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

Ph.D. THESIS

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Number of full publications**:**	**23**
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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. 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 . 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. 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 mucosal healing (MH) and give the most hope for the modification of the natural disease course. Evidence shows that MH is associated with long-term remission and lower cancer risk, thus highlighting the importance of achieving MH in clinical practice.

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. 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. 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. However, the rate of MH was 41.1% and 25% at weeks 8 and 52. 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. 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. The approval of CT-P13 was based on randomized clinical trials conducted in patients with rheumatoid arthritis , supplemented by a clinical and a pharmacokinetic study on ankylosing spondylitis. 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.

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. A substantial number of patients show only partial response, and approximately 20-45% of the primary responders show loss of efficacy, which poses significant clinical problem for IBD management. 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. In UC, detectable IFX trough level (TL) is associated with higher rate of clinical remission and endoscopic improvement and with lower risk of colectomy . ATI is reported to develop in up to 60% of IBD patients during maintenance IFX therapy. 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. 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.

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 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. 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. M2 pyruvate kinase (PK) is a biochemical form of PK which is a key enzyme in cancer cell metabolism. M2PK is expressed in normal proliferating cells, embryonic cells, adult stem cells, and cancer cells. Elevated levels of M2PK have been detected in colonic adenocarcinoma. Calprotectin is a calcium-binding and zinc-binding protein complex that is abundant in the cytosol of inflammatory cells. Fecal calprotectin (FC), a biomarker of intestinal inflammation, has been in clinical use for years in IBD. 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 . 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. 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.

2. AIMS

1. To prospectively evaluate the correlation between clinical and endoscopic disease activities of UC defined by activity scores.
2. To prospectively evaluate the efficacy of CT-P13 induction therapy on mucosal healing in patients with UC.
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.
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.

3. PATIENTS AND METHODS

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

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.

Clinical activities were defined by two activity indices: the CAI and the partial Mayo (pMayo) score. 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). 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.

3.2. 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. 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. 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. MH was defined as Mayo endoscopy subscore of 0 or 1. Complete MH 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. 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.

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.

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. 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.

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.

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.

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. 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. RESULTS

4.1. 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

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.

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). 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.

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 $p = 0.026$).

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. 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 .

4.2. 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). 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%). 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 received ADA. None of the patients with primary non-response developed ATI. One patient underwent colectomy; three patients needed dose intensification throughout the induction phase of CT-P13 therapy. Sigmoidoscopy revealed MH in 38 patients (60.3%), steroid-free MH was shown in 30 patients (47.6%). Complete MH 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. Both the Mayo score and eMayo score decreased significantly in responders at week 14 compared to baseline ($p<0.001$ and $p<0.001$). 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 MH 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 MH at week 14. TL of CT-P13 were significantly higher in patients who achieved MH or steroid-free MH and vs. patients who did not achieve endoscopic remission (mean values: 5.72, 6.35 and 2.85 μg/ml, $p=0.02$ and $p=0.008$). Mean values of CT-P13 TL 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 MH. No statistical difference was shown between the two groups (serum CT-P13 levels in Hungarian and Czech patients who achieved steroid-free MH 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 MH (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 MH (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 MH.

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.

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. 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.

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; $p=0.055$). 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%. 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. 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. 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.

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. 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%). Prediction rate in the logistic regression model was 77.1%, which correlated with the results of ROC analysis.

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). 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.

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.

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. 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.

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.

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. 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).

5. 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 MH. 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. 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. 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 ADA in induction and maintenance of clinical remission in patients with moderate-to-severe UC who received concurrent treatment with oral corticosteroids or immunosuppressants , while the PURSUIT trials examined the efficacy of golimumab as induction and maintenance therapy in anti–TNF-α-naive moderately-to-severely active UC. 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. 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, 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. 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 ADA and 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. 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. 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 . This multicentre study retrospectively evaluated the efficacy, safety and interchangeability of CT-P13 in IBD patients; however, its ability to assess MH was limited. 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 . 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. MH was achieved in 60.3% by week 14; the rate of steroid-free MH was 47.6%. Moreover, almost half of these patients showed complete MH with an eMayo score 0 at the time of control endoscopy. Our results also revealed a significant association between higher IFX TL, steroid-free MH 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. 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 MH but not in clinical outcome, showing that the rate of MH is more common in acute, severe UC than in chronic disease activity. Today, MH 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 MH and, if not, the therapy may be escalated . Achieving early MH is even more important given that MH itself does not predict sustained clinical remission in UC patients in whom IFX therapy had been stopped after achieving endoscopic remission .

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. 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. 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 MH (RR 3.0, 95% CI 1.4-6.5, p=0.004). 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 IFX w0 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. Use of concomitant immunosuppressants such as azathioprine and methotrexate may result in a 50% reduction in the risk of developing ATI (p<0.00001). 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 . 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. 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 and fear may all limit the appeal of this screening technique. 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 guaiac-based FOBT (gFOBT) is the oldest and most commonly used non-invasive test for detecting CRC. 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. 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%). 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 4 U/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 . 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. 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%. 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. MMP-9 is an important member of the gelatinases involved in the development of several human malignancies. 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. 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 MH in UC. In this cohort, two-thirds of the patients achieved MH and almost half of the patients achieved steroid-free MH 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 MH.

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 of M2PK, iFOBT, and FC may be valuable in the detection of large adenomas.

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