DIAGNOSTIC AND PROGNOSTIC VALUE OF FECAL, SERUM AND ENDOSCOPIC MARKERS IN INFLAMMATORY BOWEL DISEASE AND COLORECTAL CANCER

Ph.D. THESIS

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LIST OF FULL PAPERS RELATED TO THE SUBJECT OF THE THESIS

I. **Rutka M**, Milassin Á, Szepes Z, Szűcs M, Nyári T, Bálint A, Bor R, Molnár T, Farkas K Is mucosal healing more common than clinical remission in ulcerative colitis? - Is it the truth or only a myth coming from the studies?
Scand J Gastroenterol. 2015 Aug;50(8):985-90.
IF: 2,199

II. Farkas K, Rutka M, Golovics PA, Végh Z, Lovász BD, Nyári T, Gecse KB, Kolar M, Bortlik M, Duricova D, Machkova N, Hruba V, Lukas M, Mitrova K, Malickova K, Bálint A, Nagy F, Bor R, Milassin Á, Szepes Z, Palatka K, Lakatos PL, Lukas M, Molnár T. Efficacy of Infliximab Biosimilar CT-P13 Induction Therapy on Mucosal Healing in Ulcerative Colitis.
J Crohns Colitis. 2016 Nov;10(11):1273-1278.
IF: 5,813

III. Bor R, Farkas K, Fábián A, Bálint A, Milassin Á, **Rutka M**, Matuz M, Nagy F, Szepes Z, Molnár T. Clinical role, optimal timing and frequency of serum infliximab and anti-infliximab antibody level measurements in patients with inflammatory bowel disease.
PLoS One. 2017 Mar 31;12(3):e0172916.
IF: 2,806

IV. **Rutka M**, Bor R, Bálint A, Fábián A, Milassin Á, Nagy F, Szepes Z, Szűcs M, Tiszlavicz L, Farkas K, Molnár T. Diagnostic Accuracy of Five Different Fecal Markers for the Detection of Precancerous and Cancerous Lesions of the Colorectum**.**
Mediators Inflamm. 2016; 2016:2492081. doi: 10.1155/2016/2492081.
IF: 3,232

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

I. Bálint A, Farkas K, Szepes Z, Nagy F, Szűcs M, Tiszlavicz L, Bor R, Milassin Á, **Rutka M**, Fábián A, Molnár T. How disease extent can be included in the endoscopic activity index of ulcerative colitis: the panMayo score, a promising scoring system. *BMC Gastroenterol.* 2018 Jan 8;18(1):7.
IF: 2,212*

II. **Rutka M**, Molnár T, Bor R, Farkas K, Fábián A, Győrfi M, Bálint A, Milassin Á, Szűcs M, Tiszlavicz L, Nagy F, Szepes Z. Efficacy of the population-based pilot colorectal screening program. Hungary, Csongrád county, 2015.
Orv Hetil. 2017 Oct;158(42):1658-1667. Hungarian.
IF: 0,349*

III. Bor R, Fábián A, Bálint A, Farkas K, Szűcs M, Milassin Á, Czakó L, **Rutka M**, Molnár T, Szepes Z. Endoscopic management of complications of self-expandable metal stents for treatment of malignant esophageal stenosis and tracheoesophageal fistulas.
Therap Adv Gastroenterol. 2017 Aug;10(8):599-607.
IF: 3,648*

IV. Milassin Á, Sejben A, Tiszlavicz L, Reisz Z, Lázár G, Szűcs M, Bor R, Bálint A, **Rutka M**, Szepes Z, Nagy F, Farkas K, Molnár T. Analysis of risk factors - especially different types of plexitis - for postoperative relapse in Crohn's disease.
World J Gastrointest Surg. 2017 Jul 27;9(7):167-173.
IF: -

V. Gonczi L, Kurti Z, **Rutka M**, Vegh Z, Farkas K, Lovasz BD, Golovics PA, Gecse KB, Szalay B, Molnar T, Lakatos PL. Drug persistence and need for dose intensification to adalimumab therapy; the importance of therapeutic drug monitoring in inflammatory bowel diseases.
BMC Gastroenterol. 2017 Aug 8;17(1):97. doi: 10.1186/s12876-017-0654-1.
IF: 2,212*

VI. Bálint A, **Rutka M**, Végh Z, Kürti Z, Gecse KB, Banai J, Bene L, Gasztonyi B, Kristóf T, Lakatos L, Miheller P, Palatka K, Patai Á, Salamon Á, Szamosi T, Szepes Z, Tóth GT, Vincze Á, Bor R, Milassin Á, Fábián A, Nagy F, Kolar M, Bortlik M, Duricova D, Hruba V, Lukas M, Mitrova K, Malickova K, Lukas M, Lakatos PL, Molnár T, Farkas K. Frequency and characteristics of infusion reactions during biosimilar infliximab treatment in inflammatory bowel diseases: results from Central European nationwide cohort.
Expert Opin Drug Saf. 2017 Aug;16(8):885-890. Epub 2017 May 26.
IF: 3,439*

VII. Bor R, Farkas K, Bálint A, Szűcs M, Ábrahám S, Milassin Á, **Rutka M**, Nagy F, Milassin P, Szepes Z, Molnár T. Prospective Comparison of Magnetic Resonance Imaging, Transrectal and Transperineal Sonography, and Surgical Findings in Complicated Perianal Crohn Disease.

J Ultrasound Medicine 2016 35(11): 2367-2372.

IF: 1,547

VIII. Fábián A, Bor R, Bálint A, Farkas K, Milassin Á, **Rutka M**, Tiszlavicz L, Nagy F, Molnár T, Szepes Z. Neoadjuvant treatment as a limiting factor to rectal ultrasonography.

Orv Hetil. 2016 Jul;157(30):1193-7. doi: 10.1556/650.2016.30432. Hungarian.

IF: 0,349

IX. Farkas K, Chan H, **Rutka M**, Szepes Z, Nagy F, Tiszlavicz L, Nyári T, Tang W, Wong G, Tang R, Lo A, Cheung C, Wong S, Lui R, Molnár T, Ng SC . Gastroduodenal Involvement in Asymptomatic Crohn's Disease Patients in Two Areas of Emerging Disease: Asia and Eastern Europe..

J Crohns Colitis. 2016 Dec;10(12):1401-1406. Epub 2016 Jun 9.

IF: 5,813

X. **Rutka M**, Bálint A, Farkas K, Palatka K, Lakner L, Miheller P, Rácz I, Hegede G, Vincze Á, Horváth G, Szabó A, Nagy F, Szepes Z, Gábor Z, Zsigmond F, Zsóri Á, Juhász M, Csontos Á, Szűcs M, Bor R, Milassin Á, Molnár T. [Long-term adalimumab therapy in ulcerative colitis in clinical practice: result of the Hungarian multicenter prospective study].

Orv Hetil. 2016 May 1;157(18):706-11. Hungarian.

IF: 0,349

XI. Fábián A, Bor R, Farkas K, Bálint A, Milassin Á, **Rutka M**, Tiszlavicz L, Wittmann T, Nagy F, Molnár T, Szepes Z. Rectal Tumour Staging with Endorectal Ultrasound: Is There Any Difference between Western and Eastern European Countries?

Gastroenterol Res Pract. 2016;2016:8631381. Epub 2015 Dec 24.

IF: 1,863

XII. Gönczi L, Gecse KB, Vegh Z, Kurti Z, **Rutka M**, Farkas K, Golovics PA, Lovasz BD, Banai J, Bene L, Gasztonyi B, Kristóf T, Lakatos L, Miheller P, Nagy F, Palatka K, Papp M, Patai A, Salamon A, Szamosi T, Szepes Z, Tóth GT, Vincze A, Szalay B, Molnar T, Lakatos PL. Long-term Efficacy, Safety, and Immunogenicity of Biosimilar Infliximab After One Year in a Prospective Nationwide Cohort.

Inflamm Bowel Dis. 2017 Nov;23(11):1908-1915.

IF: 4,525*

XIII. Farkas K, **Rutka M**, Ferenci T, Nagy F, Bálint A, Bor R, Milassin Á, Fábián A, Szántó K, Végh Z, Kürti Z, Lakatos PL, Szepes Z, Molnár T. 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 Opin Biol Ther. 2017 Nov;17(11):1325-1332. Epub 2017 Aug 18.
IF: 3,684

XIV. Gönczi L, Vegh Z, Golovics PA, **Rutka M**, Gecse KB, Bor R, Farkas K, Szamosi T, Bene L, Gasztonyi B, Kristóf T, Lakatos L, Miheller P, Palatka K, Papp M, Patai Á, Salamon Á, Tóth GT, Vincze Á, Biro E, Lovasz BD, Kurti Z, Szepes Z, Molnár T, Lakatos PL. Prediction of Short- and Medium-term Efficacy of Biosimilar Infliximab Therapy. Do Trough Levels and Antidrug Antibody Levels or Clinical And Biochemical Markers Play the More Important Role?
J Crohns Colitis. 2017 Jun 1;11(6):697-705.
IF: 5,813*

XV. Annaházi A, Ábrahám S, Farkas K, Rosztóczy A, Inczefi O, Földesi I, Szűcs M, **Rutka M**, Theodorou V, Eutamene H, Bueno L, Lázár G, Wittmann T, Molnár T, Róka R. A pilot study on faecal MMP-9: a new noninvasive diagnostic marker of colorectal cancer.
Br J Cancer. 2016 Mar 29;114(7):787-92. Epub 2016 Feb 23.
IF: 6,176

XVI. Farkas K, **Rutka M**, Bálint A, Nagy F, Bor R, Milassin Á, Szepes Z, Molnár T. Efficacy of the new infliximab biosimilar CT-P13 induction therapy in Crohn's disease and ulcerative colitis - experiences from a single center.
Expert Opin Biol Ther. 2015;15(9):1257-62. Epub 2015 Jul 2.
IF: 3,438

XVII. Daróczi T, Bor R, Fábián A, Szabó E, Farkas K, Bálint A, Czakó L, **Rutka M**, Szűcs M, Milassin Á, Molnár T, Szepes Z. [Cost-effectiveness trial of self-expandable metal stents and plastic biliary stents in malignant biliary obstruction].
Orv Hetil. 2016 Feb 14;157(7):268-74. Hungarian.
IF: 0,349

XVIII. Bálint A, Farkas K, Palatka K, Lakner L, Miheller P, Rácz I, Hegede G, Vincze Á, Horváth G, Szabó A, Nagy F, Szepes Z, Gábor Z, Zsigmond F, Zsóri Á, Juhász M, Csontos Á, Szűcs M, Bor R, Milassin Á, **Rutka M**, Molnár T. Efficacy and Safety of Adalimumab in Ulcerative Colitis Refractory to Conventional Therapy in Routine Clinical Practice.
J Crohns Colitis. 2016 Jan;10(1):26-30. Epub 2015 Sep 20.
IF: 5,813

XIX. Bor R, Balanyi Z, Farkas K, Bálint A, **Rutka M**, Szűcs M, Milassin Á, Szepes Z, Nagy F, Molnár T. [Comparison of symptoms, laboratory parameters and illness perception in patients with irritable bowel syndrome and inflammatory bowel disease]. *Orv Hetil.* 2015 Jun 7;156(23):933-8. Hungarian.
IF: 0,291

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

5-ASA: 5-aminosalicylic acid

ANOVA: analysis of variance

anti-TNF-α: anti tumor necrosis factor alpha

ATI: antibody-to-IFX

AUC: area under the ROC curve

CAI: Rachmilewitz Activity Index

CD: Crohn's disease

CDAI: Crohn's Disease Activity Index

CRC: Colorectal cancer

CRP: C-reactive protein

EI: Rachmilewitz Activity Index

ELISA: Enzyme-linked immunosorbent assay

EMA: Europe, European Medicines Agency

eMayo: endoscopic Mayo subscore

FC: Fecal calprotectin

gFOBT: guaiac fecal occult blood test

Hb/Hp: hemoglobin/haptoglobin

HTC: Haematocrit

IBD: Inflammatory bowel diseases

iFOBT: immune fecal occult blood test

IFX: Infliximab

M2PK: M2 pyruvate kinase

MH: Mucosal healing

MMP: Matrix metalloproteinase

pMayo: partial Mayo score

ROC: receiver operating characteristic

TL: trough level

UC: ulcerative colitis

W2aTL: 2 weeks trough level

W6aTL: 6 weeks trough level

1. SUMMARY

Background. The most important goals of the recent therapies of inflammatory bowel disease (IBD) are to induce and maintain clinical remission and mucosal healing (MH), which can be achieved with anti-TNF-α biological therapy. CT-P13 is the first biosimilar to infliximab (IFX) that has been approved for the same indications as its originator IFX, but no data was available on the effect of IFX biosimilar on mucosal healing. Serum IFX and antibody-to-infliximab (ATI) levels are objective parameters, that may have a great role in the therapeutic decisions during maintenance biological therapy in the use of original and biosimilar agents. The aims of this thesis were, to evaluate (I) the correlation between clinical and endoscopic disease activities of UC defined by activity scores. (II) the efficacy of CT-P13 induction therapy, (III) to assess the correlation between serum IFX and ATI levels, the response to IFX therapy, and to determine the accuracy of serum drug concentration measurement in the prediction of the long-term clinical response. The last part of this thesis evaluates (IV) the diagnostic accuracy of different fecal markers in the detection of colorectal adenomas and cancer. Biomarkers are important in not only studying IBD, but may be important in colorectal cancer (CRC) as well. As CRC is the second deadliest malignancy worldwide, the simple and early detection methods are critical for theeffective management of this disease. Methods Clinical and endoscopic activities were evaluated in 100 consecutive UC patients. Clinical activities were defined by two activity indices: the Rachmilewitz Activity Index (CAI) and the partial Mayo score. They graded the findings both according to the endoscopic part of the Rachmilewitz Endoscopic Activity Index (EI) and the Mayo endoscopic subscore. MH was defined as Mayo endoscopic subscore and EI of 0. Histological activity was scored by the Riley score. Sixty-three UC patients who underwent CT-P13 induction therapy were enrolled in the second study. Sigmoidoscopy was performed after the end of the induction therapy at week 14. Mucosal healing was defined as Mayo endoscopic subscore 0 or 1. Complete mucosal healing was defined as Mayo endoscopic subscore 0. Trough level (TL) of CT-P13 was measured at week 14. Fourty-eight IBD patients receiving maintenance IFX therapy were prospectively enrolled and divided into adequate and inadequate groups. Blood samples were collected just before (trough level) and two (W2aTL) and six weeks (W6aTL) after the administration of IFX. Stool samples of patients referred to colonoscopy were collected, from 95 non-IBD patients, for the analysis of tumor M2 pyruvate kinase (M2PK), human hemoglobin (Hb), hemoglobin/haptoglobin (Hb/Hp) complex, fecal calprotectin (FC), and matrix metalloproteinase-9 (MMP-9). Results Clinical and endoscopic activities showed strong

correlations using both scoring systems ($p = 0.0029$ and $p = 0.0001$). Endoscopic disease activity also correlated with the histological activity ($p < 0.001$). Significant correlation was shown between the clinical activity and MH ($p = 0.0012$ and $p < 0.001$). Regarding CT-P13 induction therapy, cumulative clinical response and steroid-free remission at week 14 were achieved in 82.5% and 47.6% of the patients, respectively. Sigmoidoscopy revealed steroid-free mucosal healing in 47.6% of the patients, and complete mucosal healing was present in 27%. Mayo endoscopic subscore decreased significantly at week 14 compared to baseline. In this part of the thesis, we evaluated serum IFX levels, the results were single measurement of ATI titers 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 (5.0% vs 35.7%; $p = 0.016$). Sensitivity and specificity for predicting the therapeutic response were 85.0% and 71.4% based on the cut-off value of TL 2.0 μg/ml. In non-IBD patients, sensitivity and specificity of M2PK for adenomas sized > 1 cm were 60% and 67.5% and for CRC were 94.7% and 67.5%. Sensitivity and specificity of iFOBT for adenomas sized ≥ 1 cm were 80% and 72.5% and for CRC were 94.7% and 72.5%. Sensitivity and specificity of Hb/Hp complex for adenomas sized≥ 1 cm were 80% and 52.9% and for CRC were 100% and 52.9%. Sensitivity of FC andMMP-9 for CRC was 77.8% and 72.2%. Combined use ofM2PK, iFOBT, and FC resulted in a sensitivity and specificity of 95% and 47.5% for the detection of adenomas sized ≥ 1 cm. Conclusion Assessment of MH is very important for guiding therapy and for the evaluation of remission in patients with UC. Our results showed good correlation between the clinical, endoscopic, and histological activities of UC focusing on the importance of evaluating the endoscopic activity of the patients. The efficacy of CT-P13 induction therapy on mucosal healing in UC was proven. The results indicate that mucosal healing is achieved in two thirds of UC patients by the end of the induction treatment with CT-P13. 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 with indeterminate response to the therapy. In CRC, sensitivity of M2PK, iFOBT, and Hb/Hp complex proved to be high. Combined use of M2PK, iFOBT, and FC may be valuable in the detection of large adenomas.

2. INTRODUCTION

Inflammatory bowel diseases (IBD) consisting of Crohn's disease (CD) and ulcerative colitis (UC) are chronic IBDs with unknown etiology. Both entities typically present with relapsing-remitting course, characterized by immune-mediated inflammation of the gastrointestinal tract. In spite of the latest advances in the knowledge of the pathogenesis of IBD, the exact etiology and the mechanisms of the disease still remain unknown. The clinical course of IBD may vary from a mild form with the achievement of long-term remission to a chronic, relapsing course with frequent flares despite prolonged immunosuppressive or biological therapy (1). In the past, IBD treatments only attempted to resolve symptoms without the knowledge of the exact etiology. In the middle of the 1950's, the introduction of corticosteroids significantly reduced the mortality of UC patients. Later on, immunomodulators and biological therapies became very important components of the IBD therapeutic arsenal. By now, three anti-TNF-α agents: IFX in CD in 1997, in UC in 2006; adalimumab (ADA) in CD in 2007, in UC in 2012; and golimumab in UC in 2013 were approved in European Union for the treatment of IBD. The introduction of TNF-α antagonists, beginning with IFX, a chimeric monoclonal antibody, and ADA, a fully human monoclonal antibody, against TNF-α has dramatically changed the treatment of refractory IBD. Anti-TNF-α drugs suppress immune response by binding to both the soluble and membrane-bound TNF (2). They have been shown to induce and maintain clinical remission and promote mucosal healing (MH), which is currently considered the most desirable IBD therapeutical outcome (3,4). Recently, the therapeutic goals became more ambitious with the development of immunopathology, the goal being: to achieve endoscopic and histological remission. Biological drugs are the most effective known inductors of MH and give the most hope for the modification of the natural disease course. (5,6). Evidence shows that MH is associated with long-term remission and lower cancer risk, thus highlighting the importance of achieving MH in clinical practice (7,8). In different trials it has been shown to improve long-term outcome by preventing surgery and requiring fewer hospitalization stays (9,10,11).

The definition of MH currently used in most of the clinical trials is as follows: "complete absence of all inflammatory and ulcerative lesions"; however, the presence of "persistent erythema and friability at endoscopy" without ulceration or erosions is usually included as well (12). MH was significantly more common at weeks 8 and 30 in the infliximab-treated group than the placebo group in the Active Ulcerative Colitis Trials (ACT) 1 and 2. In post hoc

analysis of combined ACT 1 and ACT 2 data, MH at week 8 was highly predictive of clinical remission at week 30 (13). The ulcerative colitis long-term remission and maintance with Adalimumab (ULTRA) trial revealed that 18.5% of the patients receiving ADA achieved clinical remission at week 8 and 17.3% at week 52 (14, 15). However, the rate of MH was 41.1% and 25% at weeks 8 and 52 (14). Similar to the ULTRA trial, Program of Ulcerative Colitis Research Studies Utilizing an Investigational Treatment (PURSUIT) trial revealed clinical remission in 18.7% and 17.8% of patients receiving golimumab in a dose of 200/100 and 400/ 200 mg at week 6. The rate of MH was 43.2% and 45.3% at the same time. During the maintenance phase, 23.5% and 28.6% of the patients achieved sustained remission at both weeks 30 and 54, while MH was achieved in 41.8% and 43.5% of the patients (16,17). MH was defined as Mayo endoscopy subscore of 0 or 1 in both trials. It is unknown and controversial why the rate of MH is higher compared to the rate of clinical remission. MH is known to be associated with higher remission rate, however, there is discrepancy between the rate of clinical remission and MH.

In Europe, the European Medicines Agency (EMA) approved two available IFX biosimilars which include all of the indications pediatric and adult CD and UC - as the originals- CT-P13 biosimilar monoclonal antibody, similar to IFX, has been approved for the same indications as the originator in 2013 by the EU and in June 2014 in Hungary. CT-P13 is produced in the same type of cell line and has an identical amino acid sequence to the originator drug. CT-P13 and reference IFX show comparable binding affinities to monomeric and trimeric forms of human tumour necrosis factor TNF-α, and comparable TNF-α neutralizing and cytotoxic activities (18). The approval of CT-P13 was based on randomized clinical trials conducted in patients with rheumatoid arthritis (19), supplemented by a clinical and a pharmacokinetic study on ankylosing spondylitis (20). Initially several national societies have raised concerns regarding the use of biosimilars in extrapolated indications (21). Recently, favourable retrospective clinical data became available on the efficacy of CT-P13 in IBD. However, none of these studies evaluated the effect of IFX biosimilar on MH defined by endoscopic Mayo subscore 0 or 1 (22,23,24). The lack of endoscopic data on the efficacy of CT-P13 in UC leads to confusion among clinicians, which can decrease the use of CT-P13.

While endoscopic evaluation is the gold standard for the assessment of colonic mucosa, less invasive modalities for estimating inflammation are useful in clinical practice and can ease therapeutic decision making, particularly in the case of ineffective response or loss of response of biological therapy (25). A substantial number of patients show only partial response, and

approximately 20-45% of the primary responders show loss of efficacy (26,27,28,29), which poses significant clinical problem for IBD management. In the case of loss of efficacy, we should employ dose-escalation after excluding the possibility of complications requiring surgical intervention or infections. In case of the failure of dose-escalation, switching within the same drug class or swapping (switching out of the drug class) is preferable. Cessation of therapy or switching/swapping to another biological drug currently depends mainly on subjective clinical evaluation. Current modalities for assessing less invasive disease activity are available. They have a wide variety of uses and include everything from of physical parameters through routine laboratory values to more complicated laboratory tests of blood, stool or other tissue samples, such as biomarkers. Serum IFX and ATI levels are objective parameters that may help in the therapeutic decisions during maintenance biological therapy. Results of recent studies suggest that serum IFX concentration predicts long-term clinical response (30). In UC, detectable IFX trough level is associated with higher rate of clinical remission and endoscopic improvement and with lower risk of colectomy (31). ATI is reported to develop in up to 60% of IBD patients during maintenance IFX therapy (32,33). The presence of ATI is associated with lower serum IFX levels, higher rate of infusion reactions, loss of response, and it may shorten the effect of IFX infusions (33,34). Despite the proven importance of serum IFX and ATI levels in the prediction of clinical response, it is still not clearly defined when and how frequently we have to measure these titers.

It is of crucial clinical importance to monitor the patient's condition and assess disease activity, including, performing colonoscopy, examination of laboratory parameters, as well as evaluating biomarkers, because the clinical symptoms are frequently inconsistent with endoscopic findings. A biomarker was defined by the National Institutes of Health Biomarkers Definitions Working Group as " *a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.*" (35). Biomarkers detected from the stool have been correlated to gastrointestinal disorders. The identification of various feacal biomarkers has provided insight into the colorectum, because fecal biomarkers can indicate alternative conditions which are associated with different colorectal diseases, for example, inflammatory disorders and/or premalignant or malignant lesions. Previously, our workgroup studied the role of feacal biomarkers in IBDs (36,37,38), but we aimed to also assess diagnostic accuracy of different fecal markers for the detection of precancerous and cancerous lesions of the colorectum. The main driving force behind our study was the oncoming pilot colorectal

screening program in Csongrád country, which has long been planned for implementation. Because high incidence and mortality of CRC is especially characteristic to those Central European countries – including Hungary – where national screening program has not been started yet (39). It should be emphasized that mortality data in Hungary are very unfavourable compared to other European countries as well (incidence is 84.8 new cases/100,000 residents, mortality is 42.3/100,000 residents) (40,41,42). However, the long premalignant phase of sporadic CRCs provides a good opportunity for successful screening and intervention. The vast majority of CRC cases are sporadic colon cancers characterized by a multistep carcinogenic process (43). Advanced adenomas greater than 10 mm in diameter with high-grade dysplasia or with more than 20% villous component are considered to be the clinically relevant precursors of CRC. Colonoscopy is considered the gold standard of CRC screening tools. However, mainly due to the invasive nature of colonoscopy, the acceptance of this type of screening method among the general population is low. The most commonly used noninvasive screening method for CRC is the guaiac fecal occult blood test (gFOBT) based on the detection of hemoglobin peroxidase activity in the stool. However, the sensitivity and the specificity of this test are not good enough to safely rule out the presence of CRC or adenomas, which is why there is a great need for a better noninvasive marker for these conditions. In the case of proximal malignant lesions, hemoglobin/haptoglobin (Hb/Hp) detection can be superior to Hb detection alone since Hb/Hp complex remains stable over the entire course of the large bowel in comparison to Hb degraded along the digestive tract (44,45). M2 pyruvate kinase (PK) is a biochemical form of PK which is a key enzyme in cancer cell metabolism (46). M2PK is expressed in normal proliferating cells, embryonic cells, adult stem cells, and cancer cells (47). Elevated levels of M2PK have been detected in colonic adenocarcinoma (48). Calprotectin is a calcium-binding and zinc-binding protein complex that is abundant in the cytosol of inflammatory cells (49,50). Fecal calprotectin (FC), a biomarker of intestinal inflammation, has been in clinical use for years in IBD (50,51,52). FC has been shown to be elevated in CRC as well and has been suggested to be used for screening high risk groups for CRC (53). Matrix metalloproteinase (MMP) is a large family of calcium-dependent zinc-containing endopeptidases responsible for tissue remodelling and degradation of the extracellular matrix components, including collagens, elastins, gelatin, matrix glycoproteins, and proteoglycan, in multiple disease settings including malignant processes. MMP-9 subtypes are believed to play a crucial role in the progression and metastasis formation of many tumors, including CRC (54). Since the majority of the above mentioned tests are not officially recommended in the CRC screening guidelines and some of them have not been tested previously.

3. AIMS

3.1. To prospectively evaluate the correlation between clinical and endoscopic disease activities of UC defined by activity scores.

3.2. To prospectively evaluate the efficacy of CT-P13 induction therapy on mucosal healing in patients with UC.

3.3. To assess the correlation between serum IFX and ATI levels and response to IFX therapy and to determine the accuracy of serum drug concentration measurement in the prediction of the long-term clinical response.

3.4. To compare the diagnostic accuracy of different fecal markers in the detection of precancerous and cancerous lesions of the colorectum and to find the most accurate marker for CRC screening.

4. PATIENTS AND METHODS

4.1. To prospectively evaluate the correlation between clinical and endoscopic disease activities of UC defined by activity scores.

4.1.1. Patients

Data of 100 patients with UC, who sequentially underwent colonoscopy with biopsy in 2014, were analyzed in our clinic. Indication for examination included symptoms of disease activity or control endoscopy. The severity of intestinal inflammation was evaluated in detail based on total endoscopy. Colonoscopies and patient enrollments have been performed by two experienced gastroenterologists and endoscopists. One colonoscopy has been performed for each patient. At the same time, clinical activity indices were calculated during the visits.

4.1.2. Evaluation of UC clinical and endoscopic activity

Clinical activities were defined by two activity indices: the CAI and the partial Mayo (pMayo) score (55,56). CAI represents combined objective (erythrocyte sedimentation rate, body temperature, and hemoglobin) and subjective findings (endoscopy, degree of abdominal pain, amount of blood in stools, and physician's impression of disease). It also includes number of stools per week and extraintestinal manifestations. The total index score ranges from 0 to 29 points. pMayo score consists of three components: stool frequency, rectal bleeding, and the physician's global assessment. Each component is assigned a score of 0–3; the total score ranges from 0 to 9. Total Mayo score includes the pMayo score and the endoscopic Mayo (eMayo) subscore. Endoscopic findings were graded both according to the endoscopic part of the Rachmilewitz Activity Index (EI) and the eMayo subscore. Four items are included in the EI: vascular pattern, mucosal granularity, contactor spontaneous mucosal bleeding, and mucosal damage (mucus, fibrin, erosions, and ulcer) (55). This score ranges from 0 to 12: inactive disease is defined with a score between 0–4; mild activity with score 4–6; moderate activity with score 7–9; high activity with score 10–12. The eMayo subscore ranges from 0 to 3: 0 – inactive disease and normal mucosa; 1 – mild disease (erythema and mild friability); 2 – moderate disease (marked erythema, absent vascular pattern, friability, erosions), 3 – severe disease (spontaneous bleeding and diffuse ulceration). MH was defined as eMayo subscore 0 and EI of <4. Histological activity was scored by the Riley score. The original Riley scale consists of six histologic features, all scored on a 4-point scale: acute inflammatory cell

infiltrate, crypt abscesses, mucin depletion, surface epithelial integrity, chronic inflammatory cell infiltrate, and crypt architectural irregularities (56).

4.2. To prospectively evaluate the efficacy of CT-P13 induction therapy on mucosal healing in patients with UC.

This was a prospective, multicentre study carried out in three Hungarian and one Czech IBD tertiary centres. Adult patients diagnosed with UC, who were administered at least three CT-P13 infusions between June 2014 and April 2015 in the Hungarian centres and between September 2014 and May 2015 in the Czech centre, were enrolled in the study. Inpatients with acute relapse and outpatients with chronic, steroid-dependent and/or immunomodulatory refractory disease were enrolled in the study. Previous biological therapy and corticosteroid treatment were also allowed at inclusion. Patients' demographic data, clinical characteristics, smoking history, previous surgery, history of previous anti-TNF-α administration, concomitant medications, indications for CT-P13 therapy, and clinical and endoscopic response to CT-P13 were analysed. Disease phenotype was determined in accordance with the Montreal Classification (57). CT-P13 5 mg/kg was given as an intravenous infusion at 0, 2, and 6 weeks followed by a maintenance regimen of 5 mg/kg every 8 weeks. Disease activity was evaluated by Mayo Scoring System combining endoscopic and clinical scales for the assessment of the severity of UC at the beginning and at week 14 of the therapy (58). Total colonoscopy was performed for all patients at the starting point of the therapy. Only patients with Mayo endoscopic subscore of at least 2 were enrolled in the study. For control endoscopy, flexible sigmoideoscopy was performed at week 14. Mucosal healing was defined as Mayo endoscopy subscore of 0 or 1. Complete mucosal healing was defined as Mayo endoscopy subscore of 0. Clinical response was defined as >30% decrease in the Mayo score from the baseline and decrease of ≥ 3 points plus a decrease in the rectal bleeding subscore of ≥ 1 or a rectal bleeding subscore of 0 or 1(13). Remission was defined as Mayo score ≤2, with no individual subscores >1. Primary non-response was defined as a lack of response at week 14 after the induction phase. Previous anti-TNF-α therapy was allowed at inclusion but had to be stopped at least one year prior to CT-P13 therapy. According to Hungarian central regulations, only CT-P13 was allowed to be reintroduced if the patient received originator IFX previously. In the case of ADA, the decision was made by the patient and the physician. Inflammatory laboratory parameters (C-reactive protein [CRP], leukocyte and thrombocyte levels), IFX TL and antibody titers (ATI) were assessed at week 14 by quantitative ELISA kits obtained from Theradiag, France.

4.2.1. Statistical analysis

Categorical data were analyzed using Pearson's chi-squared test or Fisher's exact test. The effects of drug therapy on the Mayo score were examined with repeated measured analysis of variance (ANOVA). The changes from baseline in continuous variables (partial and endoscopic Mayo scores, TL) were compared using paired sample t-tests. Statistical tests were performed using R statistical software (R version 3.1.2), values of $p<0.05$ were considered significant.

4.3. To assess the correlation between serum IFX and ATI levels and response to IFX therapy and to determine the accuracy of serum drug concentration measurement in the prediction of the long-term clinical response.

Forty-eight consecutive, adult IBD patients receiving IFX maintenance therapy were prospectively enrolled between March 2014 and October 2015 in our tertiary medical center. All patients received detailed written and verbal information about the investigation, and they consented to participation in this study. IFX was administered intravenously with maintenance dosage of 5 or 10 mg/kg every 8 weeks as monotherapy or in combination with azathioprine, 5-aminosalicylates and/or corticosteroids. No distinction has been made between the original and biosimilar IFX because previous studies did not find any difference in terms of efficacy, safety and immunogenicity between the original and biosimilar agent (19,59,60). Patients were divided into adequate and inadequate responder groups based on their clinical response at inclusion, which was determined with pMayo and CDAI. Adequate response was defined as complete clinical remission with pMayo score of 2 or CDAI score <150 during the previous 6 months on maintenance therapy. Patients were categorized into the inadequate responder group, if: 1) they partially responded to 5 mg/kg dose IFX therapy (a decrease in pMayo score of 3 points or in CDAI score of 100 points from baseline); 2) dose escalation was required (10 mg/kg body weight) during the previous 6 months; 3) loss of response occurred at inclusion. The baseline was the time when patient received the first IFX infusion, so response corresponds to changes of scores during the biological therapy. Blood samples were collected for serum IFX and ATI measurements at inclusion–immediately prior to the administration of regular maintenance IFX infusion (TL)–, as well as 2 (W2aTL) and 6 weeks (W6aTL) afterwards. Serum samples were tested by quantitative enzyme-linked immunosorbent assay (ELISA) with LISA-Tracker (Theradiag, France). At the end of the 6-month follow-up the response to IFX therapy was re-evaluated by using pMayo and CDAI scoring system. Patients' demographic

data, clinical characteristics, previous surgery and concomitant medications were collected using an electronical medical database.

4.3.1. Statistical analysis

Statistical tests were performed using R statistical software version 3.3.1 (R Foundation) and SPSS software version 24 (SPSS Inc., Chicago, Illinois, USA), values of $p<0.05$ were considered statistically significant. Differences in continuous variables such as serum IFX level, disease duration, age at the diagnosis were assessed with Welch Two Sample t-test. Fisher's Exact Test were used to compare the proportion of categorical variables in the adequate and inadequate responder group (ATI positivity, type of disease, gender, concomitant treatment). The cut-off levels were determined by receiver operating characteristic (ROC) curve analysis, which used clinical remission as a classification variable to calculate the sensitivity, specificity and area under the ROC curve (AUC). Multivariate models were constructed using logistic regression (Overall model fit was described with Nagelkerke R2, and the goodness-of-fit by use of Hosmer and Lemeshow Test). Descriptive statistics were reported as mean, median and interquartile range, categorical variables were expressed as a percentage.

4.4. To compare the diagnostic accuracy of different fecal markers in the detection of precancerous and cancerous lesions of the colorectum and to find the most accurate marker for CRC screening.

4.4.1. Patients

Patients from the 1st Department of Medicine, University of Szeged, who were referred for colonoscopy were invited to participate in this study. Data on symptoms, smoking habits, family history, and current medication were collected. Every patient was informed about the study details and asked to sign a letter of written consent. The patients were instructed for sample collection and handling. All patients were asked to collect stool samples one day before administration of bowel preparation. Plastic containers were provided for feces collection. After bringing the samples at the lab of the clinic, they were frozen at −20∘C until further analysis. Patients did not have to keep a special diet andwere told to take their usual medications. Selection of the patient groups with adenomas sized <1 cm and ≥1 cm and CRC was based on the endoscopic and histological finding. The stool testing for M2PK, iFOBT, FC, and MMP-9 was carried out by a single trained person who was blinded to the results of the colonoscopy.

4.4.2. Colonoscopy and Histological Examination.

Diagnosis was based on the endoscopic and histopathological findings. Colonoscopies were performed by three experienced endoscopists who were blinded to fecal tests results. Carcinomas were classified according to the Dukes staging system and location. Adenomatous polyps were classified according to histopathological characteristics, size (large polyps: ≥1 cm; small polyps: <1 cm), and location. All colonoscopy biopsies were examined by an expert pathologist (LT). The diagnoses were reported using the standard WHO classification of colorectal neoplasia. In addition to their size, all polypoid lesions were classified as hyperplastic polyps or adenomas, being further classified according to their histological pattern as tubular, tubulovillous, villous, or serrate adenomas.

4.4.3. Statistical Analysis

CRCs and adenomas were analysed separately. The diagnostic value of fecal markers for detecting adenomas and CRCs was assessed by calculating the sensitivity and the specificity of the test. Correlations between FC and MMP-9 and endoscopic findings were determined by ANOVA method. The cut-off levels, specificity, and sensitivity between CRC and control groups were calculated using the receiver operating characteristic (ROC) analysis. All statistical analyses were carried out using STATA 9 (Stata Corp, TX, 2005). P values < 0.05 were considered to be statistically significant.

4.5. Measurements of fecal and serum samples

4.5.1. Measurement of serum IFX *and ATI levels*

CT-P13 TLand antibody titres (anti-infliximab antibodies [ATIs]) were assessed at week 14 by quantitative enzyme linked immunosorbent assay [ELISA] [LISA TRACKER, Theradiag, France, in Hungary; and SHIKARI Q-Inflixi, Q-ATI, Matriks Biotek, Turkey, in the Czech Republic]. With the LISA TRACKER, detectable TLwas 0.1 μg/ml for CT-P13. The measurement range was 10–200 ng/ml for antibodies [> 10 ng/ml considered positive]. With the SHIKARI kits, the lowest detectable level that can be distinguished from the zero standard was 30 ng/ml. ATIs were positive, when positivity index exceeded 3. These patients are also included in the Hungarian multicentre nationwide cohort evaluating the efficacy, safety and immunogenicity of CT-P13 in IBD.

4.5.2. Measurement of fecal Calprotectin

FC levels were determined using a lateral flow assay (Quantum Blue® Bühlmann). Stool extracts were prepared and analyzed according to the manufacturer's instructions. For the selective measurement of calprotectin antigen, the sandwich immunoassay was used. A monoclonal antibody binding highly specific for calprotectin is coated onto the test membrane. A second monoclonal detection antibody conjugated to the gold colloids is accumulated onto the conjugate pad. The calprotectin/anti-calprotectin conjugate binds to the anti-calprotectin antibody coated on the test membrane (test line) and the remaining free anti-Calprotectin gold conjugate binds to the goat anti-mouse antibody coated on the test membrane (control line). The assay procedure consists of three steps. One step is the extraction of stool samples with a ScheBo® Quick Prep faecal extraction device. After extraction let the stool extract settle for 10 minutes, we centrifuge the extracts for 5 minutes at 3'000x g. In the second step, we dilute the supernatant 1:16 with extraction buffer, the samples are mixed well then are equilibrated for at least 5 minutes. In the third step, lateral flow assay procedure is performed. 60 µl of diluted stool extract is applied onto the sample loading port of the test cassette. After 12 minutes, we can measure quantitatively the signal intensities of the test and the control line by the BÜHLMANN Quantum Blue® Reader.

4.5.3. Measurement of fecal MMP-9

Samples were thawed at 4°C. 1.0 g of each fecal sample was diluted, mixed and homogenised in 4 mL of ice cold Tris-buffer (0.15 M NaCl + 20 mM Tris-HCl, pH:8.3). After centrifugation (10 min, 4500 rpm, 4ºC), pellets were discarded and supernatants were recentrifuged (10 min, 10.000 g; 4ºC). The final supernatants were filtered by 0.8 µm pore-sized syringe filters and the aliquots were stored at -20 ºC until analyzed. MMP-9 was measured by a quantitative enzyme-linked immunosorbent assay (Quantikine, R&D Systems, Abingdon, UK).

4.5.4. Measurement of Fecal M2PK and iFOBT.

A combined rapid immunochromatographic lateral flow test was used for simultaneous detection of enzyme biomarker M2PK and human hemoglobin (combined M2PK and HB, 2 in 1 Quick Test, ScheBo_ Biotech). For these measurements, stool samples were thawed and a special stick capturing 4mg of stool was loaded. These tests are based on visual inspection of

colors at test and control lines. The result is exclusively qualitative (detection limit of M2PK was 4 U/mL; detection limit of Hb was 15 ng/mL).

4.5.5. Measurement of Fecal Hb and Hb/Hp Complex.

Hb/Hp complex was determined from stool samples with a visual immunochromatographic quick test: ColonView Hb and Hb/Hp fecal occult blood test (Biohit HealthCare; detection limit of Hb was 15ng/mL; detection limit of Hb/Hp was 4 ng/mL).

4.6. Ethical approval

Ethical approval was acquired from the National Ethical Committee 929772-2/2014/EKU [292/2014]). The protocol was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee of the University of Szeged (SZTE: 169/2011). The study was carried out under the declaration of Helsinki. The study was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee of the University of Szeged.

5. RESULTS

5.1. To prospectively evaluate the correlation between clinical and endoscopic disease activities of UC defined by activity scores.

As for the demographic characteristics of the patients, 49 males and 51 females have been enrolled. The mean age at the onset of UC was 32.5 years (range, 10–76). The mean disease duration at the time of the colonoscopy was 9.6 years (range, 0.6–47). Disease extent at the onset of UC was extensive colitis in 34 patients, left-sided colitis in 47, and proctitis in 19 patients, respectively. At the time of colonoscopy, 54 patients have been receiving therapy of 5-aminosalicylic acid (5-ASA), 17 steroids, 24 immunomodulators, 20 infliximab, and 24 local therapies such as 5-ASA and steroid suppositories and enemas. Overall, 20 patients were free of any medication at the time of the examination. Table 1 contains the demographic and clinical data of patients who participated in the study.

Patients (n=100)	
Mean age at diagnosis (years)	32.5 (10-76)
Mean age at present (years)	42.1 (18-79)
Mean disease duration (years)	9.62 (0-47)
Gender (Female/Male)	51/49
Extent	
Pancolitis	34
Left-sided colitis	47
Proctitis	19
Mean CAI	3.91
Mean EI	5.15
Mean pMayo score	3.18
Mean eMayo score	1.89
Mean Riley score	10.32
Therapy	
No therapy	20
Aminosalycilates	54
Corticostreroids	17
Thiopurin	24
Cyclosporine	2
Biologicals	22

Table 1. The demographic and clinical data of patients

5.1.1. Rate of clinical activity and disease extent

Inactive, mild, moderate, or severe disease activity was shown in 63 (mean CAI: 1.49), 23 (mean CAI: 6.57), 13 (mean CAI: 9.6), and 1 patient (CAI: 15) defined by CAI. According to the evaluation by pMayo score, inactive, mild, moderate, or severe disease was defined in 48 (mean pMayo: 0.71), 16 (mean pMayo: 3.25), 22 (mean pMayo: 5.5), and 14 patients (mean pMayo: 7.93). Proctitis was present in 19 (mean CAI: 2.95, mean pMayo: 2.32), left-sided colitis in 47 (mean CAI: 3.57, mean pMayo: 3.09), and extensive colitis in 34 patients (mean CAI: 4.91, mean pMayo: 3.79). Although the more extensive the disease was and the higher the clinical activity scores were, statistically there was no correlation shown between activity scores and disease extent.

5.1.2. Endoscopic and histological activity

According to the EI, mild disease activity was found in 29 (mean EI: 5.24), moderate activity in 23 (mean EI: 8.09), and severe activity in 11 patients (mean EI: 10.73). Mucosal healing (MH) was present in 37 patients with a mean EI of 1.59. Using the Mayo endoscopic subscore, 19 patients were diagnosed with mild, 25 with moderate, and 40 with severe disease. MH was found in 16 patients.

5.1.3. Mucosal healing and clinical remission in UC

The clinical and endoscopic activity scores of the two different indices showed significant correlations ($p=0.029$ and $p=0.0001$). Histological evaluation by Riley score assessed inactive disease in 14 (mean Riley: 1.93), mildly active in 10 (mean Riley: 7.5), moderately active in 15 (mean Riley: 11.93), and severely active disease in 28 patients (mean Riley: 14.68). Statistically, histological activity defined by the Riley score showed a stronger correlation with eMayo subscore than with EI ($p < 0.001$ and $p = 0.026$). Table 2. summarizes the clinical, endoscopic, and histological activities of patients scored by the activity indices.

Clinical activity	CAI (patient number)	CAI (mean)	pMayo (patient number)	pMayo (mean)
Inactive	63	1.49	48	0.71
Mild	23	6.57	16	3.25
Moderate	13	9.60	22	5.50
Severe	1	15.00	14	7.93
Endoscopic activity	EI (patient number)	EI (mean)	eMayo (patient number)	eMayo (mean)
Inactive	37	1.59	16	0
Mild	29	5.24	19	1
Moderate	23	8.09	25	2
Severe	11	10.73	40	3
Histological activity	Riley score (patient number)		Riley score (mean)	
Inactive	14		1.93	
Mild	10		7.50	
Moderate	15		11.93	
Severe	28		14.68	

Table 2. The clinical activities endoscopic, and histological activities of patients scored by the activity indices

5.1.4. Association between clinical activity and MH

When clinical and endoscopic activities were assessed by CAI and EI, 33 of the 62 patients with clinically inactive disease achieved complete MH. Nineteen patients showed mild, nine moderate, and one severe endoscopic activity. Four patients, who achieved complete MH without clinical remission, showed mild clinical activity (Figure 1).

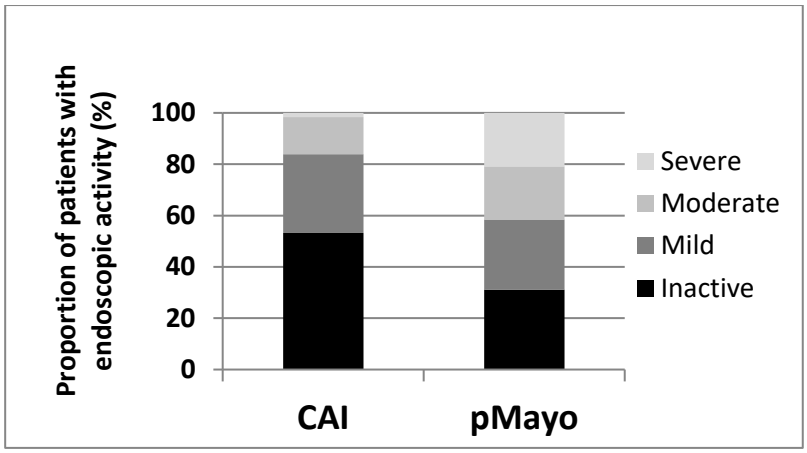


Figure 1. Distribution of the endoscopic activities in patients with clinical remission

When assessing clinical and endoscopic activities using the Mayo score, 15 of the 16 patients with complete MH achieved clinical remission, and 1 patient showed moderate clinical activity. Thirteen patients with clinically inactive disease showed mild endoscopic activities,

while 10 were moderate and 10 severe (Figure 2). Figures 3 and 4 represent the coherent clinical and endoscopic scores.

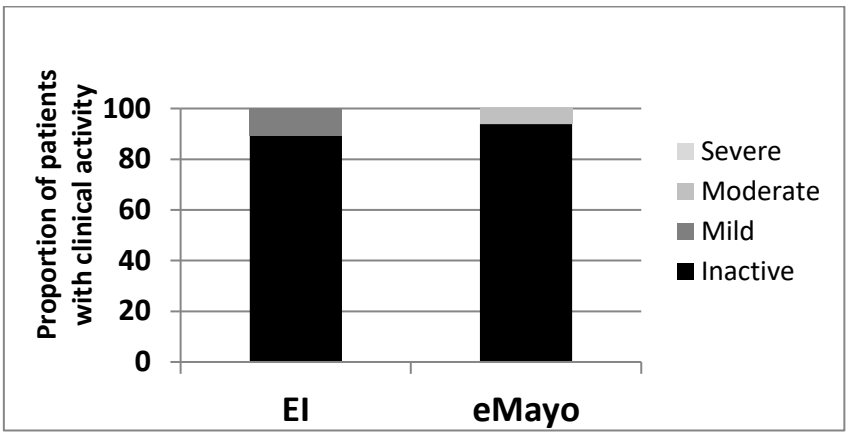


Figure 2. Distribution of the clinical activities in patients with mucosal healing.

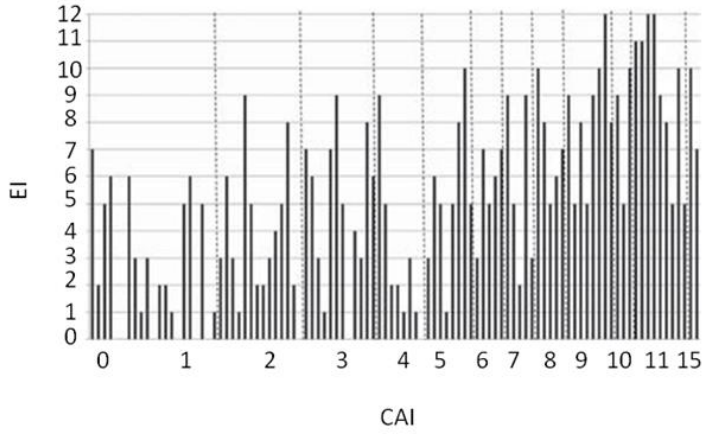


Figure 3. Coherent clinical and endoscopic scores determined by Rachmilewitz score.

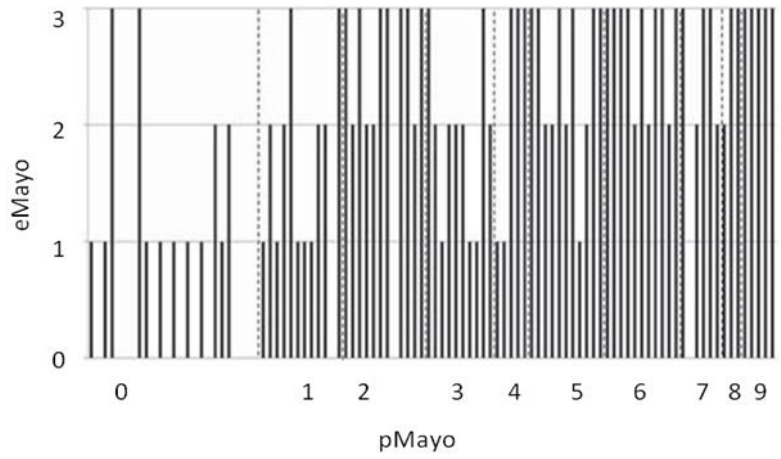


Figure 4. Coherent clinical and endoscopic scores determined by Mayo score.

5.2. To prospectively evaluate the efficacy of CT-P13 induction therapy on mucosal healing in patients with UC.

Sixty-three UC patients completed the three-dose induction therapy with CT-P13. Male-female ratio was 32:31. Mean age at diagnosis was 30.5 years (range 14-65) and mean disease duration was 5.7 years (range 0.6-22). Baseline characteristics of patients treated with CT-P13 are summarised in Table 3.

	Number of UC patients (n=63)
Gender (male/female)	32/31 (50.8/49.2%)
Mean age at the diagnosis (years)	30.5 (14-65)
Mean disease duration at CT-P13 therapy (years)	5.7 (0.6-22)
Disease extent (Montreal classification)	
Proctitis	5 (7.9%)
Left-sided colitis	23 (36.5%)
Extent colitis	35 (55.6%)
Previous anti TNF-α	5 (7.9%)
Smoking status	
Current smoker	6 (9.5%)
Previous smoker	11 (17.5%)
Never smoked	35 (55.6%)
No data	11 (17.5%)
Mean total Mayo score at inclusion	9.2
Mean endoscopic Mayo score at inclusion	2.7
Indication for CT-P13 therapy	
Acute, severe flare-up	24 (38.1%)
Chronic activity, steroid refractoriness	39 (61.9%)
Previous medications	
5-ASA	53 (84.1%)
Corticosteroid	54 (85.7%)
Azathioprine	39 (61.9%)
Concomitant medications at inclusion	
5-ASA	47 (74.6%)
Corticosteroid	32 (50.8%)
Azathioprine	27 (42.9%)

Table 3. Baseline characteristics of patients treated with CT-P13

Indications of CT-P13 therapy were acute, severe flare-up and chronic, refractory activity in 24 and 39 patients. The mean value of total Mayo score was 9.2 with mean endoscopic subscore (eMayo) of 2.7 points at the beginning of the CT-P13 therapy (21 patients with eMayo subscore of 2 and 42 patients with eMayo subscore of 3). Cumulative clinical response at week 14 was achieved in 52 patients (82.5%); the number of patients with steroid-free clinical remission was 30 (47.6%) (Figure 5).

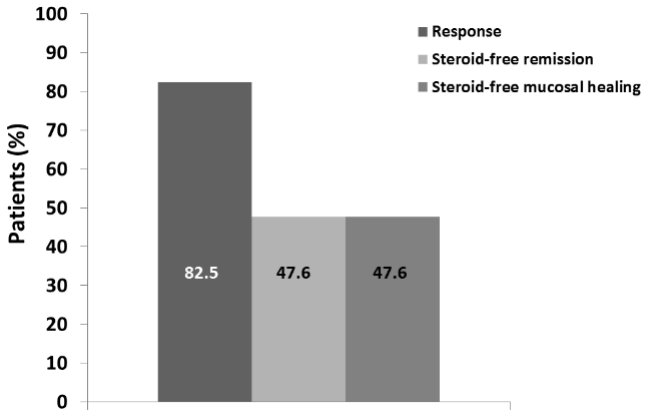


Figure 5. Proportion of patients with clinical response, steroid-free remission and steroid-free mucosal healing at week 14

At inclusion, concomitant corticosteroids were given for 11 of the 14 partially responder patients. At week 14, 4 of them could stop steroid therapy. Primary non-response occurred in 11 patients (17.5%). Three of the patients with primary non-response received previously anti-TNF-α therapy, 2 of them received originator IFX and 1 patient receivedADA. None of the patients with primary non-response developed ATI.

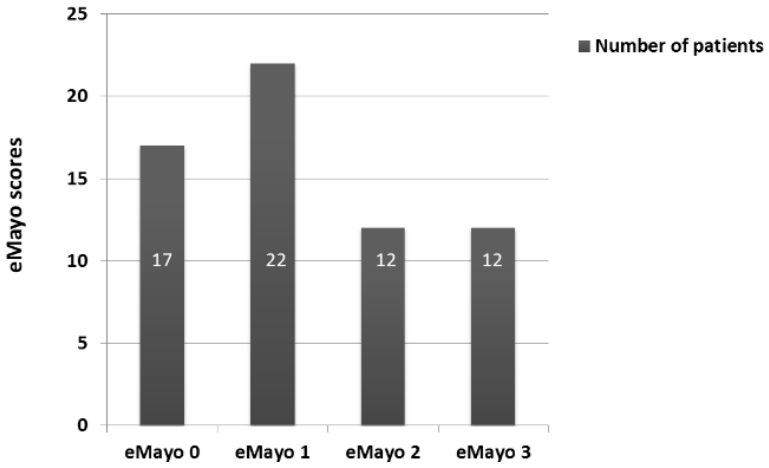
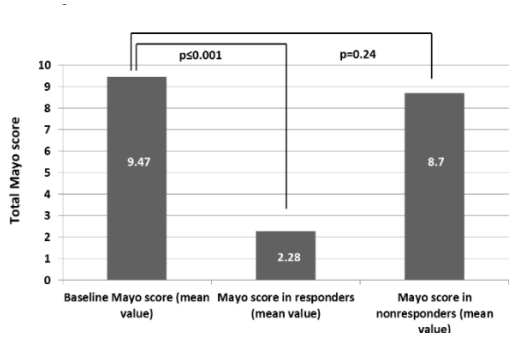


Figure 6. Number of patients with eMayo scores at week 14

One patient underwent colectomy; three patients needed dose intensification throughout the induction phase of CT-P13 therapy. Sigmoidoscopy revealed mucosal healing in 38 patients (60.3%), steroid-free mucosal healing was shown in 30 patients (47.6%). Complete mucosal healing was achieved in 17 (27%) patients at week 14. The mean value of total Mayo score was 3.4 with endoscopic subscore of 1.1 points at week 14. Figure 6 shows the number of patients

with eMayo scores. Both the Mayo score and eMayo score decreased significantly in responders at week 14 compared to baseline (p<0.001 and p<0.001) (Figure 7. and 8.).



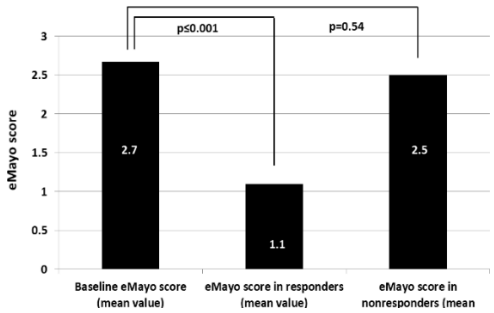


Figure 7. Change in the total Mayo score in responders vs. non-responders at week 14 compared to baseline (left)

Figure 8. Change in the endoscopic Mayo subscore in responders vs. non-responders at week 14 compared to baseline (right)

Subgroup analysis did not reveal significant difference in disease outcome at week 14 between acute, steroid refractory inpatients and outpatients with chronic activity regarding to steroid-free remission (48% vs. 51%, pMayo score: 0.44 vs. 0.45, p=0.49), and cumulative clinical response (83% vs.82.1%, tMayo score: 3.54 vs. 3.28, p=0.38). However, steroid–free mucosal healing proved to be more common in acute, steroid refractory inpatients vs. outpatients with chronic activity (41.7% vs. 51.3%, eMayo: 0.3 vs. 0.65, p=0.04). None of the examined clinical demographical data (gender, smoking status, disease extent, previous and current concomitant medications) or the laboratory parameters determined at inclusion (C-reactive protein, leukocyte count, hematocrit, thrombocyte and serum albumin levels) predicted the outcome of therapy on mucosal healing at week 14. Trough levels of CT-P13 were significantly higher in patients who achieved mucosal healing or steroid-free mucosal healing and vs. patients who did not achieve endoscopic remission (mean values: 5.72, 6.35 and 2.85 μg/ml, p=0.02 and p=0.008) (Figure 9).

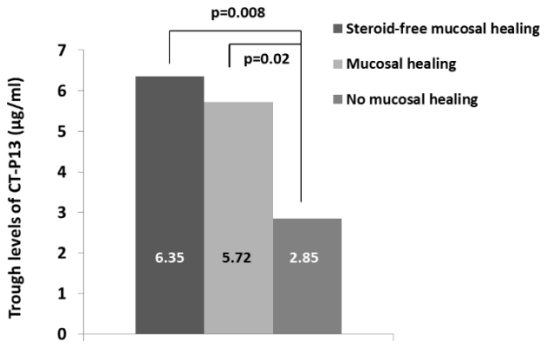


Figure 9. Trough levels of CT-P13 in patients who achieved mucosal healing and steroid-free mucosal healing vs. patients who did not achieve endoscopic remission

Mean values of CT-P13 trough levels were 3.18 µg/ml in responders and 6.15 µg/ml in patients in steroid-free remission (p=0.02). We also compared serum CT-P13 levels between the Hungarian vs. Czech patient population regarding mucosal healing. No statistical difference was shown between the two groups (serum CT-P13 levels in Hungarian and Czech patients who achieved steroid-free mucosal healing were 6.01 µg/ml and 7.21 µg/ml, p=0.35). ATI was detectable in 7 cases at week 14. ATI positive patients presented with undetectable TL. None of these patients received anti-TNF-α therapy previously.

Overall, 5 patients had received anti-TNF-α before starting on CT-P13 therapy - ADAwas given for 2 patients and originator IFX for 3 patients. Notably, at least one year elapsed between stopping previous anti-TNF-α therapy and restarting biological therapy. Previous anti-TNF-α therapy was discontinued because of central regulations in Hungary. According to the central authorities' decision, due to financial reasons, after a successful one-year treatment period of anti-TNF-α therapy resulting in clinical and endoscopic remission, biological therapy is recommended to be stopped. However, use of previous anti-TNF-α therapy did not prove to be statistically predictive to loss of response in this cohort. Two of the patients achieved mucosal healing (eMayo of 1) and 3 patients had moderate disease activity on control sigmoidoscopy despite the clinical response to CT-P13 therapy. According to the ROC analyses, the cut-off value was revealed to be 3.15 µg/ml both for steroid-free clinical remission and mucosal healing (AUC=0.65; AUC=0.69) with a sensitivity and specificity of 71% and 64% for clinical remission and with a sensitivity and specificity of 66% and 61% for mucosal healing (Figure 10).

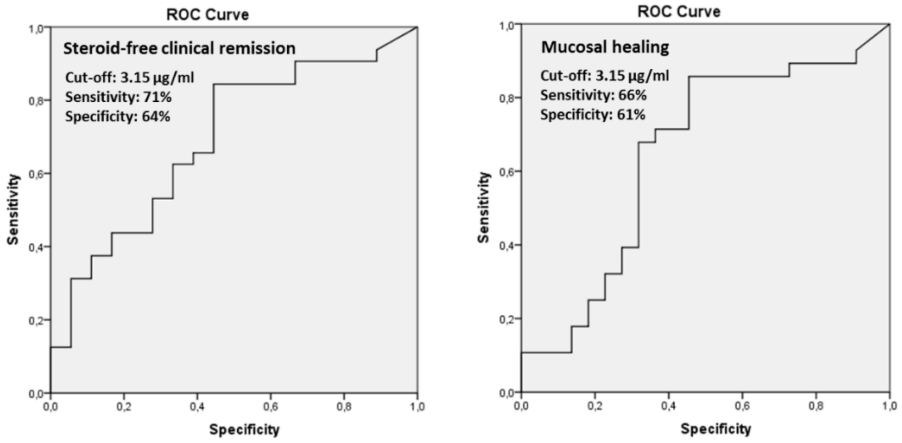


Figure 10. ROC of CT-P13 trough levels associated with steroid-free remission and mucosal healing

5.3. To assess the correlation between serum IFX and ATI levels and response to IFX therapy and to determine the accuracy of serum drug concentration measurement in the prediction of the long-term clinical response.

The adequate responder group consisted of 20 patients being in sustained clinical remission on maintenance IFX therapy. The inadequate responder group (n=28) was heterogeneous: 8 patients showed chronic activity during the last 6 months of IFX maintenance therapy with mild (n=7) or moderate (n=1) disease activity. Fourteen patients required dose escalation (10 mg/kg) in the last 6 months: 3 of them were in remission, in 10 cases mild, and in one case moderate activity was observed at the time of inclusion. Six patients had relapsed at the time of the first sampling (TL). Forty-two patients received original and 6 patients received biosimilar IFX (4 inadequate and 2 adequate responders). IFX monotherapy was applied in only one third of patients (n=16), in the remaining cases it was complemented by azathioprine (n=26), 5-aminosalicylates (n=10), local (n=3) and/or systemic corticosteroids (n=6). There was no significant proportional variance regarding gender, mean age at the diagnosis, and disease duration between the groups (Table 4.). Rate of Crohn's disease (CD) patients were higher in the inadequate responder group, but the difference was not statistically relevant, and the demographic and clinical characteristics between UC and CD patients did not differ significantly.

Clinical and demographic data of patients (N=48)		
	Adequate responder (n=20)	Inadequate responder (n=28)
Female/male (N°)	10/10	13/15
UC/CD (N°)	9/11	7/21
Ulcerative colitis		
pancolitis	4 (20%)	4 (14.3%)
left-sided colitis	5 (25%)	3 (10.7%)
Crohn's Disease		
ileal (L1)	-	2 (7.1%)
colonic (L2)	4 (20%)	11 (39.3%)
ileocolonic (L3)	7 (35%)	8 (28.6%)
non stricturing, non penetrating (B1)	4 (20%)	8 (28.6%)
stricturing (B2)	2 (10%)	3 (10.7%)
penetrating (B3)	5 (25%)	10 (35.7%)
perianal (p)	7 (35%)	9 (32.1%)
Age at the diagnosis (years)	25.00±9.21	26.29±9.78
Disease duration (years)	9.14±5.32	7.40±5.35
Duration of infliximab therapy		
< 1 year	7 (35%)	5 (17.9%)
1 - 2 years	8 (40%)	11 (39.3%)
> 2 years	5 (25%)	12 (42.9%)
Previous surgery (N°; %)	7 (35%)	14 (50%)
Seton drainage	6 (30%)	9 (32.1%)
Intraabdominal fistula	-	2 (7.1%)
Ileocecal resection	1 (5%)	3 (15%)
Right hemicolectomy	-	1 (3.6%)
Concomitant therapy (N°; %)		
Azathioprine	14 (70%)	12 (42.7%)
Local steroid	1 (5%)	2 (7.1%)
Systemic steroid	1 (5%)	5 (17.9%)
5-aminosalycilate	7 (35%)	3 (15%)

Table 4. Clinical and demographic data of 48 enrolled inflammatory bowel disease patients

5.3.1. Serum IFX levels

Serum IFX level was measured three times (TL, W2aTL, W6aTL) during the administration of regular maintenance infusion. The mean value of serum TL was significantly higher in the adequate vs. inadequate responder group (3.11±1.64 vs.1.19±1.11; $p<0.001$). Mean IFX levels did not differ between the groups at week 2 (18.87±39.05 vs. 16.99±27.65; $p=0.854$) and week 6 (3.69±3.96 vs. 1.74±2.15; $p=0.055$) (Figure 11).

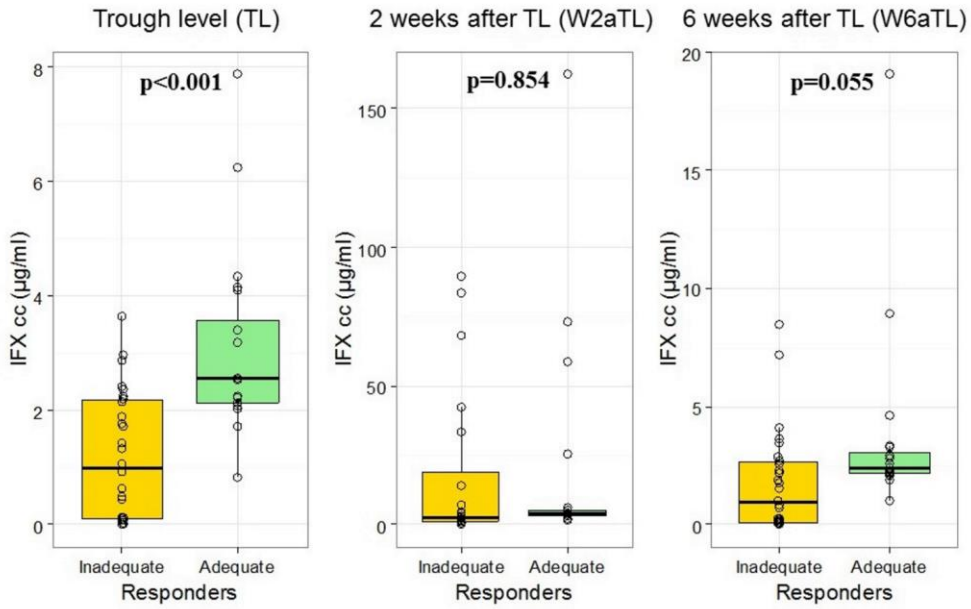


Figure 11. Serum IFX levels immediately prior the administration of regular maintenance infliximab (IFX) infusion (trough level, TL), as well as 2 (W2aTL) and 6 weeks (W6aTL) after wards in the adequate and inadequate responder group.

Therefore, W2aTL and W6aTL levels were not suitable for the prediction of therapeutic response. According to ROC analysis, the cut-off value of TL for predicting therapeutic response was 2.0 µg/ml with 85.0% sensitivity and 74.1% specificity. The AUC was 84.7% (Figure 12).

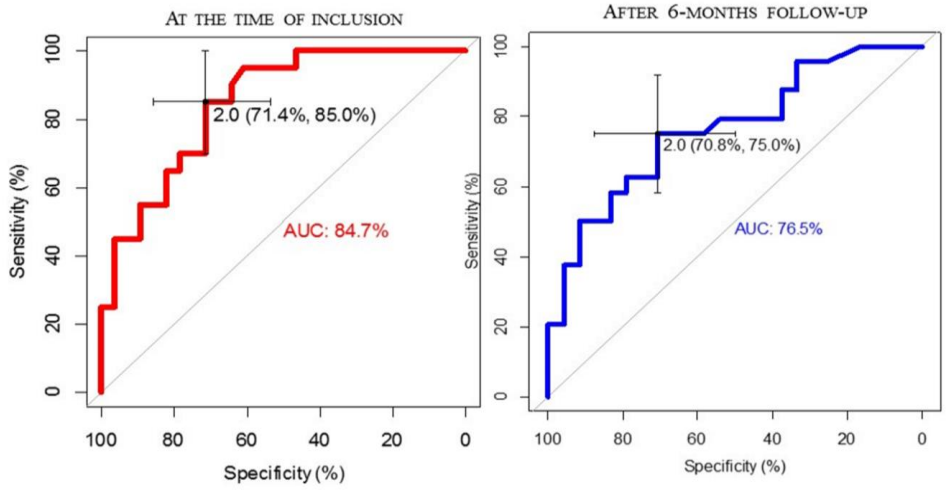


Figure 12. ROC analysis of IFX trough levels (TL) associated with current and long-term response.

In the inadequate responder group, ≥2.0 µg/ml TL was measured in 8 cases: six patients received intensified IFX therapy (10mg/kg every 8 weeks) from which five patients responded to the dose escalation. One of the three adequate responders with low IFX level and ATI positivity developed an allergic reaction, the remaining two patients with low IFX level without ATI positivity were in clinical remission. (Figure 13.)

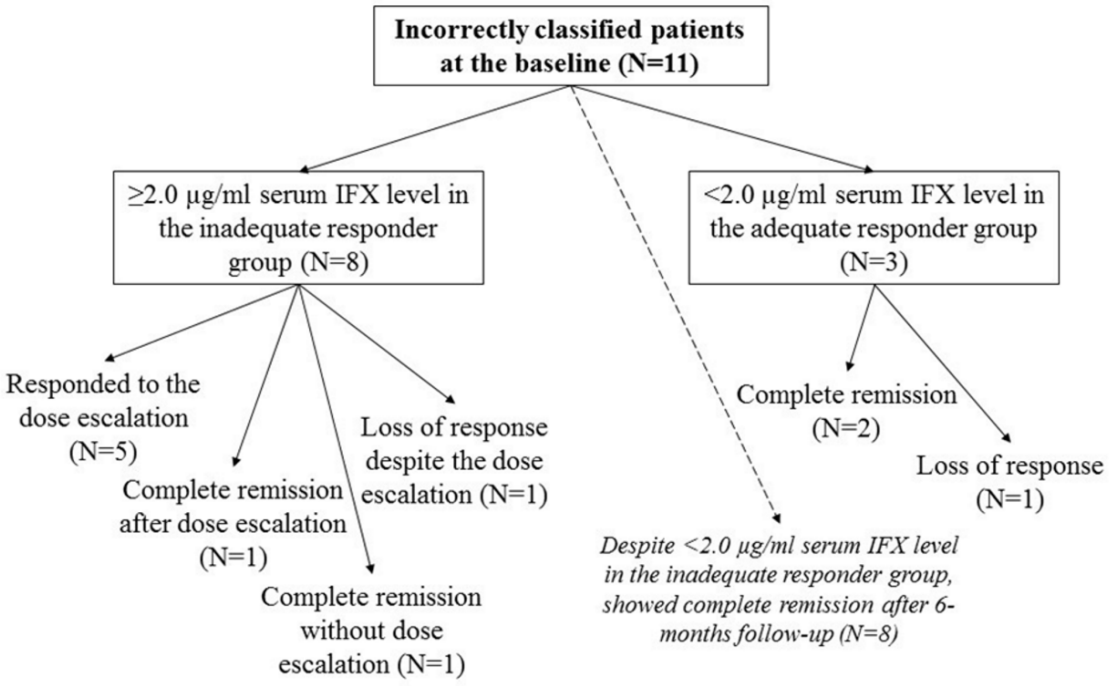


Figure 13. Incorrectly classified cases by serum infliximab trough levels after 6-months follow-up.

The results of multivariate analysis (TL, W2aTL, W6aTL levels and ATI positivity) performed by logistic regression revealed prediction rate of 85.4% for the current response (Table 5). It showed high similarity with the results of ROC analysis, which assessed only the TL. Therefore, measurement of W2aTL and W6aTL levels did not improve the accuracy of prediction of therapeutic response.

Logistic regression for prediction Currents response (Overall model fit: Nagelkerke R^2 = 0.668; Goodness-of-fit: Hosmer and Lemeshow Test p = 0.300; Classification table: Correctly predictions = 85.4%)						
	B	S.E.	df	p	OR=Exp(B)	95% CI for OR
TL	1.81	0.64	1	0.005	6.137	1.75-21.53
W2aTL	-0.07	0.03	1	0.013	0.928	0.88-0.99
W6aTL	0.41	0.33	1	0.217	1.506	0.79-2.88
ATI	0.18	1.44	1	0.900	1.198	0.07-20.03
Constant	-3.94	1.37	1	0.004	0.019	
Logistic regression for prediction long-term response (Overall model fit: Nagelkerke R^2=0.438; Goodness-of-fit: Hosmer and Lemeshow Test p = 0.221; Classification table: Correctly predictions = 77.1%)						
	B	S.E.	df	p	OR=Exp(B)	95% CI for OR
TL	1.26	0.48	1	0.008	3.515	1.38-8.96
W2aTL	-0.05	0.02	1	0.020	0.953	0.92-0.99
W6aTL	0.15	0.22	1	0.505	1.160	0.75-1.8
ATI	0.46	0.99	1	0.644	1.585	0.23-11.18
Constant	-1.99	0.87	1	0.022	0.136	

Table 5. Results of logistic regression analysis for prediction current (at inclusion) and long-term response (after 6-months follow-up). B: regression coefficient; S.E.: standard error; df: degree of freedom; OR: odds ratio; CI: confidence interval; TL: serum infliximab [IFX] trough level; W2aTL: serum IFX level 2 weeks after TL; W6aTL: serum IFX level 6 weeks after TL; ATI: antibody-to-infliximab

Response to biological therapy was reevaluated after the 6-month follow-up. Five inadequate responders were re-classified into the adequate responder group. In one of them, optimal serum IFX level was measured without ATI positivity. The clinical data of the patient suggested an ongoing infection at the time of the inclusion, which resolved after the administration of antibiotics. In two cases with dose escalation at inclusion, serum W2aTL level was higher than 2 μg/ml, but the drug concentration dropped rapidly to an almost undetectable level by week 6. In these cases, ATI expression was also detectable, which suggests an accelerated drug elimination from the circulation. Despite TL not reaching the cut-off value (1.71 μg/ml and 0.83 μg/ml), two patients showed complete clinical remission. No ATI expression was detectable in these cases. (Figure 19) ROC analysis was performed to calculate the accuracy of previously determined 2.0 μg/ml cut-off value of TL for prediction of long-term therapeutic response. Serum IFX levels showed better correlation with the current status than with the long-term efficacy. The sensitivity and specificity in the prediction of long-term

response was 70.8% and 75.0% (AUC: 76.5%). (Figure 18) Prediction rate in the logistic regression model was 77.1%, which correlated with the results of ROC analysis. (Table 5.)

5.3.2. ATI positivity

ATI was identified in 11 patients with low serum IFX levels (<1 μg/ml). In 9 cases, antibodies were not detectable in all of the three consecutive blood samples, suggesting that the expression of ATI in the blood was transient. Single sampling of ATI showed a nonsignificant trend for the correlation with the therapeutic response. The proportion of ATI positivity in the adequate and inadequate responder groups was 5.0% vs. 28.5% (p=0.060) immediately prior administration of regular maintenance IFX infusion, but two and six weeks after the biological therapy it was 5.0% vs. 7.1% (p=0.684) and 5.0% vs. 21.0% (p=0.089). Using the three points' measurements, ATI expression showed significant difference between the adequate and inadequate responder groups (5.0% vs 35.7%; p=0.016). (Figure 14)

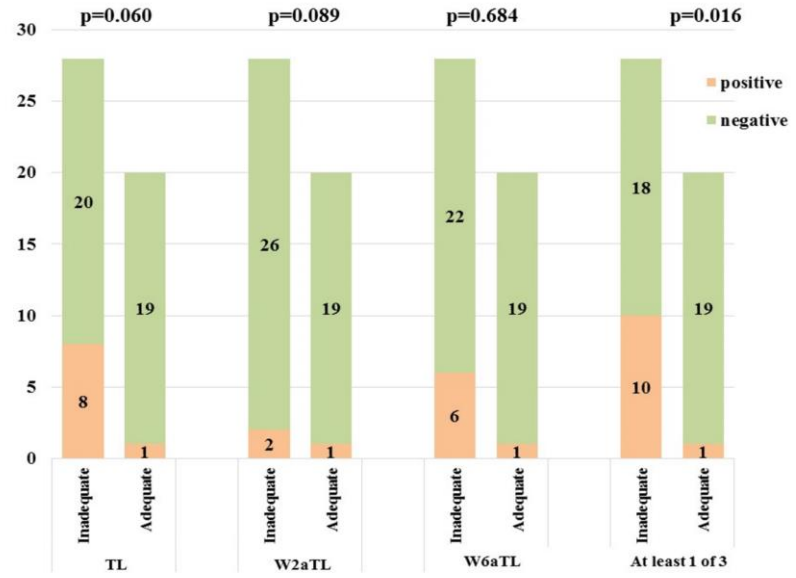


Figure 14. Proportion of ATI positivity in the adequate and inadequate responder groups.

In one of the ATI positive, adequate responder patients, an allergic reaction occurred during the subsequent regular IFX infusion. After the 6-months follow-up clinical remission was achieved in three cases, when IFX 5 mg/kg therapy was combined with perianal surgical treatment (seton drainage). Four patients showed partial response to biological therapy. In three cases acute flare-up was observed, requiring surgery or switching to another biological agent. (Figure 15.)

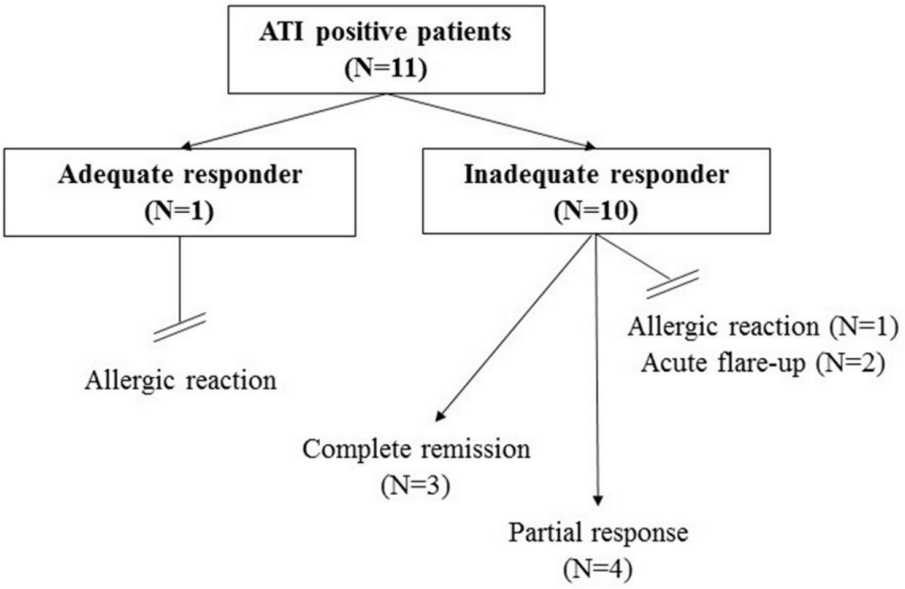


Figure 15. Antibody-to-infliximab (ATI) positive cases after 6-months follow-up.

5.4. To compare the diagnostic accuracy of different fecal markers in the detection of precancerous and cancerous lesions of the colorectum and to find the most accurate marker for CRC screening.

Ninety-five consecutive in- and outpatients admitted for total colonoscopy between September 2014 and April 2015 were prospectively enrolled in the study. Indications for colonoscopies were abdominal complaints, bloody stool, family history of CRC, and prior colorectal adenoma. Patients with active gastrointestinal bleeding, menstruation, and past history of total colectomy were excluded from the study. Study groups were defined on the basis of the result of colonoscopy and histological evaluation. Mean age was 67 years (range: 21–92) in study population. 57 female and 38 male patients were in these three groups, respectively. Demographic characteristics of the study population are summarized in Table 6.

Demographic data	All patients (95)	Control group (40)	Adenoma group (36)	Cancer group (19)
Female/Male	38/57	19/21	14/22	5 14
Age (years)	67 (21-92)	67 (21-87)	68 (51-81)	65 (44-92)
Current smokers	13 (13.7 %)	4 (10 %)	5 (13.9)	4 (21.1)
Comorbidities				
Hypertension	54 (56.8 %)	23 (57.5 %)	22 (61.1 %)	9 (47.4 %)
Diabetes mellitus	21 (22.1 %)	7 (17.5 %)	8 (22.2 %)	6 (31.6 %)
Hyperlipidaemia/hypercholesterinaemia	22 (23.2 %)	9 (22.5 %)	11 (30.6 %)	2 (10.5 %)
Cardiovascular disease	25 (26.3 %)	11 (27.5 %)	10 (27.7 %)	4 (21.1 %)
Cerebrovascular disease	13 (13.7 %)	6 (15 %)	4 (11.2 %)	3 (15.8 %)
Hyper/hypothyroidism	13 (13.7 %)	5 (12.5 %)	7 (19.4 %)	1 (5.3 %)
Pulmonary disease	6 (6.3 %)	4 (10 %)	2 (5.6 %)	0
Gout	11 (11.6 %)	5 (12.5 %)	5 (13.9 %)	3 (15.8 %)
Autoimmune disease	4 (4.2 %)	0	3 (8.3 %)	1 (5.3 %)
Malignant disease (simultaneously)	3 (3.2 %)	1 (2.5 %)	2 (5.6 %)	0
Hepatitis (B,C)	2 (2.1 %)	1 (2.5 %)	1 (2.8 %)	0
Diverticulum	24 (25.3 %)	11 (27.5 %)	10 (27.8 %)	3 (15.8 %)
Nodus hemorrhoidales	20 (21.1 %)	11 (27.5 %)	7 (19.4 %)	2 (10.5 %)

Table 6. Demographic characteristics of the study population.

Family history of CRC was reported by 26 patients. Considering therapy, 26 patients received aspirin or clopidogrel and 4 received acenocoumarol or heparin at the time of the investigation.

5.4.1. Colonoscopic and Histological Findings.

Forty of the 95 patients included in the study represented the control group without any remalignant or malignant findings on endoscopy. Nine of the control patients presented with initial diverticulosis without any sign of inflammation. Colonoscopic findings in the remaining patients of the control group were totally normal. Thirty-six patients were diagnosed with adenomas (adenoma group). In the adenoma group, 16 patients presentedwith adenomas sized <1 cm and 20 with adenomas sized ≥1 cm. Adenomas sized <1cm were equally located at the proximal and the distal part of the colon. The location of adenomas sized ≥1 cm in the majority (65%) of the patients was the proximal part of the colon. In twenty-three adenomatous cases, a histologic sample was obtained. In the remaining thirteen cases, the samples were less than 1 cm and did not suggest the presence of malignancy. Based on the histological assessment of the samples (n = 23), in 78.3% of the cases (in 18 patients), the adenomas were with low grade dysplasia; in 13% (in 3 patients), adenomas were with high-grade dysplasia; and in 8.7% (in 2 patients) there were hyperplastic polyps. In 56.5% of the patients the adenomas were of the tubular type, in 4.3% they were of the villous type, and in 30.4%they belong to the tubulovillous

type. Cancer was found in 19 cases, and, according to their histological evaluation, the tumors were identified as adenocarcinomas.

In 89% of the patients, the cancer was located in the distal colon (in 10 patients in the rectumand in 7 patients in the sigmoid colon). In the remaining 2 cases, the tumor was located in the distal part of the transverse colon. 28.8% of these patients had a family history of CRC. The numbers of patients having different stages of cancer according to Dukes classification are shown in Table 7.

Dukes stage	**Patients**
Carcinoma in situ	1
Dukes A	3
Dukes B	9
Dukes C	1
Dukes D	5

Table 7. The numbers of patients having different stages of cancer according to Dukes classification.

5.4.2. Diagnostic Accuracy of Fecal Markers in Adenomas and CRCs.

M2PK was positive in 32.5% of the patients with normal colonoscopy, in 43.7% with adenomas sized <1 cm, in 60% with adenomas sized ≥1 cm, and in 94.7% with CRCs. M2PK sensitivity for adenomas sized >1 cm was 60%, and specificity was 67.5%. Sensitivity and specificity for CRC were 94.7% and 67.5%. Sensitivity and specificity for iFOBT for adenomas sized ≥1 cm were 80%and 72.5%and for CRC were 94.7% and 72.5%. The Hb/Hp (Hb and Hb/Hp ColonView Biohit test) complex was positive in 47.1% of the patients with normal colonoscopy, in 50% with hyperplastic polyps, in 54% with adenomas sized <1 cm, in 80% with adenomas sized ≥1 cm, and in 100%with CRC. Sensitivity and specificity of Hb/Hp complex for adenomas sized ≥1 cm was 80% and 52.9% and for CRC were 100% and 52.9%.FC andMMP-9 differed significantly between the control and CRC group (p = 0.022; p < 0.001); however, no difference was found in FC and MMP-9 concentrations between the control and the adenoma groups. FC was significantly lower in adenomas sized <1 cm compared to CRCs but did not differ when compared to adenomas sized ≥1 cm with CRCs (p = 0.022, p = 0.089). MMP-9 proved to be significantly lower compared to either adenomas sized<1 cm with CRCs or adenomas sized ≥1 cm with CRCs ($p \leq 0.001$ and $p \leq 0.001$). Sensitivity of FC for CRC was 77.8%, while specificity for CRC was 70%. The cut-off value of FC for the detection of CRC was 128.5 μg/g (AUC = 0.77, p = 0.001). Sensitivity of MMP-9 for CRC was 72.2%, while

specificity was 95%. The cut-off value ofMMP-9 for the detection of CRC was 1.12 ng/g (AUC = 0.77, $p < 0.001$). Using combinations of fecal markers, the highest sensitivity for detection of adenomas sized ≥1 cm was revealed when combining M2PK, iFOBT, and FC (with the cut-off of 128.5 μg/g) resulting in a sensitivity and specificity of 95% and 47.5% for the detection of adenomas sized ≥1 cm. Sensitivities, specificities, and positive and negative predictive values of the fecal markers are summarized in Table 8. We did not find any relationship between platelet aggregation inhibitor therapy and positive results of the different hemoglobin tests (logistic regression: HbScheBo $p = 0.4$; Hb/HpBiohit $p = 0.609$).

Parameters	M2-PK ScheBo	HB SchBo	HB BIOHIT	HB/HP BIOHIT	Calprotectin	MMP-9
Sensitivity						
Adenoma sized ≥ 1 cm	60	80	66.6	80.0		
CRC	94.7	94.7	100.0	100.0	77.8	72.2
Adenoma sized ≥ 1 cm + CRC	76.9	87.2	84.8	90.9	60.5	65.8
Specificty						
Adenoma sized ≥ 1 cm	67.5	72.5	52.9	52.9		
CRC	67.5	72.5	52.9	52.9	70.0	95.0
Adenoma sized ≥ 1 cm + CRC	67.5	72.5	52.9	52.9	70.0	75.0
PPV (%)						
Adenoma sized ≥ 1 cm	80	59.2	38.5	42.9		
CRC	85.7	62	52.9	52.9	53.8	86.6
Adenoma sized ≥ 1 cm + CRC	69.7	75.5	63.6	65.2	65.7	71.4
NPV (%)						
Adenoma sized ≥ 1 cm	77.1	96.6	78.3	85.7		
CRC	96.4	96.6	100	100.0	87.5	88.3
Adenoma sized ≥ 1 cm + CRC	75	85.3	78.2	85.7	65.1	69.7

Table 8. Sensitivities, specificities, and positive and negative predictive values of the fecal markers.

DISCUSSON

The introduction of biological therapies led to a paradigm shift in our approach to IBD therapy. The therapeutic goals shifted from simply improving symptoms to the goal of achieving mucosal healing. Anti-TNF-α therapies have greatly improved outcomes in terms of reduction of relapses and complications in IBD, although they are not universally effective in all patients. A considerable proportion of initial responders lose response over time, while others may become intolerant to these agents. Therefore, there is a definite need for new therapies. However, we have long-term experience with anti-TNF-α agents and an appropriate patient selection for the therapy may increase the number of responders. Moreover, the early use of anti-TNF-α agents may change the natural course of the disease and/or the first time, the anti-TNF-α drugs provided opportunity to achieve and sustain healing of mucosal lesions for about a half of IBD patients (61). In patients with UC undergoing colonoscopy in our prospective observational study revealed that the rate of clinical and endoscopic remission was 63% and 37% if the activities were scored by CAI and EI, and 48% and 16% if the activities were evaluated by the Mayo scoring system. Although the proportion of the clinical and endoscopic remissions defined by the two different activity scores was different, clinical remission was definitively higher than MH independently from the activity indices. Significant correlation was revealed between the different scoring systems and between endoscopic and histological activities independently from the various scores. The background of the present study originated from surprising results of some recent large biological trials. ULTRA trials studied the efficacy of ADAin induction and maintenance of clinical remission in patients with moderate-to-severe UC who received concurrent treatment with oral corticosteroids or immunosuppressants (14,15), while the PURSUIT trials examined the efficacy of golimumab as induction and maintenance therapy in anti–TNF-α-naive moderately-to-severely active UC (16,17). Both studies revealed a higher percentage of patients with sustained clinical remission and MH compared to patients who received placebo. However, the rate of MH was even more than two times higher than clinical remission in the ULTRA and PURSUIT trials. Notably, endoscopic findings were assessed by local endoscopists and not by a central endoscopy reading center in both studies, which can explain the difference between the rate of clinical and endoscopic findings. Discrepancies between the rates of clinical remission and MH can also be explained by IBS-like symptoms that have been published to be about two to three times higher in UC patients in remission than in controls (62,63). In the ULTRA and PURSUIT studies, Mayo score was used for the assessment of disease activity. Patients with moderate-to-severe

disease activity were defined as a Mayo score of 6–12, with an endoscopic subscore 2. Clinical remission was defined as a Mayo score ≤ 2 points, with no individual subscore >1, and MH was defined as a Mayo endoscopy subscore of 0 or 1. The main difference compared to our study was in the use of two different activity scores and also in the assessment of MH, since eMayo subscore 1 represents an inflamed mucosa with erythema, decreased vascular pattern, and mild friability. Considering that the obligate aim of the current therapy is to achieve complete clinical remission and MH, it is questionable whether this definition of MH is adequate in these settings. In our study, MH was defined as eMayo subscore 0 and EI of <4 that presumably make more accurate evaluation of endoscopic remission. Although many scoring systems exist to assess the endoscopic activity of UC, the Mayo score is one of the most widely used. Interestingly, the Mayo score that was used in ULTRA and PURSUIT trials has not undergone appropriate validation or rigorous reliability assessment. Recently, new activity indices have been created and validated, like Ulcerative Colitis Endoscopic Index of Severity (64) and UC Colonoscopic Index of Severity (65). Moreover, scoring systems related to the assessment of clinical activity are based on signs and symptoms for which, unfortunately, no standard definitions have been developed. Truelove and Witts Severity Index, Powell-Tuck Index, Rachmilewitz Index, Seo Index, and Simple Clinical Colitis Activity Index are based on clinical and biochemical parameters. Several scoring systems exist, evaluating endoscopic disease activity, like Baron score, modified Baron score, Rachmilewitz Endoscopic Index, Sigmoidoscopic Index, and Mayo Score Flexible Proctosigmoidoscopy Assessment. Mayo score and Sutherland Index incorporate both clinical and endoscopic parameters. All of the scoring systems have multiple limitations with no exact validation of several definitions used in the activity scores. This is why none of the used activity scores are ideal. In our study, the proportion of clinical remission was about two times higher than MH proportion. The rate of clinical remission was about 90% in patients with MH, while the proportion of MH was about 30–50% in patients with clinically inactive disease. This data suggests that the assessment of the endoscopic activity seems to provide a better image of the patients' clinical activity than vice versa. This study was performed in a single center, which is a limitation of our work; however, the ratings were performed by gastroenterologists, specialized in IBD. We did not evaluate this index across different centers or amongst physicians with varying experience. Nowadays, the Mayo subscore is the most widely used activity score in clinical trials (58). In the previously mentioned trials, eMayo subscore of 0 or 1 was used for the indication of MH. In our study, MH was defined strictly as a score of 0 or EI of <4. Our data showed that endoscopic activity correlated well with disease activity measures and that MH was strongly associated with clinical remission.

Significant association was also found between endoscopic and histological activities. Assessment of MH is very important for guiding therapy and for evaluation of remission in patients with UC. The study was inspired by surprising and illogical findings of large clinical trials with ADAand golimumab that MH was more common than remission determined by clinical indices. We believe that our data is useful to highlight the importance of the accurate definition of MH and the correlation between clinical remission and MH and the weaknesses of the commonly used clinical indices.

CT-P13 is the first biosimilar monoclonal antibody of reference IFX that has been approved for use in all indications in which reference IFX is approved. CT-P13 had to undergo a clinical evaluation program including efficacy and clinical safety studies in order to demonstrate its similarity to the reference biological medicine. According to the EMA statement, if clinical similarity can be shown in a key indication, extrapolation of efficacy and safety data to other indication(s) of the reference product may be possible under certain conditions (66). However, concerns have been raised about the use of biosimilars in such extrapolated indications and many medical societies, have specifically recommended against use of biosimilars in extrapolated indications (67,68). The review by Feagan et al. addressed factors such as clinical sensitivity, mechanism of action, immunogenicity, safety, pharmacokinetics, sites of action and pathophysiology of disease to consider when evaluating indications for extrapolation for biosimilars (21). Recently, favourable retrospective clinical data became available on the efficacy of CT-P13 in IBD. In a recently published Korean study, CT-P13 showed comparable efficacy and safety relative to its originator in the treatment of moderate to severe Crohn's disease and UC (23). This multicentre study retrospectively evaluated the efficacy, safety and interchangeability of CT-P13 in IBD patients; however, its ability to assess mucosal healing was limited (23). In the study of Kang et al., including case series, only two of the enrolled nine UC patients underwent control colonoscopy at week 8 of CT-P13 therapy (24). Our multicentre, prospective study examined the outcome of induction therapy with the IFX biosimilar CT-P13 focusing on endoscopic healing in active UC patients. CT-P13 induction therapy resulted in an 82.5% clinical response and 47.6% steroid-free clinical remission. Steroids could be tapered and stopped in 60% of the patients receiving systemic corticosteroids at inclusion. Mucosal healing was achieved in 60.3% by week 14; the rate of steroid-free mucosal healing was 47.6%. Moreover, almost half of these patients showed complete mucosal healing with an eMayo score 0 at the time of control endoscopy. The originator drug, ACT-1 revealed clinical response, remission, and mucosal healing rates of IFX

to be 69%, 39%, and 62%, respectively, at week 8, while in ACT-2 they were 65%, 34%, and 60% at week 8. Note that although this is not a comparison study, evaluation of the efficacy of biosimilar biologics in clinical practice is important. Response and remission rates of our cohort proved to be higher than those in the ACT trials; however, our observation that real life data represent better outcomes than clinical studies is not a new one. Although randomized controlled trials are considered the gold standard for the evaluation of the efficacy of a drug, real-life data provide more insight into factors that might influence therapy outcomes. In the retrospective analysis of Lee et al., the rates of clinical response and remission were 87% and 45%, respectively, at week 8 (69). Zhou et al. revealed clinical response and remission rates of 91.3% and 73.9%, respectively, at week 6. (70). In paediatric patients, IFX induced a response in 73.3% at week 8 (71). Results regarding endoscopic healing in the ACT trials were highly similar to our findings assessed at week 14. A subanalysis of ACT trials showed that patients who had a week 8 endoscopic subscore of 0 or 1 had much lower rates of hospitalization or surgery and higher rates of steroid-free remission over the next 6–12 months. On the other hand, patients who did not achieve mucosal healing during the induction period had considerably higher rates of subsequent colectomy.14 Additionally, steroid-free remission rates were higher in patients who achieved complete mucosal healing compared to patients with a subscore of 1 at week 8. (3) Furthermore, ACT-1 and ACT-2 trials showed a direct correlation with serum IFX concentration both for clinical response and for mucosal healing (13).

Our results also revealed a significant association between higher IFX TL, steroid-free mucosal healing and clinical remission. The study has some limitations, including the relatively small patient number, the heterogeneous patient population including acute, severe UC patients and patients with chronic disease activity, use of corticosteroid therapy at inclusion, and different types of assays used for the detection of serum CT-P13 levels. Although corticosteroids may influence assessment of the outcome of biological therapy, in most the cases such as these, steroids cannot be avoided for use as an adjunctive therapy in clinical practice. Moreover, in the ACT studies, 50–60% of the patients were on steroid therapy at inclusion. In this study LISA TRACKER and Matriks Biotek kits were used to measure IFX biosimilar TL and antidrug antibody. In Hungary, ELISA measurements were centralized and performed at the Department of Laboratory Medicine, Semmelweis University, Budapest. The LISA TRACKER assay, developed to reduce low-affinity binding of immune complexes or interfering molecules, was used in the study of Paul et al. (72) This type of ELISA is able to assess antibody levels independently from IFXtrough concentrations. Paul et al. revealed an

association between antibody levels and loss of response to IFX. When examining the impact of therapeutic drug monitoring on dose intensification, they found that patients with antibody levels >200 ng/ml did not respond to IFX optimization, whereas all patients with IFX TL <2 mg/ml and antibody levels <200 ng/ml responded to IFXdose intensification. Crossimmunogenicity between the originator and biosimilar IFX was an interesting topic when assessing the value of drug monitoring in CT-P13 therapy. The study by Ben-Horin et al. (59) revealed that antibodies to the originator IFX in IBD patients similarly recognize and cross-react with CT-P13, supporting a similar immunogenic profile for originator and biosimilar IFX. A Czech study compared three ELISAs (Matriks Biotek [Turkey], Theradiag [France] and R-Biopharm [Germany]) for IFX detection in the measurement of CT-P13 TL and revealed a perfect agreement in qualitative and quantitative results for the majority of the samples. These observations suggest that substitution between the assay methods evaluated in the study may be possible. (73) Notably, a slight but statistically significant difference was also shown during the subanalysis between acute, steroid refractory inpatients and outpatients with chronic activity in steroid-free mucosal healing but not in clinical outcome, showing that the rate of mucosal healing is more common in acute, severe UC than in chronic disease activity. Today, mucosal healing should be considered as the main goal of therapy in IBD. UC patients may be recommended to be reevaluated with sigmoidoscopy at the end of induction therapy to ascertain whether they have achieved mucosal healing and, if not, the therapy may be escalated (74). Achieving early mucosal healing is even more important given that mucosal healing itself does not predict sustained clinical remission in UC patients in whom IFX therapy had been stopped after achieving endoscopic remission (75,76).

Our prospective study of IBD patients receiving maintenance IFX therapy aimed to determine the optimal timing and frequency of serum IFX and ATI measurements for the prediction of therapeutic response. We found that determination of serum IFX level prior to the administration of regular IFX infusion and ATI positivity showed strong correlation with disease activity and predicted the at least 6-months-long response. Measurement of serum IFX 2 or 6 weeks after the infusion did not result in further elevation in the prediction rate. Most studies have suggested that the measurement of serum IFX levels immediately after induction or during maintenance therapy may help to optimize biological treatment since it may help to decide about the necessity of dose escalation, cessation of therapy or the switching to another biological drug. A multicenter retrospective study of 16 severe and 16 moderately severe UC patients has detected significantly lower IFX TL in the acute severe UC group compared to the

moderately severe group (77). The post hoc analysis of ACCENT I study carried out by *Cornillie et al.* revealed higher median week 14 serum IFX TL in patients with sustained response to scheduled maintenance IFX 5 mg/kg without dose escalation compared to those who lost response during the 54-week follow-up: 4.0 vs 1.9 μg/ml. The optimal cut-off value for predicting therapeutic response was ≥3.5 μg/ml at week 14 (78). This study did not confirm whether serum IFX level predicts therapeutic response in patients receiving IFX 10 mg/kg. On the contrary, *Paul et al.* found significant increase in IFX TL (considered as a positive delta IFX) in patients who responded to dose escalation. The delta IFX after drug optimization was 2.2 μg/ml versus 0.2 μg/ml in the responder and nonresponder groups. The 0.5 μg/ml cut-off delta IFX was independently associated with mucosal healing (likelihood ratio 2.02; 95% CI, 1.01–4.08; p=0.048) (72). In the majority of previous studies timing of measurement is not uniform. Week 14, after induction therapy is one of the most accepted sampling time, but it is applicable only in case of newly administered IFX therapy to predict long-term response especially in questionable cases. In other studies, samples were taken in various times (at week 22, 30, 52 or 54) or at the time of relapse. Currently there is no evidence-based recommendation about the optimal timing of measurement of serum IFX levels in patients who receive maintenance biological therapy. The meta-analysis of 22 studies carried out by *Moore et al.* in 2016 has found that the >2μg/ml cut-off trough IFX level during maintenance therapy is associated with greater probability of clinical remission (risk ratio RR 2.9, 95% CI 1.8-4.7, p<0.001) and mucosal healing (RR 3.0, 95% CI 1.4-6.5, p=0.004) (79). The main limitation of this analysis was that inclusion criteria and the time of sampling was not uniform. In our study we found that the measurement of serum IFX level was effective in the prediction of therapeutic response only prior to the administration of regular IFX infusion, and that multiple sampling (W2aTL and W6aTL) did not result in further increase in the prediction rate. The 2.0 μg/ml cut-off IFXw0 value showed slightly better correlation with the current condition than with long-term response: sensitivity and specificity were 71.4% and 85% vs. 70.8% vs. 75.0%. It is important to highlight that partial response or loss of response were observed only in three patients with ≥2.0 μg/ml TL during 5 or 10 mg/kg IFX maintenance therapy. It suggests that the measurement of serum IFX levels may be a great predictor of response both in case of normal dose of IFX therapy and after dose escalation. Antibody formation against IFX may be observed in 60% of patients with episodic administration and in 6-25% of cases with scheduled biological therapy (33,80). Based on the results of *Ungar et al.* ATI-free survival can be achieved by 42% of patients by 4-years follow-up, and in 90% of the cases the antibody appears within the first 12 months of therapy (81). Use of concomitant immunosuppressants such as

azathioprine and methotrexate may result in a 50% reduction in the risk of developing ATI ($p<0.00001$) (82). The assessment of 13 studies with data of 1378 patients found that the risk of loss of response to IFX therapy in ATI positive IBD patients is elevated: risk ratio was 3.2 (95% CI: 2.0–4.9, $p<0.0001$), when compared with the ATI negative group (83). ATI formation was associated with lower serum IFX levels. The standardized mean difference in trough serum IFX levels between groups was −0.8 (95% CI: −1.2, −0.4, $p<0.0001$). Furthermore, the presence of ATI increases the rate of infusion reactions and serum sickness–like reactions (83). In the study of *O'Meara et al.* the risk ratio of any acute infusion reaction and severe infusion reactions was 2.4 (95% CI: 1.5–3.8, $p<0.001$) and 5.8 (95% CI: 1.7–19, $p=0.004$) in ATI positive patients when compared with patients without ATI, but the rate of delayed hypersensitivity reactions did not differ significantly between the groups (32). *Baert et al.* determined that the optimal cut-off serum ATI concentration for the prediction of shorter duration of response and infusion reactions is 8.0 μg/ml (84). In our study ATI formation was observed in 11 patients and was associated with lower serum IFX levels in all of the cases. The proportion of ATI was higher in the inadequate responder group, but only the three points' measurement was able to establish significant difference between the groups. ATI formation may increase the risk of loss of response but could not exclude the opportunity of clinical remission particularly after dose escalation or during combined surgical and medical therapy. Therefore, in case of ATI positivity overall assessment of symptoms, serum IFX levels and therapeutic response considering subjective judgment is required.

CRC is a major health problem worldwide. Despite being a good candidate for screening due to its detectable premalignant lesions, mortality rates of CRC are still significant in Hungary (85). Early detection by an accurate, noninvasive, cost-effective, simple-to-use screening technique is central to decrease the incidence and mortality of this disease. Patient discomfort, invasiveness, fear, and high cost may all limit the appeal of this screening technique. Furthermore, the expertise and equipment required for the procedure, and the increasing number of examinations place a huge burden on the gastroenterologists. Thus, there is still an unmet need for suitable noninvasive biomarkers to screen for CRC. In our prospective colonoscopy-controlled study, we assessed the sensitivity, specificity, and positive and negative predictive values of different noninvasive fecal markers for the detection of adenomas and CRC. For adenomas sized ≥1 cm, iFOBT showed the highest sensitivity and M2PK the highest specificity. For CRC, M2PK and Hb/Hp complex showed the highest sensitivity and fecal MMP-9 the highest specificity. FC and fecal MMP-9 concentrations did not differ between the control and

the adenoma group, although they proved to be beneficial mainly in the detection of adenomas sized ≥1 cm and CRC. In CRCs, the sensitivities of FC and MMP-9 were 78% and 72%, with specificities of 70% and 95%. The combination of M2PK, iFOBT, and FC increased their sensitivity for the detection of adenomas sized ≥1 cm up to 95%. The study has some limitations. First, we collected stool samples before performing colonoscopy; thus, we were blinded to the findings and the number of high-grade adenomas finally proved to be low. We do not know whether there would be associations between adenomas and fecal markers if the number of adenomas with high-grade dysplasia would be higher. Second, M2PK and Hb tests and the Hb/Hp complex were all qualitative tests based on a chromatographic method interpreted visually which may limit their assessment in case of borderline results. Therefore, it may be difficult to compare the results with those of FC and MMP-9. However, these tests are simple, do not require specific laboratory equipment, and therefore are less expensive than the quantitative methods. The guaiac-based FOBT (gFOBT) is the oldest and most commonly used non-invasive test for detecting CRC (86,87). Although the test is relatively inexpensive and easy to perform, false-positive and false-negative results compose its main limitation resulting in limited sensitivity for detecting cancer and advanced adenomas (88)[20]. The Hb/Hp complex shows higher stability against degradation than Hb itself. Sieg et al. revealed that Hb/Hp complex has a comparable sensitivity to fecal Hb for CRCs (87% for both) and higher sensitivity for adenomas (76% versus 54%) (44). However, these tests are based on the bleeding property of the adenomas. Since early stage cancers or advanced adenomas are unlikely to bleed continuously, 100% of clinical sensitivity cannot be achieved with the use of these tests. That is why the identification of novel fecal-based biomarkers is important. M2PK is expressed by proliferating cells, in particular the tumor cells being direct target of several oncoproteins. Among the first studies assessing the sensitivity of M2PK for the detection of CRC, Shastri et al. revealed that fecal M2PK assay had sensitivity and specificity of 81.1 and 71.1% for diagnosing CRC at a cut-off value of 4U/mL whereas FOBT showed a sensitivity of 36.5% and specificity of 92.2% for CRC. They concluded that M2PK is a poor screening biomarker, due to its low specificity (89). However, a metaanalysis including 17 studies performed between 2006 and 2010 found the mean fecal M2PK sensitivity and specificity to be 80.3% and 95.2% for CRC and a sensitivity of 44% for adenomas >1 cm (90). According to our results, M2PK, Hb, and Hb/Hp tests show better sensitivity in the detection of CRC than advanced adenomas. The study by Kimet al. revealed that the sensitivity of iM2PK, an immunochromatographic qualitative method for fecal M2PK for CRC, was 92.8% and for adenomatous lesions the sensitivity was 69.4% (91). Compared with M2PK ELISA, iM2PK

exhibited significantly enhanced sensitivity for CRC (97.5% versus 80%, p = 0.03). FC is valuable in differentiating functional and organic bowel diseases. FC was shown to be more sensitive (79%) but less specific (72%) for CRC and adenomatous polyps as a combined group than gFOBT (92). MMP-9 is an important member of the gelatinases involved in the development of several human malignancies (93). Yang et al. found that MMP-9 expression in colon cancer tissues was significantly higher than that in corresponding distal normal mucosa tissue (54). However, the sensitivity of MMP-9 detected in feces has not been examined previously. Our results revealed a moderate sensitivity of 72%and a good specificity of 95%for fecal MMP-9 in CRC. However, neither FC nor fecal MMP-9 provided valuable information on the detection of adenomas. In this study, we compared the sensitivity and specificity of several fecal markers for the detection of CRCs. The strengths of this study are the design that allowed directly calculating sensitivity and specificity of the different fecal markers, since every patient underwent colonoscopy after stool sample collection. This was the first time when five biomarkers were simultaneously studied. Fecal M2PK has the advantage that it detects both bleeding and nonbleeding tumors and adenoma. Conversely, fecal M2PK does not have false-positive results due to various noncancerous sources of bleeding. Furthermore, FC, MMP-9, and fecal M2PK are also sensitive to intestinal inflammation (inflammatory bowel disease, diverticulitis) increasing the proportion of false positive cases. In this study, we performed examinations for patients with GI symptom(s) not as a part of screening process because by this method we could disclose false positive results and could determine specificity data as well. In our cohort, the highest sensitivity and specificity were achieved by the use of combined M2PK and iFOBT test in the detection of CRC. FC seems to be a useful adjuvant to the investigation of patients at high risk for colorectal neoplasia, while fecal MMP-9 may be a promising factor for detection of CRC. Although, in CRC, sensitivity of M2PK, iFOBT, and Hb/Hp complex proved to be high, in adenomas sized ≥1 cm, sensitivity decreased significantly. Therefore, none of these markers are unique for detection of precancerous lesions of the colorectum. However, our result revealed that combined use of M2PK, iFOBT, and FC may be valuable in the detection of large adenomas. We recommend these non-invasive fecal tests in low-risk patients and in patients who do not have comorbidities. Results of FOBT maybe false positive if the source of bleeding is not an adenoma or a malignant disease (diverticulitis, hemorrhoids, and anticoagulant therapy). However, inflammatory diseases of the colon (diverticulitis, different infections, and IBD) and extraintestinal cancer (cancer in the hepatobiliary tract, pancreas) or inflammation (hepatitis) may affect the results of the inflammatory marker test; thus, in these cases, we recommend colonoscopy as a one-step investigation. High-risk patients (who had at

least one relative with early CRC or adenoma or had at least two relatives with CRC or adenoma) with symptoms or patients who have early (under the age of 60) CRC or adenoma among their relatives should also undergo colonoscopy. However, it is not questionable whether continued efforts are needed to discover effective tests to identify patients with nonhereditary risk factors and to develop invasive and cost-effective screening modalities.

Conclusion

Our results revealed that clinical remission was higher than MH and also showed significant correlation among the clinical, endoscopic, and histological activities of UC focusing on the importance of evaluating the endoscopic activity of the patients.

In our multicentre, prospective study, we examined induction therapy of IFX-biosimilar, CT-P13, regarding mucosal healing in UC. In this cohort, two-thirds of the patients achieved mucosal healing and almost half of the patients achieved steroid-free mucosal healing at week 14. IFX biosimilar CT-P13 represents a promising treatment option for patients with UC not only regarding clinical activity, but also in achieving mucosal healing.

Our results suggest that the simultaneous measurement of IFX TL and ATI titers significantly increase the diagnostic accuracy for the therapeutic decision in uncertainly responding patients. The measurement of W2aTL and W6aTL levels does not improve further the accuracy of the prediction of therapeutic response, but results in substantially elevated costs. Then expression of ATI in the circulation may be transient, therefore single sampling is supposed to be insufficient for predicting the therapeutic response. It increases the risk of loss of response, but does not exclude the optimal response to normal or escalated dose of IFX. We recommend simultaneous assessment of serum IFX and ATI levels together with the clinical condition ofpatients. Clinical response based on the subjective judgment of the attending physician always takes priority over the results of measurement.

In our non-IBD cohort, the highest sensitivity and specificity were achieved by the use of combined M2PK and iFOBT test in the detection of CRC. FC seems to be a useful adjuvant to the investigation of patients at high risk for colorectal neoplasia, while fecal MMP-9 may be a promising factor for detection of CRC. Although, in CRC, sensitivity of M2PK, iFOBT, and Hb/Hp complex proved to be high, in adenomas sized ≥1 cm, sensitivity decreased significantly. Therefore, none of these markers are unique for detection of precancerous lesions of the colorectum. However, our result revealed that combined use ofM2PK, iFOBT, and FC may be valuable in the detection of large adenomas.

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REFERENCES

1. Lichtenstein GR, Hanauer SB, Sandborn WJ. et al Management of Crohn‘s disease in adults. *Am J Gastroenterol* 2009; 104: 465-483; quiz 464, 484
2. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death Differ*. 2003; 10: 45–65
3. Schnitzler F, Fidder H, Ferrante M, et al. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn’s disease. *Inflamm Bowel Dis* 2009; 15: 1295–1301
4. Colombel JF, Rutgeerts P, Reinisch W, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology* 2011; 141: 1194–1201
5. Truelove SC, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J* 1955;2:1041-1048.
6. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med.* 2010;362:1383-95.
7. Solberg IC, Lygren I, Jahnsen J, Vatn M, Mourn B. Mucosal healing after initial treatment may be a prognostic marker for long-term outcome in inflammatory bowel disease. Gut 2008;57:A15.
8. Magro F, Portela F. Management of inflammatory bowel disease with infliximab and other anti-tumor necrosis factor alpha therapies. BioDrugs 2010;24:3–14.
9. Frøslie KF, Jahnsen J, Moum BA et al Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. *Gastroenterology*. 2007 Aug;133(2):412-22.
10. Lichtenstein GR, Yan S, Bala M, et al Infliximab maintenance treatment reduces hospitalizations, surgeries, and procedures in fistulizing Crohn's disease. *Gastroenterology*. 2005 Apr;128(4):862-9.
11. Schnitzler F, Fidder H, Ferrante M, et al Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. *Inflamm Bowel Dis*. 2009 Sep;15(9):1295-301.
12. D’Haens G, Sandborn WJ, Feagan BG, et al. A review of activity indices andefficacyendpointsfor clinicaltrialsofmedicaltherapyin adults with ulcerative colitis. *Gastroenterology* 2007;132: 763–86.
13. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005;353:2462–76.
14. Reinisch W, Sandborn WJ, Hommes DW, et al. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* 2011;60:780–7.
15. 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;142:257–65; e1-3.
16. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2014;146:85–95; quiz e14-5.

17. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2014;146:96–109; e1.
18. Rinaudo-Gaujous M, Paul S, Tedesco ED, Genin C, Roblin X, Peyrin-Biroulet L. Review article: biosimilars are the next generation of drugs for liver and gastrointestinal diseases. Aliment Pharmacol Ther 2013;38:914–24.
19. Yoo DH, Hrycaj P, Miranda P, Ramiterre E, Piotrowski M, Shevchuk S, et al. A randomised, double-blind, parallel-group study to demonstrate equivalence in efficacy and safety of CT-P13 compared with innovator infliximab when coadministered with methotrexate in patients with active rheumatoid arthritis: the PLANETRA study. Ann Rheum Dis 2013;72(10):1613-20.
20. Park W, Hrycaj P, Jeka S, Kovalenko V, Lysenko G, Miranda P, et al. A randomised, double-blind, multicentre, parallel-group, prospective study comparing the pharmacokinetics, safety, and efficacy of CT-P13 and innovator infliximab in patients with ankylosing spondylitis: the PLANETAS study. Ann Rheum Dis 2013;72(10):1605-12.
21. Feagan BG, Choquette D, Ghosh S, et al. The challenge of indication extrapolation for infliximab biosimilars. Biologicals 2014;42: 177-83
22. Farkas K, Rutka M, Balint A, et al. Efficacy of the new infliximab biosimilar CT-P13 induction therapy in Crohn's disease and ulcerative colitis – experiences from a single center. Expert Opin Biol Ther 2015;15: 1257–62.
23. Jung YS, Park DI, Kim YH, et al. Efficacy and safety of CT-P13, a biosimilar of infliximab, in patients with inflammatory bowel disease: A retrospective multicenter study. J Gastroenterol Hepatol 2015;30:1705–12.
24. Kang YS, Moon HH, Lee SE, Lim YJ, Kang HW. Clinical experience of the use of CT-P13, a biosimilar to infliximab in patients with inflammatory bowel disease: A case series. Dig Dis Sci 2015;60:951–6.
25. Mosli MH, Zou G, Garg SK,et al. C-Reactive Protein, Fecal Calprotectin, and Stool Lactoferrin for Detection of Endoscopic Activity in Symptomatic Inflammatory Bowel Disease Patients: A Systematic Review and Meta-Analysis.Am J Gastroenterol. 2015;110:802-19.
26. Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 2002; 359(9317): 1541–1549. doi: 10.1016/S0140-6736(02)08512-4
27. Schnitzler F, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, et al. Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. Gut 2009; 58(4): 492–500. doi: 10.1136/gut.2008.155812 [PubMed]
28. Sands BE, Blank MA, Patel K, et al. Long-term treatment of rectovaginal fistulas in Crohn's disease: response to infliximab in the ACCENT II Study. Clin. Gastroenterol. Hepatol. 2004; 2(10): 912–920.
29. Molnár T, Lakatos PL, Farkas K, Nagy F, Szepes Z, Miheller P, 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): 225–233. doi: 10.1111/apt.12160

30. Levesque BG, Greenberg GR, Zou G, Sandborn WJ, Singh S, Hauenstein S, et al. A prospective cohort study to determine the relationship between serum infliximab concentration and efficacy in patients with luminal Crohn's disease *Aliment Pharmacol Ther*. 2014 May;39(10):1126-35.
31. Seow CH, Newman A, Irwin SP, Steinhart AH, Silverberg MS, Greenberg GR. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut 2010; 59 (1):49–54.
32. O'Meara S, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm. Bowel Dis.* 2014; 20(1): 1–6. https://doi.org/10.1097/01.MIB.0000436951.80898.6d PMID: 24280879
33. Weisshof R, Ungar B, Blatt A, Dahan A, Pressman S, Waterman M, et al. Anti-infliximab Antibodies with Neutralizing Capacity in Patients with Inflammatory Bowel Disease: Distinct Clinical Implications Revealed by a Novel Assay. *Inflamm. Bowel Dis*. 2016; 22(7): 165516–61.
34. Nanda KS, Cheifetz AS, Moss AC. Impact of antibodies to infliximab on clinical outcomes and serum infliximab levels in patients with inflammatory bowel disease *Am J Gastroenterol*. 2013 Jan;108(1):40-7; quiz 48.
35. Biomarkers Definition Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Therapeutics*. 2001; 69:89–95.
36. Farkas K, Bálint A, Bor R, et al Faecal matrix metalloprotease-9 is a more sensitive marker for diagnosing pouchitis than faecal calprotectin: results from a pilot study. *Expert Rev Gastroenterol Hepatol.* 2015 Mar;9(3):387-92.
37. Farkas K, Saródi Z, Bálint A et al The diagnostic value of a new fecal marker, matrix metalloprotease-9, in different types of inflammatory bowel diseases. *J Crohns Colitis.* 2015 Mar;9(3):231-7.
38. Annaházi A, Molnár T, Farkas K, et al Fecal MMP-9: a new noninvasive differential diagnostic and activity marker in ulcerative colitis. *Inflamm Bowel Dis*. 2013 Feb;19(2):316-20
39. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent Jet al., "Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012," *European Journal of Cancer*, vol. 49, no. 6, pp. 1374–1403, 2013.
40. Hungarian Central Statistical Office [A Központi Statisztikai Hivatal (KSH) adatai.] [Hungarian]
41. Altobelli E, Lattanzi A, Paduano R, et al. Colorectal cancer prevention in Europe: burden of disease and status of screening programs. *Prev Med*. 2014; 62: 132–141.
42. World Health Organization, International Agency for Research on Cancer: World Cancer Report, 2014,
43. Arvelo F, Sojo F, Cotte C.Biology of colorectal cancer. *Ecancermedicalscience*. 2015 Apr 9;9:520.
44. Sieg A, Thoms C, Luthgens K, M. R. John, and H. Schmidt-Gayk, "Detection of colorectal neoplasms by the highly sensitive hemoglobin-haptoglobin complex in feces," *International Journal of Colorectal Disease*, vol. 14, no. 6, pp. 267–271, 1999.

45. Vasilyev S, Smirnova E, Popov D et al., “Anew-generationfecal immunochemical test (FIT) is superior to quaiac-based test in detecting colorectal neoplasia among colonoscopy referral patients,” *Anticancer Research*, vol. 35, no. 5, pp. 2873–2880, 2015.
46. Tamada M, Suematsu M, Saya H, “Pyruvate kinase M2: multiple faces for conferring benefits on cancer cells,” *Clinical Cancer Research*, vol. 18, no. 20, pp. 5554–5561, 2012.
47. Christofk HR, Vander Heiden MG, Harris MH et al., “The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth,” *Nature*, vol. 452, no. 7184, pp. 230–233, 2008.
48. Eigenbrodt E, Basenau D, Holthusen S, et al “Quantification of tumor type M2 pyruvate kinase (Tu M2-PK) in human carcinomas,” Anticancer Research, vol. 17, no. 4, pp. 3153–3156, 1997.
49. Poullis A, Foster R, Mendall MA, et al “Emerging role of calprotectin in gastroenterology,” Journal of Gastroenterology and Hepatology, vol. 18, no. 7, pp. 756–762, 2003.
50. Tibble J, Teahon K, Thjodleifsson B et al., “A simple method for assessing intestinal inflammation in Crohn’s disease,” *Gut*, vol. 47, no. 4, pp. 506–513, 2000.
51. Wagner M, Peterson C. G B, Ridefelt P, et al “Fecalmarkers of inflammation used as surrogatemarkers for treatment outcome in relapsing inflammatory bowel disease,” *World Journal of Gastroenterology*, vol. 14, no. 36, pp. 5584–5589, 2008.
52. Tibble JA, Sigthorsson G, Bridger S, et al “Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease,” *Gastroenterology*, vol. 119, no. 1, pp. 15–22, 2000.
53. Johne B, Kronborg O, Ton HI, J. et al. “A new fecal calprotectin test for colorectal neoplasia: clinical results and comparison with previous method,” *Scandinavian Journal of Gastroenterology*, vol. 36, no. 3, pp. 291–296, 2001.
54. B. Yang, F. Tang, B. Zhang, Y. et al “Matrixmetalloproteinase-9 overexpression is closely related to poor prognosis in patients with colon cancer,” *World Journal of Surgical Oncology*, vol. 12, no. 1, article 24, 2014.
55. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. *BMJ* 1989;298:82–6.
56. Riley SA, Mani V, Goodman MJ, Dutt S, Herd ME. Microscopic activity in ulcerative colitis: what does it mean? *Gut* 1991;32:174–8.
57. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut* 2006;55:749–53.
58. 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;317:1625–9.
59. Ben-Horin S, Yavzori M, Benhar I, Fudim E, Picard O, Ungar B, et al. Cross-immunogenicity: antibodies to infliximab in Remicade-treated patients with IBD similarly recognise the biosimilar Remsima. *Gut* 2016; 65(7): 1132±1138. ttps://doi.org/10.1136/gutjnl-2015-309290 PMID: 25897019

60. Park W, Lee SJ, Yun J, Yoo DH. Comparison of the pharmacokinetics and safety of three formulations of infliximab in healthy subjects: a randomized, double-blind, three-arm, parallel-group, single-dose. *Expert Rev. Clin. Immunol*. 2015; 11(supplement 1): 25±31.
61. Rutgeerts P, Diamond RH, Bala M, et al Scheduled maintenance treatment with infliximab is superior to episodic treatment for the healing of mucosal ulceration associated with Crohn's disease. *Gastrointest Endosc*. 2006 Mar;63(3):433-42; quiz 464.
62. Simren M, Axelsson J, Gillberg R, et al Quality of life in inflammatory bowel disease in remission: the impact of IBS-like symptoms and associated psychological factors. *Am J Gastroenterol* 2002;97:389–96.
63. Ansari R, Attari F, Razjouyan H, et al. Ulcerative colitis and irritable bowel syndrome: relationships with quality of life. *Eur J Gastroenterol Hepatol* 2008;20:46–50.
64. Travis SP, Schnell D, Krzeski P, Abreu MT, Altman DG, Colombel JF, et al. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the Ulcerative Colitis Endoscopic Index of Severity (UCEIS).*Gut* 2012;61:535–42.
65. Samuel S, Bruining DH, Loftus EV Jr, Thia KT, Schroeder KW, Tremaine WJ, et al. Validation of the ulcerative colitis colonoscopic index of severity and its correlation with disease activity measures. *Clin Gastroenterol Hepatol* 2013;11:49–54; e1.
66. Online, G. Generics and Biosimilars Initiative. Efficacy, extrapolation and interchangeability of biosimilars. 2014; www.gabionline.net/Biosimilars/Research/Efficacy-extrapolation-and interchangeability-of-biosimilars].
67. Online, G. Generics and Biosimilars Initiative. Extrapolation of biosimilar infliximab indications to inflammatory bowel disease. 2014; www.gabionline.net/Biosimilars/Research/Extrapolation-of-biosimilar-infliximab-indications-to-inflammatory-bowel-disease].
68. Online, G. Generics and Biosimilars Initiative. ABPI issues updated position paper on biosimilars. 2014; www.gabionline.net/Biosimilars/General/ABPI-issues-updated-position-paper-on-biosimilars].
69. Lee KM, Jeen YT, Cho JY, et al. Efficacy, safety, and predictors of response to infliximab therapy for ulcerative colitis: a Korean multicenter retrospective study. J Gastroenterol Hepatol 013;28:1829–33.
70. Zhou YL, Xie S, Wang P, et al. Efficacy and safety of infliximab in treating patients with ulcerative colitis: experiences from a single medical center in southern China. J Dig Dis 2014;15:483–90.
71. Hyams J, Damaraju L, Blank M, et al. Induction and maintenance therapy with infliximab for children with moderate to severe ulcerative colitis. Clin Gastroenterol Hepatol 2012;10:391–9.e1.
72. Paul S, Del Tedesco E, Marotte H, et al. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. *Inflamm Bowel Dis* 2013;19:2568–76.

73. Malickova K, Duricova D, Bortlik M, et al. Serum trough infliximab levels: A comparison of three different immunoassays for the monitoring of CT-P13 (infliximab) treatment in patients with inflammatory bowel disease. *Biologicals* 2016;44:33–6.
74. Advances in IBD: Current developments in the treatment of inflammatory bowel diseases. *Gastroenterol Hepatol* (N Y) 2009;5:830–3.
75. Dai C, Liu WX, Jiang M, Sun MJ. Mucosal healing did not predict sustained clinical remission in patients with IBD after discontinuation of oneyear infliximab therapy. *PLoS One* 2014;9:e110797.
76. Farkas K, Lakatos PL, Szucs M, et al. Frequency and prognostic role of mucosal healing in patients with Crohn's disease and ulcerative colitis after one-year of biological therapy. *World J Gastroenterol* 2014;20:2995–3001.
77. Ungar B, Mazor Y, Weisshof R, Yanai H, Ron Y, Goren I, et al. Induction infliximab levels among patients with acute severe ulcerative colitis compared with patients with moderately severe ulcerative colitis. *Aliment. Pharmacol Ther*. 2016; 43(12): 1293±1239.
78. Cornillie F, Hanauer SB, Diamond RH, Wang J, Tang KL, Xu Z, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut* 2014; 63(11): 1721±1727.
79. Moore C, Corbett G, Moss AC. Systematic Review and Meta-Analysis: Serum Infliximab Levels During Maintenance Therapy and Outcomes in Inflammatory Bowel Disease. *J. Crohns. Colitis* 2016; 10(5): 619±625.
80. Hanauer SB, Wagner CL, Bala M, Mayer L, Travers S, Diamond RH, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. *Clin. Gastroenterol. Hepatol*. 2004; 2(7): 542±553.
81. Ungar B, Chowers Y, Yavzori M, Picard O, Fudim E, Har-Noy O, et al. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. *Gut* 2014; 63(8): 1258±1264.
82. Lee LYW, Sanderson JD, Irving PM. Anti-infliximab antibodies in inflammatory bowel disease: prevalence, infusion reactions, immunosuppression and response, a meta-analysis. *Eur. J. Gastroenterol. Hepatol*. 2012; 24(9): 1078±1085.
83. Rutgeerts P, Van Assche G, Vermeire S. Optimizing anti-TNF treatment in inflammatory bowel disease. Gastroenterology 2004; 126(6): 1593±1610.
84. Baert F, Noman M, Vermeire S, Van Assche G, D'Haens G, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N. Engl. J. Med.*2003; 348(7): 601±608.
85. Boncz I, Brodszky V, Péntek M et al., "The disease burden of colorectal cancer in Hungary," *European Journal of Health Economics*, vol. 10, no. 1, pp. S35–S40, 2010.
86. Kronborg O, Fenger C, Olsen J, et al "Randomised study of screening for colorectal cancer with faecal-occult-blood test," *The Lancet*, vol. 348, no. 9040, pp. 1467–1471, 1996.
87. Kewenter J, Brevinge H, Engarás B, "Results of screening, rescreening, and follow-up in a prospective randomized study for detection of colorectal cancer by fecal occult

blood testing: results for 68,308 subjects," *Scandinavian Journal of Gastroenterology*, vol. 29,no. 5, pp.468-473, 1994.
88. Duffy MJ, Van Rossum LGM, Van Turenhout ST et al "Use of faecal markers in screening for colorectal neoplasia: a European group on tumor markers position paper," *International Journal of Cancer*, vol. 128, no. 1, pp. 3–11, 2011.
89. Shastri YM, Naumann M, Oremek GM et al., "Prospective multicenter evaluation of fecal tumor pyruvate kinase type M2 (M2-PK) as a screening biomarker for colorectal ecoplasia," *International Journal of Cancer*, vol. 119, no. 11, pp. 2651–2656, 2006.
90. Tonus C, Sellinger M, Koss K, Neupert G, "Faecal pyruvate kinase isoenzyme typeM2 for colorectal cancer screening: a meta-analysis," World Journal of Gastroenterology, vol. 18, no. 30, pp. 4004–4011, 2012.
91. Kim YC, Kim JH, Cheung DY et al., "The usefulness of a novel screening kit for colorectal cancer using the immunochromatographic fecal tumor M2 pyruvate kinase test," Gut and Liver, vol. 9, no. 5, pp. 641–648, 2015.
92. Tibble J, Sigthorsson G, Foster R, et al "Faecal calprotectin and faecal occult blood tests in the diagnosis of colorectal carcinoma and adenoma," Gut, vol. 49, no. 3, pp. 402–408, 2001.
93. Groblewska M, Siewko M, Mroczko B et al "The role of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in the development of esophageal cancer," Folia Histochemica et Cytobiologica, vol. 50, no. 1, pp. 12–19, 2012

FIGURES

TABLES

ANNEX