Early and simple laboratory markers of poor outcome in children with IBD.

Keywords

children, predictors, Crohn's disease, biological treatment, ulcerative colitis

Abstract

Introduction

Personalized medicine in inflammatory bowel disease (IBD) aims to achieve maximum effectiveness through rapid induction and maintenance of remission. To achieve this goal, reliable predictors of disease course are needed. The aim of this study was to identify early markers of IBD's poor course understood as the need for anti-tumor necrosis factor (anti-TNF α) treatment.

Material and methods

We analyzed the clinical, laboratory, radiological and endoscopic data of children with IBD. These parameters were assessed at the time of diagnosis (T0) and 8-12 weeks (T1) after the start of induction therapy. The results of patients who did not require anti-TNF α treatment were compared to children who needed such treatment during the 2-year observation time.

Results

58.14% of patients with Crohn's disease (CD) and 31.71% of patients with ulcerative colitis (UC) required biological therapy. Patients with CD and UC receiving biological therapy, compared with those without, differed in selected clinical and laboratory parameters both at T0 and T1. In multivariate analysis, the risk of anti-TNF α therapy in patients with CD was associated with the lack of normalization of MCV and PCDAI. In patients with UC higher albumin levels reduced this risk.

Conclusions

In children with IBD, disease activity and concentrations of selected biochemical parameters assessed within 3 months after diagnosis may be helpful in predicting a poor outcome of CD and UC.

Early and simple laboratory markers of poor outcome in children with IBD.

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are idiopathic, inflammatory processes predominantly affecting the gastrointestinal tract, recognized as inflammatory bowel disease (IBD). In general, the course of IBD is chronic, lifelong, with episodes of exacerbations and remissions. Approximately 20-30% of children with CD already have complications at the time of initial diagnosis, i.e. structuring and/or penetrating disease [1]. Similarly, the course of UC in children is more severe compared to adult patients with more frequent exacerbations, hospitalizations and the need for colectomy [2]. 10% of all CD patients and 15-20% of all UC cases, are particularly at risk of unfavorable outcome due to long-term exposure to pathogenic factors such as malnutrition, anorexia and chronic inflammation [2, 3]. The goal of treating children with IBD is to achieve rapid and sustained remission, cure mucosal lesions, ensure quality of life and avoid adverse drug reactions. For this reason, patients with pediatric IBD require an individualized approach based on reliable predictors of good and poor treatment outcomes. A number of clinical, laboratory and endoscopic parameters are being analyzed for their usefulness in assessing the course of IBD in children. According to current guidelines, the combination of fecal calprotectin (fCal) and C-reactive protein (CRP) is better than fCal alone in the evaluation of children with CD [4]. What is more, fCal assessed in 14 weeks after initiation of biological treatment in children with CD was a predictor of clinical remission and endoscopic healing after 1 year of treatment with infliximab (IFX) [5]. The Paediatric Crohn's Disease Activity Index (PCDAI), as another marker studied, did not predict the need for steroid treatment at week 12 when assessed at disease onset [6].

Treatment regimens for IBD in children have changed significantly over the decades. First remission induction therapy in UC depends on disease activity and includes 5-aminosalicylic acid (5-ASA) or steroids. On the other hand, in patients with CD until 2020, the type of treatment to induce remission also depended on the severity of the disease, and now relies on the presence of prognostic factors for poor disease outcome, and when these are absent, exclusive enteral nutrition (ENN) or steroids are used. When remission is achieved, then maintenance treatment is used as 5-ASA in UC or thiopurine in CD as a monotherapy or combination with partial enteral nutrition/exclusion diet. If treatment fails, escalation therapy with monoclonal antibody against tumor necrosis factor α (anti-TNF α) is recommended as the first choice in children. Based on current guidelines, consideration of early anti-TNF α therapy applies to CD patients with severe growth retardation, failure to achieve remission after induction therapy, deep colonic ulceration, stenotic and penetrating disease at diagnosis and severe perianal disease. In pediatric patients with UC, biological therapy is dedicated to

chronically active or steroid-dependent UC uncontrolled by 5-ASA and thiopurines and a second-line treatment for acute, severe UC [2, 7, 8]. Nowadays, there is a growing body of literature in both children and adults in which anti-TNF α treatment is more effective when given earlier in the course of the disease ("top-down" strategy) [9-11]. In adults' studies, the CALM and REACT trials, which enrolled patients with active CD, found that earlier escalation to anti-TNF α therapy resulted in fewer major adverse outcomes than treatment with conventional step-up strategies [12]. Moreover, recent data did not show a biomarker utility (CRP and fCal) assessed at diagnosis in treatment choice and top-down treatment was suggested as a standard of care for patients with newly diagnosed active CD [13]. Despite a growing amount of data on the benefits of a "top-down" strategy, no data on non-invasive prognostic markers for the early use of biological therapy in children with IBD are available.

The purpose of this study was to identify predictors of the progressive course of CD and UC requiring treatment escalation (anti-TNF α) at two-year follow-up, based on clinical, biochemical and endoscopic parameters assessed at initial diagnosis and during early follow-up. In this study, a progressive course of IBD was defined as one that required a stepwise approach with definitive use of anti-TNF α during the first 2 consecutive years of disease.

Materials and methods

Patient's characteristics and study design

We retrospectively analyzed the clinical, laboratory, radiological and endoscopic data of children with IBD diagnosed and treated at our department between 2015 and 2021. Due to the need for two-year follow-up of patients in our Department, the maximum age of inclusion in the study was 16 years. The diagnosis of IBD was made based on European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines [14]. For the purpose of the study, two assessment time points were chosen: at the time of diagnosis (TO) and 8-12 weeks after diagnosis and remission-inducing treatment (T1).

Both the biochemical parameters and clinical disease activity were evaluated at two timepoints. Among laboratory parameters, complete blood count, CRP, erythrocyte sedimentation rate (ESR), albumin, ferritin, fCal were assessed in all participants according to standard laboratory practice. Laboratory norms for selected parameters were: WBC 4-10 x10⁹/L, neutrophil count 1500-7000 x10⁹/L, Hb 12-15g/dL, MCV >75 fl, PLT 150-450 x10⁹/L, CRP <5 mg/dL, ESR <20 mm/h, albumin >3.5 g/dL, ferritin >30 mg/dL and fCal <200 ug/g.

Disease activity was assessed according to the Pediatric Crohn's Disease Activity Index (PCDAI) in children with CD and defined as: remission < 10 points, mild disease 10-27.5 points, moderate

disease 30-37.5 points and severe disease > 40 points. The Pediatric Ulcerative Colitis Activity Index (PUCAI) was used in children with UC and rated as: remission (<10 points), mild (10-34 points), moderate (35–64 points) or severe (>64 points) [15-17].

Data obtained during colonoscopy, which was performed only at the time of diagnosis (T0), were also used for analysis. The phenotype of CD and UC was assessed using the Paris classification. In UC it was assessed as: E1—proctitis, E2—distal to splenic flexure colitis, E3—extensive, distal to hepatic flexure and E4—pancolitis (18). CD type was evaluated as follows: age at diagnosis (A), disease location (L), disease behavior (B) and growth retardation (G) [9]. The Mayo Endoscopic Scale was used for UC children assessed as: 0—normal colonic mucosa, 1—erythema, decreased vascular pattern, mild friability, 2—marked erythema, erosions, no vascular pattern, friability, 3—ulceration, spontaneous bleeding [15, 19]. Endoscopic assessment of CD activity was performed using the Simple Endoscopic Scale for CD (SES-CD), which evaluates the size of ulceration, the proportion of the area that has been ulcerated, the proportion of the area affected, stenosis, and each item is scored by segment on a scale from zero to three. Patients with active moderate to severe forms of the disease unresponsive to conventional treatment were qualified for biologic treatment after other therapeutic options were exhausted. Once the diagnosis was made, the patients were treated according to current recommendations for that time [2, 7, 8, 20, 21].

For the next two years, patients were followed up at the same medical center where the initial diagnosis was made. The entire cohort was then divided into two groups according to the course of the disease, i.e. Group I with stable disease without intensification of therapy for two years follow-up and Group II with progressive complications requiring biological drugs (anti-TNF α) during two years follow-up. Children diagnosed with IBD received appropriate induction treatment, and in UC it was 5-ASA in combination (n=32) or without steroids, and in CD it was exclusive enteral nutrition (EEN) with (n=10) or without steroids. In Group II all outcomes analyzed at T1 were taken before the initiation of biological therapy. Exclusion criteria included other autoimmune comorbidities, perianal lesions (fistula or abscess) in CD cases at the time of diagnosis, as well as parental and patient noncompliance with medication, changing the residence, significant data gaps (Figure 1 suppl.).

Statistical analysis

Statistical tests, calculations and graphics were performed using STATISTICA software. Continuous variables were summarized as median (min-max) and compared using the Mann-Whitney U test; categorical variables were presented as number and percentage and compared using the Chi-square test. Both tests involved comparing patients in Groups I and II at the same time point (T0 or T1). The

paired Wilcoxon rank sum test was used to assess statistical differences within the same groups at T0 and T1. A paired Wilcoxon rank sum test was also used to compare the effect sizes (R) of different variables. Interpretation values for R were 0.1 and < 0.3 as small effect, > 0.3 and < 0.5 as moderate effect and \ge 0.5 as large effect. The diagnostic value of biomarker concentrations was estimated using receiver operating characteristic (ROC) curve analysis. Cut-off values were selected to calculate sensitivity, specificity and accuracy of indicators based on the maximization criterion of Youden's J statistic. Spearman's correlation test was used to analyze the correlations between variables assessed at T1. Odds ratio (OR) and 95% confidence intervals (95% C.I.) for factors associated with biological treatment were calculated using both univariate and multivariate logistic regression. Following the rule of 10 per factor in the multivariate regression analysis, power was sufficient to account for 2 variables in patients with UC and 2 variables in patients with CD. All candidate variables were initially evaluated using univariate logistic regression. Variables that demonstrated statistical significance were subsequently entered into a multivariable logistic regression model. A significant area under the curve (AUC) value for the model was >0.7. p < 0.05 was considered statistically significant.

Ethical consideration

The study protocol was approved by Local Ethics Committee under the registration number R-I-002/308/2014. All methods were carried out in accordance with relevant guidelines and regulations.

Results

Between 2015 and 2021, the total number of IBD diagnoses in our Department was 148, including 56 children with CD and 92 children with UC. 12 patients did not meet the inclusion criteria for the study. The demographic and clinical characteristics of the study groups were summarized in Table I suppl.

Ultimately, 54 children with CD (median age: 14 years, males: 59.26%) and 82 children with UC (median age: 14 years, males: 43.9%) were included in the study (Figure 1 suppl.). Almost half of children with CD (48.15%) received anti-TNF α therapy including infliximab (IFX) or adalimumab (ADA) compared to one third of children with UC treated with IFX (31.71%). The median time from diagnosis to initiation of biological treatment was 6 months in CD cases and 8 months in UC cases.

1. Patients with CD

1.1. At the time of diagnosis (TO)

The demographic, clinical, laboratory and endoscopic data of Group I and Group II were shown in Table I. Patients in both groups did not differ in terms of BMI, while discrepancies in the values of clinical, laboratory and endoscopic parameters were noticeable at TO. Group I consisted mainly of boys, and the patients were older than those in Group II. Mild CD activity assessed by PCDAI was more frequently observed in Group I (71.42% vs 34.62%, p=0.007), while severe activity was more frequently observed in the group of patients who received biologic treatment during the 2-year follow-up (50.00% vs 14.29%, p=0.005). At the time of diagnosis, patients from Group II had higher ESR, PLT, PLT/albumin ratio, PCDAI, SES-CD and lower Hb and MCV compared to Group I. PLT >450x10⁹/L, Hb <12 g/dL and MCV <75fL were observed in more children in Group II than in Group I (Table I). In addition, children with ileocecal localization of disease (L3) significantly often required anti-TNF α treatment during the 2 consecutive years (50.00% vs 14.30%, p=0.004). Induction treatment, results of other laboratory and radiological findings were comparable in both groups (Table I).

1.2. At 8-12 weeks after diagnosis and remission-inducing treatment (T1)

Despite the trend toward normalization of clinical and laboratory parameters after the induction treatment in children with CD, significant differences were observed in several biochemical parameters between Group I and Group II after 8-12 weeks of follow-up (Table I, Table II supp., Figure 1). Severe disease, based on PCDAI score, was noted in 3 (about 12%) children from Group II despite the treatment. Significantly higher ESR, CRP, PLT/albumin, PCDAI and lower Hb, MCV and albumin were observed in Group II compared to Group I. Moreover, significantly more children in Group II had CRP>5mg/dL (57.69% vs 21.43%, p=0.006), ESR≥20 mm/h (53.87 vs 25.00%, p=0.03), Hb<12g/dL (50.00% vs 28.57%, p=0.01) and MCV<75 fL (61.54% vs 25.00%, p=0.007) despite significant improvements of these outcomes based on calculated size effect (Table II suppl.). Only the results for fCal and neutrophil count showed no significant changes in Group II compared to Group I. However, the result for the neutrophil count was on the verge of statistical significance (Table I, Table II suppl.). We decided to check if abnormal results of more than one marker could be more often observed in Group II (Table I). For analysis we choose PCDAI>10, CRP>5mg/dL and MCV<75 fL based on results of regression analysis and AUC from ROC analysis presented below (due to the presence of ESR and albumin in the PCDAI, both parameters were not included in the regression analysis). It should be noted that combination of two markers was not useful in differentiating patients who will require biologic treatment in the next two years. However only one patient (3.57%) from Group I had abnormal results for the three markers simultaneously (PCDAI, Hb and MCV) in contrast to 9 patients from Group II (34.62%) (p=0.003) (Table I).

Due to the possible effect of steroids on WBC and neutrophil count values, patients receiving steroids from Groups I and II were compared, with no significant differences observed in the values of the parameters (data not shown).

Among patients who required anti-TNF α treatment within 2 years follow-up, 14 children (56.00%) received it within the first 6 months of diagnosis. There were no significant differences in clinical presentation or additional test results between the groups receiving biological treatment within and after 6 months (data not shown).

1.3. ROC analysis

ROC analysis was performed to select the best marker for predicting the need for treatment intensification in the next two years (Table II). At T0 the best diagnostic value with AUC > 0.7 was obtained for Hb (AUC 0.773; sensitivity 87.0%; specificity 64.5% with a cut-off of 12.1 g/dL) and MCV (AUC 0.763; sensitivity: 82.6%; specificity: 71.0% with a cut-off of 75.6 fL), PCDAI (AUC 0.706, sensitivity 65.2%, specificity 66.7% with a cut-off 30 points) and SES-CD (AUC 0.714; sensitivity 73.9%; specificity 67.7% with a cut-off of 11 points). At T1 the best differentiation markers were ESR (AUC 0.794; sensitivity: 82.6%; specificity: 67.7% at a cutoff point of 17 mm/h), CRP (AUC 0.754; sensitivity: 65.2%; specificity: 80.06% at a cutoff point of 5.08 mg/dL), Hb (AUC 0.717; sensitivity: 87.0%; specificity: 51.6% at a cutoff point of 12.8 g/dL), MCV (AUC 0.725; sensitivity: 78.3%; specificity: 74.2% at a cutoff point of 75.4 fL), albumin (AUC 0.775; sensitivity: 73.9%; specificity: 90.3% at a cutoff point of 4.23 g/dL) and PCDAI (AUC 0.788; sensitivity: 60.9%; specificity: 86.7% at a cutoff point of 17.5).

1.4. Spearman correlation

Spearman correlation was used to check for the collinearity of the PCDAI and laboratory parameters assessed in T1. The following significant correlations were observed: CRP and ESR (R=0.57, p=0.002), CRP and neutrophil count (R=0.47, p=0.001), CRP and PCDAI (R=0.54, p=0.004), ESR and PLT (R=0.52, p=0.007), ESR and albumin (R=-0.66, p<0.001), ESR and PCDAI (R=0.48, p=0.01), Hb and albumin (R=0.44, p=0.02), PLT and WBC (R=0.63, p<0.001), PLT and fCal (R=0.50, p=0.02), WBC and neutrophil count (R=0.82, p<0.001).

1.5. Univariate and multivariate analysis

Parameters that were significantly associated with the need for anti-TNFα therapy in CD patients shown in the univariate regression model are listed in Table III. The best multivariate regression model with an AUC=0.86 was noted for PCDAI and MCV (Table III). Considering the components of PCDAI, ESR, CRP and albumin were not analyzed. PCDAI had a stimulating effect on the risk of treatment

intensification (OR 1.122, 95% CI 1.034-1.217), while an increase in MCV levels reduced this risk (OR 0.803, 95% CI 0.671-0.960) (Table III).

2. Patients with UC

2.1. At the time of diagnosis (TO)

Demographic parameters, PUCAI and endoscopic findings revealed no significant differences between Group I and Group II (Table IV). Analysis of serum and stool markers determined at T0 showed only significant higher ESR levels in children requiring anti-TNF α treatment. In addition, more children in Group II had CRP>5md/dL (46.15% vs 19.64%, p=0.01), PLT >450x10⁹/L (30.77% vs 14.29%, p=0.03) and Hb<12 g/dL (61.54% vs 32.14%, p=0.01). Induction treatment was comparable in both groups.

2.2 At 8-12 weeks after diagnosis and remission-inducing treatment (T1)

In contrast to T0, more differences between Group I and II in clinical and laboratory parameters were observed at T1 (Table IV, Table III suppl., Figure 2). Significant differences in ESR, WBC, neutrophil count, PLT, Hb, MCV, albumin, PLT/albumin ratio, fCal and PUCAI were noted. Because of the effect of systemically administered steroids on WBC and neutrophil counts, patients receiving steroids in Group I and Group II were compared; no significant differences in WBC or neutrophil counts were observed between the two groups (p>0.05) (data not shown). In Group I the significant improvement of most serum markers (CRP, WBC, neutrophil count, albumin) and fCal and PUCAI with a large R (≥ 0.5) was noted. In contrast, in Group II the significant improvement in results at moderate R was observed only for PLT, ESR and PLT/albumin ratio, while no significant normalization was observed for CRP, WBC, neutrophil count, Hb, MCV, albumin and fCal. In Group II, significantly more children had WBC>10x10⁹/L (30.77 % vs 8.93 % vs, p=0.02), Hb<12g/dL (61.54 % vs 17.86 % vs, p<0.001), MCV <75fL (23.08% vs 7.14%, p=0.04) and fCal >200ug/g (84.62 % vs 57.14 % vs, p=0.02). PLT count >450x10⁹/L was noted in 30.77% of patients in Group II, while this abnormality was not found in Group I. Moreover, clinical remission was achieved by 67.86% children in group I (p=0.005). Severe disease according PUCAI was noted in 15.38% of children requiring biological treatment over the following two years. It is worth noting that no patient in Group I had a severe course of disease at T1. We then performed a combination analysis of the best parameters based on AUC value (PUCAI >10, PLT/albumin ratio ≥ 83.22 and Hb<12g/dL) to see how many patients had more than one abnormal marker result. In contrast to patients in the CD group, only ROC analysis was relied on due to the borderline statistical significance obtained in the regression model. Then, among the parameters with the highest AUC, those whose cut-off point was not within the laboratory norm were selected. The presence of abnormal three (PUCAI >10 + PLT/albumin ratio \ge 83.22 + Hb<12g/dl) markers was significantly more frequent in children with progressive disease (p=0002). Other clinical and laboratory findings were comparable in both groups (Table IV).

12 (46.15 %) children with UC were treated with biological therapy within the first 6 months of diagnosis. The only parameter differentiating the group with early (within the first 6 months after diagnosis) and late (within 7-24 months after diagnosis) induction of anti-TNF α treatment was a lower albumin concentration at T0 in the first subgroup (4.18 vs 4.36, p=0.04; data not shown).

2.3 ROC analysis

To assess the discriminatory diagnostic ability of the markers AUC value was calculated for TO and T1 (Table V). The only parameter that achieved statistical significance at T0 was ESR (AUC 0.640; sensitivity 96.2%; specificity 30.4% with a cut-off point of 8 mm/h). At T1, the best diagnostic markers with AUC > 0.7 were albumin (AUC 0.769; sensitivity 88.5%; specificity 60.7% with a cut-off point of 4.58 g/dL), Hb (AUC 0.761; sensitivity 57.7%; specificity 89.3% with a cut-off point of 11.3 g/dL) and PUCAI (AUC 0.739; sensitivity 57.7%; specificity 83.9% with a cut-off 15 points).

2.4 Spearman correlation

Spearman correlation was used to check for the collinearity of the PUCAI and laboratory parameters assessed in T1. The following significant correlations were observed: WBC and PLT (R=0.66, p<0.001), WBC and Hb (R=-0.47, p=0.02), WBC and neutrophil count (R=0.85, p<0.001), WBC and albumin (R=-0.47, p=0.02), PLT and Hb (R=-0.60, p=0.001), PLT and MCV (R=-0.49, p=0.01), PLT and neutrophil count (R=0.65, p<0.001), PLT and albumin (R=-0.41, p=0.04), PLT and ESR (R=0.50, p=0.009), Hb and MCV (R=0.43, R=0.03), Hb and neutrophil count (R=-0.41, p=0.04), Hb and albumin (R=0.64, p<0.001), neutrophil count and albumin (R=-0.45, p=0.02), CRP and ESR (R=0.47, p=0.02), CRP and PUCAI (R=0.55, p=0.003), ESR and fCal (R=0.40, p=0.04), PUCAI and fCal (R=0.52, p=0.007), PUCAI and albumin (R=-0.43, p=0.03).

2.5. Univariate and multivariate analysis

We observed that certain parameters were significant in univariate analyses as shown in Table VI. The best multivariate regression model with an AUC=0.86 was noted for albumin and neutrophil count (Table VI). In a multivariate logistic regression model, predictors of treatment intensification with biological therapy were higher neutrophil count (OR 1.38, 95% CI 1.017-1.873) and lower albumin concentration (OR 0.117, 95% CI 0.016-0.862) assessed after remission-inducing treatment.

Discussion

Our study showed that evaluation of commonly used clinical and laboratory parameters within the first 8-12 weeks of diagnosis could be helpful to select patients with progressive IBD who will require close monitoring of the course of the disease and early escalation of therapy with anti-TNF α monoclonal antibody. To the best of our knowledge, there are only a few published studies dedicated to analyzing markers useful in predicting the progressive course of IBD requiring anti-TNF α treatment in pediatric patients at time of diagnosis and after treatment initiation [6, 22-25]. We compared children with CD as well as UC separately. In addition, patients in other studies were evaluated at different time points (3-20 months assessed during the use of various therapeutic regimens, including biological treatment). In our study, the moment of follow up was clearly defined as 8-12 weeks from the moment of diagnosis, after remission-inducing treatment, but before the initiation of anti-TNF α therapy. We observed that assessment of readily available biochemical parameters used in daily clinical practice in the first few weeks of treatment can predict which patients will require close monitoring in subsequent months of disease and earlier escalation of treatment.

Considering the studies involving children with CD, we found anti-TNF α treatment in almost 50% of patients, similar to the observation by Olbjørn et al. [22]. Olbjørn et al. evaluated ESR, CRP, fCal at diagnosis and after 12-24 months of biologic treatment. A high ESR at diagnosis was identified as a predictor of further biologic treatment as in our report. Moreover, we noted higher PLT, PCDAI and lower Hb, MCV in this group of patients. In our study, results after 8-12 weeks showed that more than half of the patients who required biologic therapy at two-year follow-up had elevated CRP and ESR, compared to only a quarter of patients in the other group. Abnormal results for both PCDAI (includes ESR and albumin), MCV and CRP were noted in about 35% of patients who received intensified treatment, compared to about 4% of patients who did not require intensified treatment. In addition, the multivariable regression analysis showed the significant value of PCDAI and MCV as predictors of risk of intensification of treatment in next two years. The low MCV is related to deficiency anemia often seen in children with IBD [26, 27]. To the best of our knowledge, MCV has not been evaluated in published studies in the first months of disease as a predictor of poor outcome of IBD. In addition, low Hb (<12g/dL) at time T1 was found in less than 4% of children in the group without CD progression in next two years and no concomitant abnormal PCDAI, CRP and Hb values were observed in this group. As mentioned previously, the combination of fCal and CRP is better than fCal alone in the evaluation of children with CD [7]. In our study, we did not include fCal in the analysis due to its lack of normalization after 8-12 weeks of treatment. Turner et al. showed that fCal decreases irrespective of

treatment (steroids or exclusive enteral nutrition) within 8 weeks of starting treatment in both CD and UC, indicating that assessing fCal over a shorter period of time may not be helpful in clinical practice [28]. Furthermore, Levine et al. observed a reduction in fCal in 23-50% of children with CD regardless of the type of initial therapy (steroids, EEN, 5-ASA) [6]. Similarly, Matuszczyk et al. reported a reduction in fCal <250 ug/g in less than one-third of children with CD after 12 weeks of treatment of Crohn's disease with an exclusion diet combined with partial enteral nutrition [29]. Our results suggest that finding still abnormal PCDAI (>10), CRP (>5mg/dL) and MCV <75fL results at the follow-up time point is more useful in predicting which patients will require close monitoring. More studies are needed to assess the usefulness of the combinations of different factors in daily clinical practice

Regarding children with UC, only a few publications are available that focus on early markers of poor prognosis of the disease and the need for biological therapy [24, 25]. Among our patients, nearly 32% required biological treatment within two consecutive years. At the time of diagnosis, only ESR values significantly differentiated study groups. Hyams et al. noted that factors leading to escalation of therapy included low levels of Hb, vitamin D3, and eosinophil counts in rectal biopsy specimens at the time of diagnosis [24]. On the other hand, Gasparetto et al. noted that children with severe UC (requiring biologic treatment and/or major surgery within 18 months) had lower albumin and Hb values at diagnosis compared to mild disease [25]. In contrast, ESR values did not differ significantly between patients groups [25].

Interestingly, in our study, more differences in assessed parameters between the groups requiring and not requiring biological treatment were found after 8-12 weeks of remission-inducing treatment. The group with UC progressing despite treatment had significantly higher PUCAI values and most inflammatory parameters (ESR, PLT, fCal) and lower albumin and Hb, MCV levels after 8-12 weeks compared to the group not requiring biological treatment. Although PUCAI scores decreased over 8-12 weeks of follow-up in both groups, clinical remission (PUCAI<10) was achieved by the majority of children in the group without biological treatment, while there were a few cases with severe disease in the other group. Similar results were obtained by Gasparetto et al., who noted that the clinical assessment with PUCAI of patients 3 months after diagnosis is important for predicting the severity of the disease [25]. They observed that clinical remission without steroids, as well as PUCAI assessed at month 3 of the disease, are the most reliable clinical predictors of long-term remission in children in subsequent years. However, no laboratory parameter was evaluated during follow-up in this study, making it impossible to compare most of our results. In our study, significantly lower fCal values at T1 was observed in children not requiring biologic treatment. However, it should be noted that only less than half of the patients had fCal <200 ug/g. Perhaps the reasons for the lack of normalization of fCal in our study were the measurement of fCal too soon after the inclusion of remission-inducing treatment and the more severe form of the disease at the time of diagnosis. It is now emphasized that the goal of treating patients with IBD is not only to achieve clinical remission, but also mucosal healing, which is a long-term process [30]. Therefore, monitoring fCal over a longer period seems to be more important. The use of multivariate regression analysis in our study revealed that the high predictive value of weak improvement in albumin levels after 2-3 months may be associated with a higher risk of a more severe disease course requiring treatment with TNF α inhibitors. However, it should be noted that in the ROC analysis, the cut-off point for albumin differentiating both study groups was 4.58 g/dL, which is within the laboratory norm. Moreover, the albumin values were within normal laboratory limits in majority of patients at the follow-up time point. Perhaps in the group of children with UC, different norm values of albumin should be considered. To the best of our knowledge, published studies have not assessed the usefulness of albumin assessed 8-12 weeks after diagnosis of UC to the need for treatment intensification. The only study by Hyams et al. reported that a higher baseline albumin level of 1 g/dL in the <12 age group could indicate remission without CS 12 weeks after diagnosis in children with UC [31]. In adults, the positive value of the neutrophil-to-albumin ratio in determining the response to IFX treatment and long-term maintenance of remission has been observed. The higher peripheral neutrophil counts compared to healthy controls were positively correlated with the severity of endoscopic lesions according to the Mayo score [32, 33]. However, in both studies, the neutrophil to albumin ratio was assessed just before IFX was included (up to 1 week earlier). Finally, we determined the usefulness of a combination of two or three selected clinical and laboratory markers (PUCAI, Hb and PLT/albumin ratio) in predicting which UC patients would require anti-TNF α treatment, and the results were comparable to the CD group (evaluated PCDAI, MCV and CRP).

The most important outcome of our study was to demonstrate the usefulness of common biochemical parameters assessed after 8-12 weeks of remission-inducing treatment in predicting which patients with CD or UC will require close monitoring in subsequent years of disease and more rapid intensification of treatment. To date, only a few studies in the pediatric population have focused on outcomes not only at the time of diagnosis, but also at specific time points. Another strength of this study is that our department was the main pediatric endoscopy center with histopathological examination in northeastern Poland, diagnosing all IBD in children in recent years, and all patients remained under monitoring until the age of 18. It should be noted that the markers we are analyzing are readily available and inexpensive. The present study also has some limitations. First, the small number of participants does not allow us to generalize our results and reduces statistical power. The number of patients included in the study was low due to the time frame and monocentric nature of the study. We recognize that our results may be subject to errors of omission (type II error) and did not interpret non-significant results as underlying a true null difference. Another limitation is the retrospective analysis, which involves potential errors. Further research without these limitations would be useful.

Conclusion

Based on our results, we have shown that the assessment of patients' clinical and routine biochemical parameters not only at the time of diagnosis, but also shortly after initial remission-inducing treatment seems to be a promising benchmark for predicting the course of the disease over the next two years. In a multivariate regression model, the risk of anti-TNF α therapy in patients with CD was associated with failure to normalize MCV and PCDAI after remission-inducing treatment. In contrast, in patients with UC, higher albumin levels reduced this risk of such therapy. Further studies in a larger group of patients are needed to confirm the usefulness of the markers we analyzed. Perhaps long-term studies of new genetic, serological or microbiological markers would provide more information about the course of the disease [34, 35].

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Competing interests

The author(s) declare no competing interests.

Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.



Table I. Comparison of demographic parameters and selected clinical parameters at the time of diagnosis of CD between the group not requiring biological treatment (Group I) and the group requiring such treatment (Group II) during two years of follow-up.

	Group I (n=28)	Group II (n=26)	p		
ТО					
Age (years) (median, range)	15 (9-16)	14 (6-16)	0.04		
Male (n, %)	21 (75.0%)	11 (42.31%)	0.01		
BMI (pc) (median, range)	29 (1-80)	19 (1-87)	0.60		
Growth G1/G0 (n, %)	5 (17.90%) / 23 (82.1%)	8 (30.77%) /18 (69.23%)	0.27		
PCDAI (median, range)	22.5 (10.0-55.0)	40.0 (10.0-65.0)	0.01		
IBD activity:	1				
Remission (n, %)	0 (0.0%)	0 (0.0%)	NA		
Mild (n, %)	20 (71.42%)	9 (34.62%)	0.007		
Moderate (n, %)	4 (14.29%)	4 (15.38%)	0.91		
Severe (n, %)	4 (14.29%)	13 (50.00%)	0.005		
ESR (mm/h) (median, range)	36 (2-68)	46 (6-120)	0.03		
ESR ≥20 mm/h (n, %)	22 (78.57%)	23 (88.46%)	0.33		
CRP (mg/dL) (median, range)	15.6 (0.6-124.0)	24.5 (1.8-342.0)	0.08		
CRP >5 mg/dL (n, %)	20 (71.43%)	21 (80.77%)	0.42		
WBC (x10 ⁹ /L) (median, range)	7.4 (4.0-14.1)	8.9 (5.8-14.9)	0.67		
WBC >10x10 ⁹ /L (n, %)	10 (35.71%)	10 (38.46%)	0.83		
Neutrophil count (x10 ⁹ /L)	4.9 (2.0-10.9)	5.7 (3.7-11.3)	0.79		
(median, range)					
Neutrophil count > 7x10 ⁹ /L (n, %)	9 (32.14%)	8 (30.77%)	0.91		
PLT (x 10 ⁹ /L) (median, range)	335 (160-624)	462 (309-756)	0.03		
PLT >450x10 ⁹ /L (n, %)	6 (21.43%)	14 (53.85%)	0.01		
Hb (g/dL) (median, range)	12.5 (9.6-15.0)	11.1 (8.5-14.2)	<0.001		
Hb <12 g/dL (n, %)	10 (35.71%)	17 (65.38%)	0.03		
MCV (fL) (median, range)	78.6 (64.4-83.6)	69.4 (62.7-82.0)	0.001		
MCV <75 fl (n, %)	8 (28.57%)	17 (65.38%)	0.007		
Ferritin (µg/L) (median, range)	45.2 (5.3-200.3)	47.9 (12.5-182.0)	0.45		
Ferritin <30 mg/dL (n, %)	12 (42.86%)	10 (38.46%)	0.74		
Albumin (g/dL) (median, range)	4.15 (3.35-5.07)	3.97 (3.05-4.50)	0.08		
Albumin <3.5 g/dL (n, %)	3 (10.71%)	7 (26.92%)	0.13		
PLT/albumin ratio (median,	81.77 (31.56-186.27)	117.97 (72.54-223.23)	0.02		
range)					
fCal (ug/g) (median, range)	1864 (693-3479)	2280 (366-3865)	0.92		
fCal >200 ug/g (n, %)	28 (100.0%)	26 (100.0%)	NA		
SES-CD (median, range)	7 (1-31)	12(4-25)	0.008		

Location L1/L2/L3 (n, %)	17 (60.7%)/ 7 (25.0%)/ 4	11 (42.31%)/ 2 (7.69%)/13	0.18/0.09/0.004
	(14.30%)	(50.00%)	
Behavior B1/B2 (n, %)	16 (57.10%)/12 (42.90%)	13 (50.0%) /13 (50.00%)	0.60
Length of inflammatory	50 (0-150)	70 (0-210)	0.31
lesions/stenosis on MRI (mm)			
(median, range)			
Thickness of the small bowel wall	5.5 (3-10)	7 (2-10)	0.06
on MRI (mm) (median, range)			
Induction treatment:			
EEN only (n, %)	25 (89.3%)	19 (73.08%)	0.13
Steroids (+/- EEN) (n, %)	3 (10.7%)	7 (26.92%)	0.13
	T1		
BMI (pc) (median, range)	21 (1-84)	30 (1-90)	0.60
PCDAI (median, range)	5 (0-25)	20 (0-55)	<0.001
PCDAI ≥10 points (n, %)	12 (42.86%)	18 (26.23%)	0.05
IBD activity:			
Remission (n, %)	16 (57.14%)	8 (30.77%)	0.05
Mild (n, %)	12 (42.86%)	15 (57.69%)	0.28
Moderate (n, %)	0 (0.0%)	0 (0.0%)	NA
Severe (n, %)	0 (0.0%)	3 (11.54%)	NA
ESR (mm/h) (median, range)	10 (2-43)	25 (3-70)	<0.001
ESR≥ 20 mm/h (n, %)	7 (25.00%)	14 (53.85%)	0.03
CRP (mg/dL) (median, range)	0.8 (0.1-28.0)	6.4 (0.3-43.9)	0.002
CRP >5 mg/dL (n, %)	6 (21.43%)	15 (57.69%)	0.006
WBC (x 10 ⁹ /L) (median, range)	6.7 (3.9-22.9)	8.39 (4.3-16.1)	0.1
WBC >10x10 ⁹ /L (n, %)	5 (17.86%)	6 (23.08%)	0.63
Neutrophil count (x 10 ⁹ /L)	4.2 (1.5-11.7)	5.0 (1.7-10.3)	0.05
(median, range)			
Neutrophil count > 7x10 ⁹ /L (n, %)	4 (14.29%)	5 (19.23%)	0.63
PLT (x 10 ⁹ /L) (median, range)	329 (147-687)	399 (228-607)	0.14
PLT >450x10 ⁹ /L (n, %)	5 (17.86%)	10 (38.46%)	0.09
Hb (g/dL) (median, range)	13.1 (10.5-15.2)	11.9 (10.1-14.6)	0.007
Hb <12 g/dL (n, %)	8 (28.57%)	13 (50.00%)	0.01
MCV (fL) (median, range)	77.6 (64.4-83.6)	72.6 (67.6-85.7)	0.005
MCV <75 fl (n, %)	7 (25.00%)	16 (61.54%)	0.007
albumin (g/dL) (median, range)	4.64 (3.76-5.00)	4.15 (3.28-4.93)	<0.001
albumin <3.5 g/dL (n,%)	0 (0.0%)	1 (3.85%)	NA
PLT/albumin ratio (median,	67.09 (29.52-182.71)	97.95 (48.51-182.83)	0.02
range)			
fCal (ug/g) (median, range)	1391 (60-3288)	2281 (366-3865)	0.06
fCal >200 ug/g (n, %)	25 (89.29%%)	26 (100.0%)	NA

PCDAI ≥10 + MCV < 75 fl	1 (3.57%)	4 (15.38%)	0.13
PCDAI ≥10 + CRP>5 mg/dL	2 (7.14%)	4 (15.38%)	0.34
MCV < 75 fl + CRP>5 mg/dL	1 (3.57%)	1 (3.85%)	0.96
PCDAI ≥10 + MCV <75 fl + CRP>5	1 (3.57%)	9 (34.62%)	0.003
mg/dL			

BMI-body mass index, pc – percentile, ESR-erythrocyte sedimentation rate, CRP-C-reactive protein, WBC-white blood count, PLT-platelet count, Hb-hemoglobin, MCV-mean corpuscular volume, fCal-fecal calprotectin, PCDAI-Pediatric Crohn's Disease Activity Index, SES-CD-simple endoscopic score for Crohn's disease, L1-distal 1/3 ileum, L2-colonic, L3-ileocolonic, B1-non-stricturing, non-penetrating, B2-stricturing, G0-no evidence of growth delay, G1-growth delay, MRI-magnetic resonance imaging , EEN- exclusive enteral nutrition, pc-percentile, T0-at diagnosis, T1-8-12 weeks after diagnosis Table II. Analysis of the diagnostic performance of selected parameters (significantly differentiating groups I and II) as predictors of anti-TNF α treatment within 2 years of CD diagnosis.

Parameter	AUC	SE	95% C.I.(AUC)	р	Cut-off	Sensitivity	Specificity
			CD at T0				
ESR (mm/h)	0.670	0.075	(0.523-0.818)	0.02	54	52.2%	83.9%
CRP (mg/dL)	0.639	0.076	(0.490-0.788)	0.07	138	100.0%	0.0%
WBC (x10 ⁹ /L)	0.535	0.08	(0.379-0.691)	0.66	7.12	82.6%	45.2%
Neutrophil count (x10 ⁹ /L)	0.522	0.08	(0.365-0.679)	0.79	3.7	100.0%	28.8%
PLT (x 10 ⁹ /L)	0.676	0.073	(0.533-0.819)	0.02	309	100.0%	32.3%
Hb (g/dL)	0.773	0.064	(0.648-0.899)	<0.001	12.1	87.0%	64.5%
MCV (fL)	0.763	0.071	(0.625-0.901)	<0.001	75.6	82.6%	71.0%
Albumin (g/dL)	0.640	0.077	(0.489-0.790)	0.07	3.7	47.8%	83.9%
PLT/alb ratio	0.684	0.072	(0.542-0.825)	0.01	72.54	100.0%	35.5%
fCal (ug/g)	0.492	0.081	(0.333-0.650)	0.97	1989	65.2%	48.4%
PCDAI	0.706	0.072	(0.565-0.847)	0.004	30	65.2%	66.7%
SES-CD	0.714	0.071	(0.575-0.853)	0.003	11	73.9%	67.7%
		1	CD at T1				
ESR (mm/h)	0.794	0.062	(0.673-0.914)	<0.001	17	82.6%	67.7%
CRP (mg/dL)	0.754	0.069	(0.619-0.889)	<0.001	5.08	65.2%	80.06%
WBC (x10 ⁹ /L)	0.634	0.076	(0.485-0.783)	0.08	5.26	95.7%	35.5%
Neutrophil count (x10 ⁹ /L)	0.661	0.075	(0.514-0.807)	0.04	5.23	52.2%	77.4%
PLT (x10 ⁹ /L)	0.616	0.077	(0.466-0.767)	0.13	286	82.6%	41.9%
Hb (g/dL)	0.717	0.073	(0.573-0.861)	0.003	12.8	87.0%	51.6%
MCV (fL)	0.725	0.074	(0.581-0.869)	0.001	75.4	78.3%	74.2%
Albumin (g/dL)	0.775	0.070	(0.637-0.913)	<0.001	4.23	73.9%	90.3%
PLT/alb ratio	0.698	0.072	(0.557-0.838)	0.006	68.89	78.3%	56.7%
fCal (ug/g)	0.663	0.082	(0.503-0.823)	0.05	1864	63.2%	70.4%
PCDAI	0.783	0.064	(0.657-0.908)	<0.001	17.5	60.9%	87.1%

AUC-area under the curve, SE-standard error, C.I.-confidence interval, ESR- erythrocyte sedimentation rate, CRP-C-reactive protein, PLT-platelet count, Hb-hemoglobin, MCV-mean corpuscular volume, fCal - fecal calprotectin, PCDAI-Pediatric Crohn's Disease Activity Index, SES-CD-simple endoscopic score for Crohn's disease, T0-at diagnosis, T1-8-12 weeks after diagnosis Table III. Univariate and multivariate analyses yielding independently significant predictors of eventual anti-TNF α use during the first 2 consecutive years of CD.

Variable	Odds ratio (95% C.I.)	p
Univariate	·	·
ESR TO	1.036 (1.005-1.068)	0.02
MCV TO	0.817 (0.723-0.923)	0.001
Hb TO	0.498 (0.316-0.786)	0.003
Albumin T0	0.252 (0.070-0.917)	0.04
PLT/albumin ratio T0	1.015 (1.001-1.030)	0.04
PCDAI TO	1.053 (1.010-1.097)	0.01
SES-CD TO	1.093 (1.007-1.188)	0.03
CRP T1	1.133 (1.019-1.260)	0.02
ESR T1	1.076 (1.029-1.125)	0.001
Hb T1	0.528 (0.319-0.875)	0.01
MCV T1	0.854 (0.751-0.970)	0.02
Albumin T1	0.052 (0.009-0.318)	0.001
PLT/albumin ratio T1	1.020 (1.001-1.039)	0.04
PCDAI T1	1.122 (1.044-1.206)	0.002
Multivariate		
PCDAI T1	1.122 (1.034-1.217)	0.006
MCV T1	0.803 (0.671-0.960)	0.02

C.I.-confidence interval, ESR-erythrocyte sedimentation rate, CRP-C-reactive protein, PLT-platelet count, Hb-

hemoglobin, MCV-mean corpuscular volume, PCDAI-Pediatric Crohn's Disease Activity Index, T0-at diagnosis,

T1-8-12 weeks after diagnosis (T1)

Table IV. Comparison of demographic parameters and selected clinical parameters at the time of diagnosis of UC between the group not requiring biological treatment (Group I) and the group requiring such treatment (Group II) during two years of follow-up.

	Group I (n=56)	Group II (n=26)	p			
то						
Age (years) (median, range)	14 (4-16)	13 (8-16)	0.80			
Male (n, %)	26 (46.4%)	10 (38.5%)	0.50			
BMI (pc) (median, range)	39 (1-98)	66 (1-98)	0.08			
PUCAI (median, range)	45 (10-75)	40 (10-75)	0.34			
Remission (n, %)	0 (0.0%)	(0.0%)	NA			
Mild (n, %)	23 (41.07%)	6 (23.08%)	0.11			
Moderate (n, %)	23 (41.07%)	14 (53.85%)	0.28			
Severe (n, %)	10 (17.86%)	6 (23.08%)	0.58			
ESR (mm/h) (median, range)	16 (2-87)	20 (2-113)	0.04			
ESR≥ 20 mm/h (n, %)	20 (35.71%)	13 (50.0%)	0.22			
CRP (mg/dL) (median, range)	1.1 (0.1-94.9)	3.5 (0.3-18.9)	0.25			
CRP >5 mg/dL (n, %)	11 (19.64%)	12 (46.15%)	0.01			
WBC x 10 ⁹ /L (median, range)	7.47 (3.71-14.53)	7.77 (4.82-11.22)	0.69			
WBC >10x10 ⁹ /L (n, %)	11 (19.64%)	6 (23.08%)	0.72			
Neutrophil (x 10 ⁹ /L) (median,	2.34 (0.62-5.76)	2.33 (1.26-4.73)	0.17			
range)						
Neutrophil > 7x10 ⁹ /L (n, %)	5 (8.93%)	1 (3.85%)	0.41			
PLT (x 10 ⁹ /L) (median, range)	334 (148-609)	374 (197-827)	0.30			
PLT >450x10 ⁹ /L (n, %)	8 (14.29%)	8 (30.77%)	0.03			
Hb (g/dL) (median, range)	12.6 (5.8-14.9)	11.3 (6.4-16.5)	0.08			
Hb <12 g/dL (n, %)	18 (32.14%)	16 (61.54%)	0.01			
MCV (fL) (median, range)	80.8 (61.9-90.1)	80.9 (63.0-91.9)	0.57			
MCV <75 fl (n, %)	5 (8.93%)	5 (19.23%)	0.20			
Ferritin (µg/L) (median, range)	22.3 (2.0-184.5)	19.7 (2.1-122.4)	0.33			
Ferritin <30 mg/dL (n, %)	31 (55.36%)	18 (69.23%)	0.23			
Albumin (g/dL) (median, range)	4.2 (3.2-5.2)	4.3 (3.0-5.0)	0.10			
Albumin <3.5 g/dL (n, %)	3 (5.36%)	1 (3.85%)	0.77			
PLT/Alb ratio (median, range)	74.88 (30.27-159.77)	86.12 (43.78-187.95)	0.17			
fCal (ug/g) median, range	2405 (100-3975)	2147 (136-3635)	0.33			
fCal >200 ug/g (n, %)	55 (98.21%)	25 (96.15%)	0.57			
Mayo endoscopic score I/II/III (n,	10 (17.8%)/ 26 (46.4%)/ 20	5 (19.2%)/ 8(30.8%)/ 13	0.43			
%)	(35.7%)	(50.0%)				
Mayo total score (median, range)	8 (4-11)	8 (4-11)	0.89			

Extent- E1/E2/E3/E4 (n, %)	17 (30.4%)/ 16 (28.6%)/ 4	4 (15.4%)/ 10 (38.5%)/ 4	
	(7.1%)/ 19 (33.9%)	(15.4%)/ 8 (30.8%)	0.14/0.37/0.24/0.78
Induction treatment			
5-ASA (n, %)	36 (64.29%)	14 (53.8%)	0.37
Steroids (n, %)	20 (35.71%)	12 (46.2%)	0.37
	T1		
BMI (pc) (median, range)	46 (2-99)	60 (3-99)	0.39
PUCAI (median, range)	5 (0-65)	15 (0-80)	<0.001
PUCAI ≥10 points (n, %)	18 (32.14%)	17 (65.38%)	0.005
IBD activity:	1		
Remission (n, %)	38 (67.86%)	9 (34.62%)	0.005
Mild (n, %)	14 (25.0%)	11 (42.31%)	0.11
Moderate (n, %)	4 (7.14%)	2 (7.69%)	0.93
Severe (n, %)	0 (0.0%)	4 (15.38%)	NA
ESR (mm/h) (median, range)	7 (2-66)	13 (2-107)	0.03
ESR ≥20 mm/h (n, %)	11 (19.64%)	10 (38.5%)	0.07
CRP (mg/dL) (median, range)	0.6 (0.3-26.2)	0.6 (0.3-125.6)	0.80
CRP >5 mg/dL (n, %)	4 (7.14%)	5 (19.23%)	0.05
WBC (x 10 ⁹ /L) (median, range)	6.25 (3.44-12.27)	8.88 (3.75-24.09)	0.009
WBC >10x10 ⁹ /L (n, %)	5 (8.93%)	8 (30.77%)	0.02
Neutrophil (x 10 ⁹ /L) (median,	1.57 (0.52-7.83)	4.35 (1.44-13.7)	0.009
range)			
Neutrophil >7x10 ⁹ /L (n, %)	1 (1.79%)	2 (7.69%)	0.18
PLT (x 10 ⁹ /L) (median, range)	280 (161-447)	349 (178-794)	0.04
PLT >450x10 ⁹ /L (n, %)	0 (0.0%)	8 (30.77%)	NA
Hb (g/dL) (median, range)	13.1 (9.9-15.7)	11.1 (8.6-17.5)	<0.001
Hb <12 g/dL (n, %)	10 (17.86%)	16 (61.54%)	<0.001
MCV (fL) (median, range)	81.3 (66.4-92.3)	79.5 (69.0-91.2)	0.10
MCV <75 fl (n, %)	4 (7.14%)	6 (23.08%)	0.04
Albumin (g/dL) (median, range)	4.64 (3.91-5.12)	4.3 (3.4-4.8)	<0.001
Albumin <3.5 g/dL (n, %)	0 (0.0%)	1 (3.85%)	NA
PLT/albumin ratio (median,	61.97 (35.08-102.88)	80.64 (38.44-188.15)	0.04
range)			
fCal (ug/g) (median, range)	219 (10-3697)	1143 (18-3620)	0.02
fCal >200 ug/g (n, %)	32 (57.14%)	22 (84.62%)	0.02
PUCAI >10 + PLT/albumin ratio	1 (1.79%)	1 (3.85%)	0.57
≥83.22			
PUCAI >10 + Hb<12g/dL	0 (0.0%)	1 (3.85%)	NA
PLT/albumin ratio ≥83.22 +	2 (3.57%)	3 (11.54%)	0.16
Hb<12g/dl			

PUCAI >10 + PLT/albumin ratio	3 (5.36%)	8 (30.77%)	0.002
≥83.22 + Hb<12g/dL			

BMI-body mass index, ESR-erythrocyte sedimentation rate, CRP-C-reactive protein, WBC-white blood count, PLTplatelet count, Hb-hemoglobin, MCV-mean corpuscular volume, fCal-fecal calprotectin, PUCAI-Pediatric Ulcerative Colitis Activity Index, 5-ASA-5-aminosalicylic acid, pc-percentile, T0-at diagnosis, T1-8-12 weeks after diagnosis

Table V. Analysis of the diagnostic performance of selected parameters (significantly differentiating groups I and II) as predictors of anti-TNF α treatment within 2 years of UC diagnosis.

Parameter	AUC	SE	95% C.I.(AUC)	р	Cut-off	Sensitivity	Specificity
			UC at TO				
ESR (mm/h)	0.640	0.064	(0.541-0.766)	0.03	8	96.2%	30.4%
CRP (mg/dL)	0.580	0.071	(0.441-0.720)	0.26	3	69.6%	30.4%
WBC (x 10 ⁹ /L)	0.527	0.069	(0.391-0.664)	0.69	9.01	34.6\$%	75.0%
Neutrophil (x 10 ⁹ /L)	0.595	0.068	(0.462-0.728)	0.16	6.1	38.5%	83.9%
PLT (x 10 ⁹ /L)	0.572	0.071	(0.432-0.712)	0.31	339	61.5%	55.4%
Hb (g/dL)	0.621	0.069	(0.486-0.756)	0.08	12.0	65.4%	64.3%
MCV (fL)	0.539	0.072	(0.399-0.680)	0.59	75.9	26.9%	89.3%
Albumin (g/dL)	0.613	0.065	(0.486-0.740)	0.08	4.32	57.7%	66.1%
PLT/albumin ratio	0.595	0.070	(0.459-0.731)	0.17	77.17	69.2%	51.8%
fCal (ug/g)	0.432	0.069	(0.296-0.568)	0.33	136	100.0%	1.8%
PUCAI	0.566	0.068	(0.433-0.699)	0.33	35	76.9%	37.5%
	•	•	UC at T1				I
ESR (mm/h)	0.648	0.067	(0.516-0.780)	0.03	8	73.1%	53.6%
CRP (mg/dL)	0.482	0.078	(0.330-0.634)	0.82	11.21	19.2%	98.2%
WBC (x 10 ⁹ /L)	0.681	0.070	(0.545-0.818)	0.009	8.07	61.5%	78.6%
Neutrophil (x 10 ⁹ /L)	0.681	0.069	(0.545-0.816)	0.009	5.7	42.3%	92.9%
PLT (x 10 ⁹ /L)	0.643	0.073	(0.499-0.786)	0.05	459	30.8%	100.0%
Hb (g/dL)	0.761	0.063	(0.638-0.884)	<0.001	11.3	57.7%	89.3%
MCV (fL)	0.613	0.068	(0.480-0.746)	0.10	76.8	38.5%	83.9%
Albumin (g/dL)	0.769	0.053	(0.666-0.872)	<0.01	4.58	88.5%	60.7%
PLT/albumin ratio	0.692	0.069	(0.556-0.827)	0.006	83.22	50.0%	85.7%
fCal (ug/g)	0.667	0.064	(0.541-0.793)	0.009	540	65.4%	66.1%
PUCAI	0.739	0.061	(0.619-0.860)	<0.001	15	57.7%	83.9%

AUC-area under the curve, SE-standard error, C.I.-confidence interval, ESR-erythrocyte sedimentation rate, WBCwhite blood count, PLT-platelet count, Hb-hemoglobin, fCal-fecal calprotectin, PUCAI-Pediatric Ulcerative Colitis Activity Index , T0-at diagnosis, T1-8-12 weeks after diagnosis Table VI. Univariate and multivariate analyses yielding independently significant predictors of eventual anti-TNF use during the first 2 consecutive years of UC.

Variable	Odds ratio (95% C.I.)	p				
Univariate						
Hb T1	0.543 (0.386-0.764)	0.004				
Albumin T1	0.041 (0.007-0.234)	<0.001				
Neutrophil count T1	1.505 (1.162-1.950)	0.002				
WBC T1	1.354 (1.119-1.639)	0.002				
PLT T1	1.008 (1.002-1.013)	0.007				
ESR T1	1.047 (1.006-1.090)	0.02				
PUCAI T1	1.041 (1.014-1.069)	0.003				
Multivariate						
Albumin T1	0.117 (0.016-0.862)	0.04				
Neutrophil count T1	1.38 (1.017-1.873)	0.04				

C.I.-confidence interval, Hb-hemoglobin, WBC-white blood count, PLT-platelet count, ESR-erythrocyte sedimentation rate, PUCAI-Pediatric Ulcerative Colitis Activity Index, T0-at diagnosis, T1-8-12 weeks after diagnosis



Figure 1. Biochemical and clinical parameters (median, min-max) assessed at T0 (white box) and T1 (green box), which showed significant diagnostic utility in differentiating CD patients in Group I from Group II.



Figure 2. Biochemical and clinical parameters (median, min-max) assessed at (T0) and (T1), which showed significant diagnostic utility in differentiating UC patients in Group I from Group II.



Figure 1 suppl. Flow chart of the inclusion of IBD patients.