relapse in patients with clinical remission. Although serum drug level, anti-drug antibody positivity and BMI was not predictive, CRP showed only significant prediction to relapse. We hypothesized that body mass and composition may influence the anti-TNF levels and thus the therapeutic response; therefore, we performed multivariate regression analysis with serum drug level and tissue drug level as the response variables. However, we found that body composition parameters, including body mass index, total body water, as well as minerals and skeletal muscles mass had no significant impact on the serum and tissue drug levels.

Although the low number of patients may serve as a limitation of the study, to our knowledge, this is the first study that simultaneous examined the serum, tissue and faecal concentrations of anti-TNF and compared them with clinical and endoscopic activities as well as body composition. Based on our data, we can suggest that faecal drug concentration is a better predictor of endoscopic activity and loss of response. Loss of response 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 the therapeutic response and help prevent exposure of non-responders to anti-TNF therapy to enhance the safety and ensure cost-effective use of this treatment is a very important initiation in the management of the disease. Our study reports several important findings, including the importance of faecal drug

monitoring that is not being routinely used at present. Data have been presented at the United European Gastroenterology Week in 2017, at the congress of the European Crohn's Colitis Organisation and the annual meeting of the Hungarian Gastroenterology Society in 2018. The manuscript on the research proposal has already been submitted.

During the study period we conducted additional studies which were associated with our original research goals. We assessed the correlation between serum IFX and anti-infliximab antibody (ATI) levels and response to IFX therapy and tried to determine the accuracy of serum drug concentration measurement in the prediction of the long-term clinical response. Our results showed that single measurement of ATI titer was insufficient for predicting therapeutic response due to transient expression of ATI, however, using the three points' measurements, significant difference has been detected between the adequate and inadequate responder group. The mean value of TL was significantly higher in the adequate responder group without further difference on the second and sixth week. We concluded that simultaneous measurement of serum IFX level prior to administration of regular IFX infusion and ATI titers significantly increase the diagnostic accuracy for the therapeutic decision in patients uncertainly responding to the therapy. These results have been published in Plos One (Bor R, Farkas K, Fábián A, Bálint A, Milassin Á, Rutka M, et al. (2017) Clinical role, optimal timing and frequency of serum infliximab and anti-infliximab antibody level measurements in patients with inflammatory bowel disease. PLoS ONE 12(3): e0172916.)

We examined the role of CT-P13, the first biosimilar monoclonal antibody to IFX therapy in the maintenance of clinical and endoscopic remission in two additional studies. For the evaluation of the long-term efficacy and safety of CT-P13 therapy in IBD 57 CD and 57 UC patients were included who were administered CT-P13. Clinical remission was achieved in 65.5% of CD and 75.5% of UC patients at week 14. Rate of continuous clinical response was 51% in both CD and UC at week 54. We were not able to show any of the examined parameters to predict the outcome of CT-P13 therapy at week 54 neither in CD nor in UC. This was the first European prospective study that evaluated and confirmed long-term efficacy and safety of CT-P13 therapy in IBD. We have published our results in the journal of Expert Opinion on Biological Therapy in 2017 (Farkas K, Rutka M, Ferenci T 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, 2017; 17: 1325-1332).

For the evaluation of the efficacy of CT-P13 in maintaining mucosal healing in UC we analyzed the data of 61 patients. This was a multicenter, prospective 'real life' study conducted at four Hungarian and one Czech IBD center. Mucosal healing was shown in 65.5% and 62.1%, complete mucosal healing was present in 31% and 38% at week 14 and 54, respectively. The median values of CRP, leukocytes, thrombocytes, and albumin showed significant difference between baseline and week 54. Our results proved that CT-P13 therapy is effective at long-term period in everyday practice. The paper on this topic was published in the journal of Expert Opinion on Biological Therapy in 2018 (Bálint A, Rutka M, Kolar M 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, 2018; 11: 1181-1187)

Immunological reactions including immunogenicity and infusion-related reactions related to the chimeric mouse-human structure of IFX is one of the main limiting factors of the therapy. Immediate infusion reactions, developing during the course of the infusion or within 1–2 h of its completion, are reported in 5–23% of IBD patients receiving originator IFX. Our prospective, observational, multicenter study carried out in 13 Hungarian and 1 Czech IBD tertiary centers **aimed to assess the frequency, characteristics and predictors of infusion reactions during CT-P13 therapy.** Three hundred and eighty-four IBD patients were included. Twenty-eight Hungarian IBD patients (9.6%) developed infusion reaction during the treatment, 64.3% of them was previously exposed to anti TNF therapy. No infusion reaction occurred in the Czech population. CT-P13 therapy had to be stopped in 17 patients who developed infusion reaction and was switched to ADA in 12 patients. However, in 39.3% of patients developing infusion reaction CT-P13 therapy was continued with the use of premedication. Cumulative anti-drug-antibody positivity rates were 8.7%, 19.3%, and 28.0% at weeks 0, 14, and 30. Previous anti-TNF-alpha exposure (30% vs. 3.1%, $p < 0.001$, OR 6.3 (2.7–14.6)) and anti-drug antibody positivity (32.6% vs. 4.1%, $p < 0.001$, OR 19(5–73)) during the induction therapy were predictive factors for infusion reactions. This large, multicenter study was the first that evaluated the immunogenicity profile of CT-P13 in IBD in the clinical practice, characterized infusion reactions in this population and revealed predictors for infusion reaction. Our results suppose a lower immunogenicity of the biosimilar in CD and similar rates and characteristics of infusion reaction with the originator. The study

was published in the Expert Opinion on Drug Safety in 2017 (Balint A, Rutka M, Végh Z, Frequency and characteristics of infusion reactions during biosimilar infliximab treatment in inflammatory bowel diseases, 2017; 16: 885-890).

The topic of CT-P13 has also been summarized in a review article published in Immunotherapy in 2018 (Farkas K, Molnar T. A review on biosimilar infliximab, CT-P13, in the treatment of inflammatory bowel disease. Immunotherapy, 2018; 10: 107-117).

Since only limited data were available from the Eastern Europe regarding to the rate of more complicated disease course, use of biological therapy and number of surgeries, during the research proposal **we conducted a study that aimed to determine demographic features, disease phenotypes, medical and surgical therapies in our large IBD patients' registry and to identify which parameters are in association with the need of surgery and/or biologic therapy as surrogate markers of worse disease course in CD and UC.** Data of 911 IBD patients (428 CD, 483 UC) were analyzed. The median lag time between onset of symptoms and diagnosis proved to be significantly longer in UC than in CD (4.6 years vs. 2.1 years, $p = 0.01$). 40% of the patients received biological therapy, 301 patients underwent surgery required more frequently for CD than UC. Surgery was more common in CD patients with ileal location and penetrating behaviour. In UC, more severe disease onset predicted to unfavourable disease course. Higher proportion of surgery was shown in patient aged above 40 years in both CD and UC. Diagnostic delay of more than 1 year and appendectomy predicted to unfavourable disease outcome of both CD and UC. In this study we used objective measures, surgery and need of biological therapy as surrogate markers of worse disease course. Hopefully the new trends in IBD management and the optimization of therapeutic options will make possible to change the course of disease and provide better therapy and quality of life for patients suffering from IBD (Szántó K, Nyári T, Bálint A, Biological therapy and surgery rates in inflammatory bowel diseases - Data analysis of almost 1000 patients from a Hungarian tertiary IBD center, Plos One, 2018; 13(7):e0200824. doi: 10.1371/journal.pone.0200824. eCollection 2018.).