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# TGF- $\beta$ 1 and granulocyte elastase in the evaluation of activity of inflammatory bowel disease. A pilot study\*

## TGF- $\beta$ 1 i elastaza granulocytów w ocenie aktywności nieswoistych chorób zapalnych jelit. Badanie pilotażowe

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### Summary

#### Introduction:

The aim was to assess the usefulness of TGF- $\beta$ 1 and elastase in the evaluation of activity of ulcerative colitis (UC) and Crohn's disease (CD).

#### Material and Methods:

32 patients diagnosed with UC, 31 with CD and 30 healthy volunteers were enrolled in this study. Diagnosis of the disease was confirmed by videocolonoscopy and histopathological evaluation of intestinal biopsies. Disease activity was assessed by use of the Mayo Scoring System for Assessment of Ulcerative Colitis Activity in UC patients and by CDAI in CD patients. hsCRP was determined by the immunonephelometric method, TGF- $\beta$ 1 and elastase plasma concentration by ELISA. The results of the study were analyzed using Statistica and R statistical language.

#### Results:

In UC a positive correlation between disease activity and platelet level, hsCRP and TGF- $\beta$ 1 concentration was noted. Elastase concentration in UC patients was significantly higher than in CD, but there was no correlation with the activity of the disease. In CD patients we observed a positive correlation between disease activity and leukocytes, platelet levels and elastase concentration, and a very low correlation with hsCRP and TGF- $\beta$ 1.

#### Discussion:

Determination of TGF- $\beta$ 1 can be used for evaluation of inflammatory activity in UC and it is connected with elevated concentrations of CRP and platelets. To a lower extent TGF- $\beta$ 1 can also be used for evaluation of inflammatory activity in CD. Examination of elastase concentration may be useful in the assessment of CD activity. Plasma elastase concentration may be helpful in UC and CD differentiation. The preliminary results of this investigation seem promising; nevertheless, more studies are necessary.

#### Keywords:

ulcerative colitis • Crohn's disease • TGF- $\beta$ 1 • elastase

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### Abbreviations:

**CD** – Crohn's disease; **CDAI** – Crohn's Disease Activity Index; **EMT** – epithelial-to-mesenchymal transition; **hsCRP** – high sensitivity C-reactive protein; **IBD** – inflammatory bowel disease; **PLT** – platelets; **TGF- $\beta$ 1** – transforming growth factor-beta1; **TNF- $\alpha$**  – tumor necrosis factor-alpha; **UC** – ulcerative colitis; **UJ** – Jagiellonian University; **WBC** – levels of platelets and white blood cells.

## INTRODUCTION

Inflammatory bowel disease (IBD) comprises two types of chronic intestinal inflammation – ulcerative colitis (UC) and Crohn's disease (CD). The etiology is unknown; however, dysfunction of the immunological system and inappropriate production of mucosal cytokines play the major roles [7]. As the prevalence of IBD increases, it is important to find a biochemical marker that can be used in early detection of disease exacerbation, treatment monitoring and in the differential diagnosis.

Inappropriate production of mucosal cytokines and impaired balance between pro- and anti-inflammatory cytokines play a crucial role in mucosal injury in IBD. Proinflammatory cytokines, such as interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF- $\alpha$ ), are produced and released by macrophages and monocytes in inflamed mucosa. Transforming growth factor-beta1 (TGF- $\beta$ 1), interleukin-10 (IL-10) and interleukin-11 (IL-11) are the most important anti-inflammatory cytokines [3,10,11]. Transforming growth factor-beta (TGF- $\beta$ ) plays a great role as an inducer of fibrosis and myofibroblast generation and in a biological process called epithelial-to-mesenchymal transition (EMT) in colonic diseases [12]. EMT is a well-established biological phenomenon important in normal tissues and organ development and in the pathogenesis of diseases (such as chronic inflammation-related fibrosis, colorectal carcinogenesis, cancer invasion and in mucosal healing) [12]. The inhibition of EMT seems to minimize chronic inflammation-related wall fibrosis in colon [12]. In IBD, TGF- $\beta$ 1 produced and secreted from the cells in the lamina propria and the epithelium of the colon controls proliferation and takes part in healing and fibrosis [8].

Granulocyte elastase (elastase) is a neutral proteinase released from granulocytes and plays an important role in intestine injury and inflammation. Infiltration of bowel mucosa by neutrophils and eosinophilic granulocytes is a characteristic feature of chronic inflammatory bowel disease [4].

As far as we know, there are no precise data about TGF- $\beta$ 1 and elastase in patients with IBD. Moreover, some conclusions of these studies seem to be contradictory.

We performed a pilot study aiming to assess the usefulness of plasma TGF- $\beta$ 1 and elastase in the evaluation of UC and CD activity. The study was approved by the local ethical committee at the UJ.

## MATERIAL AND METHODS

We assessed plasma concentration of TGF- $\beta$ 1, granulocyte elastase, hsCRP (high sensitivity CRP), and levels of platelets (PLT) and white blood cells (WBC) in patients suffering from UC or CD and in healthy volunteers.

Thirty-two patients diagnosed with UC, 31 with CD and a group of 30 healthy volunteers matched for age and gender were enrolled in this study. Diagnosis of IBD was confirmed by videocolonoscopy and histopathological evaluation of intestinal biopsies. Patients with IBD were hospitalized in the Department of Gastroenterology, Hepatology and Infectious Diseases of the University Hospital in Cracow.

The activity of UC was assessed according to the Mayo Scoring System including stool frequency, rectal bleeding, endoscopic findings and physician's global assessment. In the group of patients with UC, 50% (16) had severe disease (activity score 10-12 points), 40.6% (13) moderate-severe disease (activity score 6-9 points) and 9.4% (3) mild disease (activity score 2-5 points).

The activity of CD was measured using the Crohn's Disease Activity Index (CDAI), which includes the number of liquid or very soft stools in one week, the sum of seven daily abdominal pain ratings, general well-being, symptoms or findings presumed related to Crohn's disease, taking loperamide or opiates for diarrhea, abnormal mass, hematocrit and weight. In the group of patients with CD, 59.4% (19) had moderate disease (activity score 220-450 points), 21.8% (7) severe disease (activity score >450 points) and 15.6% (5) mild disease (activity score 150-220 points).

All laboratory tests from peripheral blood samples were performed in the Department of Clinical Biochemistry, Jagiellonian University Medical College. Leukocytes, platelet levels, hsCRP, TGF- $\beta$ 1 and elastase concentrations were determined in all groups. hsCRP was determined by the immunonephelometric method (Behring Nephelometer 100 Analyser and reagent N High Sensitivity CRP from DADE Behring firm, Marburg, Germany).

TGF- $\beta$ 1 was measured by the immunoenzymatic ELISA method using specific ELISA kits (DIACLONE, France). Elastase was determined by the immunoenzymatic ELISA method using specific ELISA kits (Human PMN Elastase, BioVendor, Czech Republic).

Statistical analysis was performed using R statistical language (a free software environment for statistical computing and graphics) and Statistica. Variance analysis, Student's t-test, Pearson's r correlation, Wilcoxon, and Spearman's rho correlation were used.

## RESULTS

The levels of WBC, PLT and plasma concentrations of hsCRP, TGF- $\beta$ 1 and elastase were statistically higher in

patients with active UC than in the healthy controls ( $p<0.001$ ).

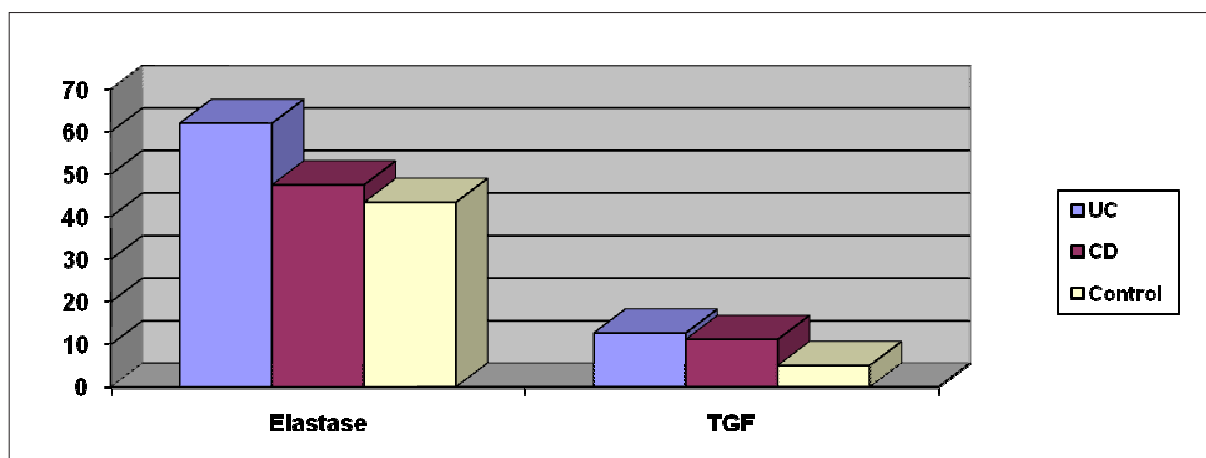
The levels of WBC and PLT, and plasma concentrations of hsCRP and TGF- $\beta$ 1 were statistically higher in patients with active CD than in the healthy controls ( $p<0.001$ ). There was no significant difference in elastase concentration between CD patients and healthy controls ( $p=0.18$ ).

There was no significant difference in the levels of WBC, PLT, hsCRP, and TGF- $\beta$ 1 between the UC and CD patients. However, there was a significant difference in the mean elastase concentration between the patients with UC and CD ( $p=0.011$ , UC:  $61.97\pm27.46$  ng/ml, CD:  $47.56\pm14.00$  ng/ml).

**Table 1.** The description of the subgroups and the comparison of the subgroups in terms of the parameters.

Parameter [reference values]	Patients with UC	Patients with CD	Control group	p UC/C	p CD/C	p UC/CD
Number of people	N=32	N=31	N=30	-	-	-
Average age [years]	32.7 (min.18, max.55)	32.5(min.21, max.50)	33.6 (min.23, max 50)	-	-	-
Female	N=16 (50%)	N=13 (42%)	N=13 (43%)	-	-	-
Smokers	N=11 (34%)	N=4 (13%)	N=17 (56%)	-	-	-
WBC [ $4\text{-}10\times 10^3/\text{mm}^3$ ]	$8.9\pm3.26$	$7.74\pm2.71$	$6.37\pm1.40$	$P=0.001$	$P=0.049$	SI
Platelets [ $125\text{-}340\times 10^3/\text{mm}^3$ ]	$382.78\pm73.32$	$349\pm109.14$	$230.50\pm44.62$	$P<0.001$	$P<0.001$	SI
hsCRP [ $<5\text{mg/l}$ ]	$41.53\pm33.30$	$40.84\pm24.81$	$2.88\pm1.29$	$P<0.001$	$P<0.001$	SI
TGF $\beta$ 1 [ $2.1\text{-}6.1$ ng/ml]	$12.69\pm6.01$	$11.25\pm5.94$	$5.12\pm2.77$	$P<0.001$	$P<0.001$	SI
Elastase [ $24.7\text{-}90.4$ ng/ml]	$61.97\pm27.46$	$47.56\pm14.00$	$43.36\pm10.13$	$P<0.001$	$P=0.18$	$P=0.011$

\* $p<0.05$ ; SI: statistically insignificant. CD: Crohn's disease. UC: ulcerative colitis. C: group of healthy control. WBC: white blood cells. CRP: high sensitivity C-reactive protein. Elastase: granulocyte elastase. TGF  $\beta$ 1: transforming growth factor-beta 1.



**Fig. 1.** Mean levels of granulocyte elastase (elastase) and transforming growth factor-beta1 (TGF, TGF- $\beta$ 1) in patients with UC (ulcerative colitis), CD (Crohn's disease) and healthy controls (Control)

We also analyzed the correlation between the measured parameters in the patients with IBD and the control group:

- in UC, a low positive correlation between increased concentration of TGF-β1 and elevated concentration of hsCRP (the correlation coefficient was 0.57;  $p<0.05$ ) and platelets (the correlation coefficient was 0.42;  $p<0.05$ ) was found.
- in CD a low positive correlation between increased concentration of TGF-β1 and elevated level of platelets was observed (the correlation coefficient was 0.46;  $p<0.05$ ). Additionally, a low positive correlation between increased concentration of elastase and increased level of platelets (0.39;  $p<0.05$ ) and hsCRP (0.36;  $p<0.05$ ) was noted.

No statistical correlation between increased concentration of TGF-β1 and elastase was found in all groups.

We also analyzed the correlation between the measured parameters and the activity of the disease in the patients with IBD:

- in UC a low positive correlation between increased concentration of TGF-β1 (correlation coefficient was 0.86;  $p<0.001$ ), CRP (0.75;  $p<0.001$ ), number of platelets (0.53;  $p=0.002$ ) and severity of the disease was found. Although elastase concentration in UC patients was

significantly higher than in CD patients, there was no correlation with the activity of the disease.

- in CD a positive correlation between increased concentration of elastase (correlation coefficient was 0.6;  $p<0.001$ ), platelets (0.75;  $p<0.001$ ) and severity of the disease was noted. A lower one was found between the concentration of TGF-β1 (0.43;  $p=0.016$ ), hsCRP (0.37;  $p=0.039$ ) and WBC (0.36;  $p=0.05$ ) and disease activity.

## DISCUSSION

The present study carries three messages that we believe to be important. Firstly, TGF-β1 might differentiate active from inactive UC, which is consistent with a few previous studies. Secondly, elastase level may be useful in the evaluation of CD activity. Finally, serum elastase level may be helpful in UC and CD differentiation. There are only a few publications concerning TGF-β1 or elastase in patients with IBD. The majority of studies have assessed cytokines in colonic mucosa or stool. However, we found no study involving plasma TGF-β1 and elastase together in such a group of patients. Some of the results are incoherent.

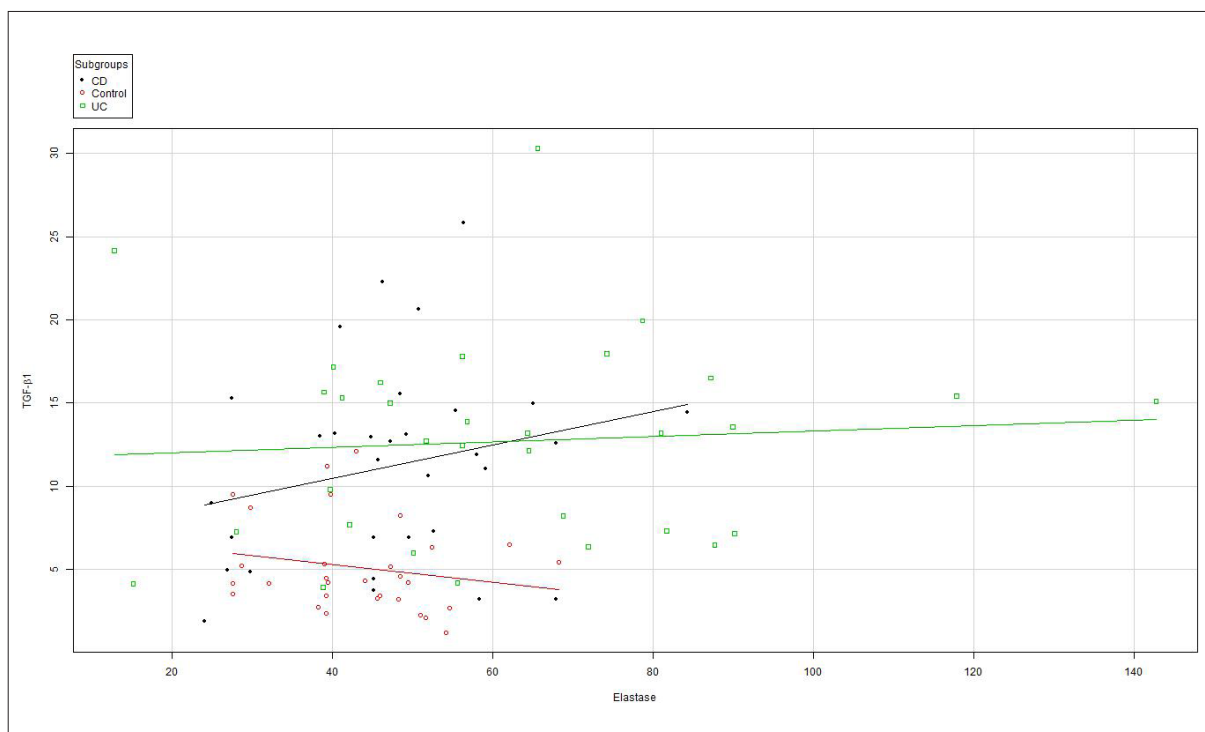
## TGF-β1

Our study has shown that TGFβ1 might be considered as a sensitive marker of UC activity. Indeed, Kiliç et al.

**Table 2.** Rho-Spearman correlation coefficients between variables in patients with UC and r-Pearson correlation coefficients between variables in CD and healthy controls.

UC	WBC	PLATELETS	hsCRP	ELASTASE	TGF-β1
WBC	-	0.14	0.02	-0.03	0.05
PLATELETS	0.14	-	0.49*	0.15	0.42*
hsCRP	0.02	0.49*	-	0.04	0.57*
ELASTASE	-0.03	0.15	0.04	-	0.10
TGF-β1	0.05	0.42*	0.57*	0.10	-
CD	WBC	PLATELETS	hsCRP	ELASTASE	TGF-β1
WBC	-	0.55*	0.25	0.17	0.34
PLATELETS	0.55*	-	0.33	0.39*	0.46*
hsCRP	0.25	0.33	-	0.36*	0.23
ELASTASE	0.17	0.39*	0.36*	-	0.24
TGF-β1	0.34	0.46*	0.23	0.24	-
CONTROL	WBC	PLATELETS	hsCRP	ELASTASE	TGF-β1
WBC	-	0.08	-0.20	0.08	-0.20
PLATELETS	0.08	-	0.01	0.15	0.41*
hsCRP	-0.20	0.01	-	0.21	-0.01
ELASTASE	0.08	0.15	0.21	-	-0.16
TGF-β1	-0.20	0.41*	-0.01	-0.16	-

\* $p < 0.05$ . CD: Crohn's disease. UC: ulcerative colitis. C: group of healthy control. WBC: white blood cells. CRP: high sensitivity C-reactive protein. Elastase: granulocyte elastase. TGF β1: transforming growth factor-beta 1.



**Fig. 2.** Scatter plot with regression lines. CD: Crohn's disease. UC: ulcerative colitis. Control: the healthy controls. Elastase: granulocyte elastase. TGF- $\beta$ 1: transforming growth factor-beta1 (TGF)

noted that in UC, the mean level of TGF- $\beta$ 1 in active disease was higher than in remission and can be used as a marker for differential diagnosis of these stages [8]. In their study they enrolled 70 patients with IBD (UC or CD) and 20 controls [8].

In a few studies TGF- $\beta$  was measured in bowel tissue by an immunohistochemical method. Kanazawa et al. studied the expression of TGF- $\beta$  (1,2,3) in paraffin-embedded samples from bowel tissue and the concentration in blood: basic fibroblast growth factor (b-FGF), endothelin-1 (ET-1) and vascular endothelial growth factor (VEGF) [7]. They examined 11 patients with UC, 11 with CD and 10 healthy controls [7]. Expression of TGF- $\beta$ 1 in the endothelial cells was not found in either the UC or the CD group [7]. They noted moderate or weak expression of transforming growth factor-beta2 (TGF- $\beta$ 2) and transforming growth factor-beta3 (TGF- $\beta$ 3) in the inflammatory cells in 5 (of 7) cases of active UC and in 4 (of 6) cases of active CD [7]. In contrast, in our study an increased concentration of TGF- $\beta$ 1 in blood was found, both in UC and CD patients.

In the earlier study also Babyatsky et al. assessed TGF- $\beta$  (and TGF- $\alpha$ ) in colonic mucosa from patients with UC, CD and healthy controls [2]. TGF- $\beta$  levels were increased in affected mucosa [2]. Scientists concluded that TGF- $\beta$  might be the key cytokine during periods of active inflammation [2]. However, there was no distinction between the types of TGF- $\beta$ .

Some studies were conducted in pediatric patients and the conclusions seem to be surprising. Kader et al. measured TGF- $\beta$ 1 level in serum of 65 children suffering from CD and 23 from UC [6]. They noted that TGF- $\beta$ 1 was significantly higher in patients with CD in remission than in active disease [6]. There was no significant difference in UC patients. In our study TGF- $\beta$ 1 level in serum was higher in both UC and CD patients. Furthermore, it correlated with IBD activity.

In another pediatric study, Wedrychowicz et al. assessed the influence of exclusive enteral nutrition on serum concentration of TGF- $\beta$ 1 and vascular endothelial growth factor (VEGF) in 39 children and adolescents with IBD (24 with CD and 15 with UC) [13]. At the baseline they found increased serum TGF- $\beta$ 1 in UC patients versus the CD group and controls [13].

### Elastase

Initial studies demonstrated that elastase level in serum may potentially be a marker for IBD. Fishbach et al. investigated plasma elastase in 44 patients with CD and UC; it was significantly higher in these patients in comparison to 7 patients with non-inflammatory bowel diseases or 53 healthy controls [4]. Moreover, elevated plasma levels were more often observed in patients with active inflammation than in those with inactive disease. However, it did not correlate with WBC level, nor with clinical indices, and not always with IBD activity [4]. Scientists concluded that elastase did not reliably indicate IBD activity.

**Table 3.** Rho-Spearman correlation coefficients between measured parameters and the degree of disease's activity in patients with UC and CD.

Parameter [reference values]	The degree of UC activity	
	p	Rho-Spearman
WBC	0.833	0.04
PLATELETS	0.002	0.53*
hsCRP	< 0.001	0.75*
ELASTASE	0.358	0.17
TGF $\beta$ 1	< 0.001	0.86*
Parameter [reference values]	The degree of CD activity	
	p	Rho-Spearman
WBC	0.050	0.36*
PLATELETS	< 0.001	0.75*
hsCRP	0.039	0.37*
ELASTASE	< 0.001	0.60*
TGF $\beta$ 1	0.016	0.43*

\*p < 0.05. CD: Crohn's disease. UC: ulcerative colitis. WBC: white blood cells. CRP: high sensitivity C-reactive protein. Elastase: granulocyte elastase. TGF  $\beta$ 1: transforming growth factor-beta1.

In our study the mean elastase concentration was significantly higher in UC, but ultimately correlated with CD activity.

To examine whether plasma elastase represents a reliable laboratory marker for establishing the activity of IBD, Gouni-Berthold et al. measured plasma elastase concentration in 61 patients suffering from CD or UC, and compared them with 40 healthy controls [5]. Plasma elastase concentration was significantly higher in patients with IBD than in healthy controls, and significantly higher in active disease than in remission [5]. They concluded that elastase may be a practical marker of IBD activity, especially for identifying patients in remission [5]. In our study, the conclusions seems less promising – only in CD patients did elastase level correlate with activity of the disease.

Andus et al. also studied plasma elastase concentration, comparing it to fecal elastase concentration in CD and UC patients [1]. According to the results, the plasma elastase level correlated with IBD and its activity; however, the fecal elastase level correlated only with UC activity [1].

Also Langhorst et al. examined fecal levels of polymorphonuclear neutrophil (PMN)-elastase, lactoferrin, calprotectin and serum CRP in 139 patients (54 with IBS, 42 with UC and 43 with CD) [9]. All patients with IBD underwent videocolonoscopy, determining disease activity with CAI or CDAI respectively [9]. Scientists found significantly higher levels of all parameters in CD and UC patients with active inflammation compared with patients with inactive inflammation or IBS. They concluded that elastase enables active IBD to be differentiated from inactive IBD or IBS, and that it is superior to CRP in its diagnostic accuracy [9].

In our study there was no significant difference in elastase between CD patients and healthy controls. However, elastase appeared to be more useful in determining CD severity.

The different results of TGF- $\beta$ 1 and elastase in CD and UC in our study may be connected with different microscopic extent of inflammation in the two diseases. Inflammation in CD evolves from superficial into transmural, resulting in deep fissuring ulcers penetrating through the muscle layer, forming fistulas and ulcers, whereas in UC, it is limited to mucosa and submucosa. The pathophysiology of this phenomenon is still unknown and may be connected with an imbalance between proteases, such as elastase, and their inhibitors.

## CONCLUSIONS

From the present study we concluded that:

- TGF- $\beta$ 1 as well as CRP, platelets and WBC can be useful in the early diagnosis of IBD exacerbation. TGF- $\beta$ 1 can be used for evaluation of inflammation activity in UC and it is connected with the elevated concentration of CRP and platelets. To a lower extent, TGF- $\beta$ 1 can also be used for evaluation of inflammatory activity in CD.
- Examination of elastase concentration as well as platelets may be useful in the assessment of CD activity.
- Plasma elastase concentration may be helpful in UC and CD differentiation.
- The preliminary results of this investigation seem promising; nevertheless, more studies are necessary to establish new diagnostic strategies that can be efficiently used in clinical practice in the near future.



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The authors have no potential conflicts of interest to declare.