Plasma viscosity: a potential predictor of both medical treatment response and clinical stage of ulcerative colitis

Ann. Ital. Chir., 2016 87: 531-543
pii: S0003469X1602618X

Yakar Tolga*, Cosar Arif Mansur**, Gokturk Hüseyin Savas***, Unler Kanat Ayse Gulhan***, Parlakgumus Alper°, Kozanoglu Ilknur°°, Serin Ender*

**Başkent University, Faculty of Medicine, Departments of Internal Medicine and Gastroenterology, Adana, Turkey*
***Karadeniz Technical University, School of Medicine, Departments of Internal Medicine and Gastroenterology, Trabzon, Turkey*
****Başkent University, Faculty of Medicine, Departments of Internal Medicine and Gastroenterology, Konya, Turkey*
°Başkent University, Faculty of Medicine, Department of General Surgery, Adana, Turkey
°°Department of Physiology, Faculty of Medicine, Baskent University, Ankara, Turkey

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AIM: *Ulcerative colitis (UC) is one of the major forms of chronic relapsing inflammatory bowel diseases. The ability to identify type, severity and responsiveness to therapy of UC using laboratory parameters has long been the aim of clinical studies. The aim of this study was to assess the relation betweenplasma viscosity (PV) and disease activity and response to medical treatment in patients with UC.*
MATERIAL AND METHODS: *The study included 105 patients with UC and 42 healthy volunteers. Blood samples were assessed for PV, erythrocyte sedimentation rate (ESR), high sensitive C-reactive protein (hs-CRP), D-dimer, and fibrinogen.*
RESULTS: *Patients with UC were grouped according to disease activity, i.e. active (n= 59) and remission (n= 46). PV was higher in those with active UC compared with those with UC in remission or healthy subjects. It was significantly higher in both UC refractory to steroid compared to UC responsive to steroid (p< 0.001) and UC refractory to cyclosporine compared to UC responsive cyclosporine (p= 0.003). IncreasedSimple Clinical Colitis Activity Index (SCCAI), Endoscopic Grading Scale (EGS), and Histological Disease Activity (HAD) scores were significantly associated with higher PV in patients with UC.*
CONCLUSION: *PV is a useful marker in predicting response to steroid or cyclosporine treatment in patients with active UC. It could be replaced by ESR or hs-CRP as a measure of the acute phase response in UC since it is sufficiently sensitive. These findings may help identify patients with active UC who will require colectomy.*

KEY WORDS: Biomarkers, Disease activity, Medical treatment, Steroid-refractory ulcerative colitis, Ulcerative colitis

Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease of the intestines. The clinical course of UC is characterized by periods of remission punctuated by clinical exacerbations. To date, no factors have been identified as being reliable predictors of relapses in UC. Diagnosis of UC is based on clinical symptoms combined with radiological and endoscopic investigations. Tests sometimes invasive are routinely performed for the diagnosis and care of patients with inflammatory bowel diseases (IBD). It is necessary to employ non-invasive biomarkers. The ability to determine type, severity and prognosis of UC and response to therapy by using biomarkers has long been the aim of clinical researchers [1,2]. Simple laboratory markers of colonoscopic activity in UC are desirable, but none has yet been identified.

Pervenuto in Redazione Settembre 2016. Accettato per la pubblicazione Ottobre 2016.
Correspondence to: Parlakgumus Alper, Başkent University, Faculty of Medicine, Departments of General Surgery, Adana, Turkey (e-mail: aparlakgumus@yahoo.com)

The main biomarkers in UC are acute-phase proteins (APP), which are released from the liver in response to cytokines, and measurement of serum concentrations offers a valuable means of assessing IBD. The laboratory tests most used to measure APP in clinical practice are serum concentrations of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Other biomarkers of inflammation in UC include platelet count, leukocyte count, and serum albumin and serum orosomucoid concentrations [3]. A good biomarker must be accurate, reproducible, standardized, and easy to be interpreted by clinicians and must have a high diagnostic sensitivity and specificity. Unfortunately, no single marker has all these features. Development of biomarkers in UC will be very important in the future. Clinical indices of disease activity, such as the ulcerative colitis activity index (UCAI) and Truelove-Witts criteria reflect the patient's well-being and the quality of life rather than the degree of mucosal inflammation.

Goals of drug treatment for UC are to treat the symptoms and to induce clinical remission. In severe UC, after three days of intensive treatment (hydrocortisone and/or cyclosporine), patients with frequent stools (>8/d or 3-8 stools/d) and CRP >45 mg/L should be identified as most of them will need to undergo colectomy [4]. Ileal-pouch anal anastomosis is an elective surgical treatment in about 20%-30% of UC patients that eventually undergo surgery. Earlier colectomy in patients with UC failing to respond to medical therapy has decreased the mortality rate from 30% to <3% [5,6]. Both steroid and cyclosporine treatments reduce success of colectomy. Moreover, significant side effects have been reported in UC patients treated with both medical treatments [7].

Viscosity is known to be resistance of fluids against flow. Hemorheological characteristics of blood include plasma viscosity (PV), red blood cell deformability and aggregation whole blood viscosity, hematocrit, and plasma fibrinogen levels. PV is a biochemically changeable content and is determined by plasma proteins, especially fibrinogen, immunoglobulins and other blood-borne proteins, lipoproteins. Elevated PV may lead to tissue injury by impairing microcirculatory flow, elevated tendency for thrombosis, and shear stress injury at the blood endothelial [8].

There have been few studies about the use of PV in clinical practice. The first biochemical application of PV measurement was reported in 1942 when its use in pulmonary tuberculosis was described, and a relation between ESR and PV was noted [9]. There is only one previous report of the use of PV in IBD [10]. It was a retrospective evaluation of PV measurement in a large series of patients with UC. PV can be used as a non-specific measure of APP [11,12] and may have several advantages over the more widely used CRP or ESR.

The aim of the present retrospective study was to reveal the role of PV and other parameters in determining the disease activity and response to medical treatment in UC by employing multiple statistical approaches as delaying colectomy means diminished success in the standard practice. Laboratory indices may be particularly helpful here, but no laboratory test has been shown to be without limitations. This is the first study to investigate PV and D-dimer.

Materials and Methods

Patients

The study included 105 patients with UC(55 females and 50 males) aged 18 to 71 years (42±15 years, mean±SD) and followed by Gastroenterology Department of Baskent University, Adana, Turkey. Only patients with a definite or strong suspicion of UCwere screened. The recruited patients were hospitalized or followed up at the outpatient clinic of Gastroenterology Department. Inclusion criteria were age of 18-75 years and a diagnosis of certain or probable UC. The patients were grouped according to disease activity; i.e. active (n= 59) or remission (n= 46). The diagnosis of UC was based on conventional clinical, radiological, endoscopic and histological criteria. Upper endoscopic and ultrasonographic examinations of the patients were obtained. The control group consisted of 42 healthy volunteers (19 females and 23 males) from the hospital staff and their family members aged 42±12 years and having normal liver functions according to standard biochemical tests. The exclusion criteria were reported age of <18 or >75 years, pregnancy and known or probable Crohn's colitis, infectious colitis or ongoing infection. Furthermore, individuals suffering from chronic or acute diseases, such as hypertension, diabetes mellitus, diseases of the liver, kidney, and endocrine and immunological disorders, cardiovascular disease, other known causes of protein-losing enteropathy, abscess formation, intestinal fistulae or obstruction, pseudomembranous colitis or other specific infective colitis, previous intestinal resection, multiple sclerosis, malignancy, alcohol-drinking subjects and patients on drugs including steroid therapy, anti inflammatory, antidepressant, anticonvulsant, antiplatelet, anticoagulation, phenytoin, ketoconazole, antitubercular therapy, thyroid hormone replacement, regular calcium and vitamin D supplementation were excluded from both patient groups and healthy controls.

Clinical, Endoscopic, and Histological Disease Activity

A complete medical history was obtained and physical examination was performed in all the UC patients. Disease activity (active or remission) in the patients with UC was assessed withSimple Clinical Colitis Activity Index (SCCAI), taking account of five clinical criteria: day and night stool frequency, urgency of defecation,

blood in the stool, general well-being, and presence of extracolonic manifestations [13]. An SCCAIof ≤2 points was defined as clinical remission. Active disease was defined as mild (SCCAI of 3-6), moderate (SCCAI of 7-11), or severe (SCCAI of ≥12).

Colonoscopy with biopsy sampling was performed in all the patients with UC to assess endoscopic severity and extent of the disease with Endoscopic Grading Scale (EGS). Endoscopic severity was measured by a modified endoscopic score on an 18-point scale [14] involving 9 parameters: erythema, vascular pattern, friability, granularity, spontaneous bleeding, occurrence and severity of ulcers, extent of ulcerated surface, and presence of mucopurulent exudates. All parameters were scored from 0 to 2 points. Four grades of endoscopic activity were considered according to the sum of all parameters: inactive disease (0-3), mild disease (4-7), moderate disease (8-12), and severe disease (13-18). The extent of the disease was recorded as proctitis, left-sided colitis, and extensive colitis.

Grading of theHistological Disease Activity (HDA)was performed with scores from 0 to 3 for four histological variables [14]: ulceration, erosion, crypt abscess, and cryptitis (HDA from 0 to 12). Four grades of histological activity were considered according to the sum of the histological variables: remission (0-3), mild disease (4-6), moderate disease (7-9), and severe disease [10-12]. The most inflamed site in the colon or rectum was taken into account for the assessment of the endoscopic and histological scores.

Clinical remission was defined as baseline bowel function with absence of blood for at least one year. Endoscopic remission was defined as normal endoscopic features, i.e., grade 0 or 1 using a previously reported grading score. Grade 0 corresponded to normal, vascular pattern clearly visible, grade 1 erythema with loss of vascular pattern, grade 2 erythema with loss of vascular pattern plus contact bleeding, grade 3 erythema with loss of vascular pattern plus spontaneous bleeding and grade 4 erythema with loss of vascular pattern plus obvious ulceration [15]. UC was considered to be in remission when the combination of clinical, endoscopic, and histological grading was suggestive of inactive disease.

Sample Collection and Analysis

All subjects fasted for at least 12 hours. Venous blood from all the study subjects was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes without any additives between 8 and 10 a.m. The samples were sealed to prevent any evaporation and consequent concentration, and transported on ice to the laboratory for analysis. Plasma and serum were separated by low-speed centrifugation (at 4 °C, at 1200g for 15 minutes), which were all performed later the same day. Grossly hemolyzed or lipemic specimens were not used.

Measurement of Plasma Viscosity

PV was determined using standard technology in a programmable rotational viscometer (Brookfield Programmable Rheometer model DV-III plus, Stoughton, MA, USA), which uses a cone-and-plate measuring head requiring 0.5 mL of the sample fluid. The cone is coupled to a motor by a spring, the rotational speed of which can be preset, and determines the shear rate. All measurements were performed at 37 °C, with a shear rate of 450 s^{-1}. The interassay coefficient of variation was 2%. The viscosity measurement results were expressed in mPa-s.

Other Measurements

Serum fasting blood glucose, total protein, albumin, blood urine nitrogen (BUN), creatinine, serum lipids including total cholesterol, triglyceride, aspartate aminotransferase, alanine aminotransferase, and total bilirubin were measured in an Abbott AEROSET autoanalyzer using Abbott kits (Abbott Laboratories, Abbott Park, IL, USA).

Plasma D-Dimer (quantitative) and fibrinogen levels were measured by an immunoturbidimetric method with Diagnostic Stago START analyzer using Diagnostic StagoFibri-Prest 2 kit (Rankin Biomedical Corporation, Holly, MI, USA). Prothrombin time (PT) was measured by Sysmex CA-1500 System (Siemens Healthcare Diagnostics, Marburg, Germany). A complete blood count [hemoglobin concentration, total white blood cell (WBC) count, platelet count, mean platelet volume (MPV)] was measured (Coulter counter; Coulter electronics, Luton). ESR was measured by the Westergren method. High-sensitive CRP (hs-CRP) was detected by rate nephelometry (Behringnephelometer; Hoechst UK, Hounslow). D-Dimer, fibrinogen, PV, CRP, and ESR were measured at baseline, on the third day, and in the third month.

This study was approved by BaskentUniversity institutional review board and ethics committee. Written informed consent was obtained from all participants at inclusion in accordance with the Helsinki Declaration.

Statistical Analysis

All parametric results were expressed inmean±standard deviation (SD). The Statistical Package Program for Social Sciences (SPSS, version 21 for windows; SPSS Inc., Chicago, Illinois, USA) was used to perform all statistical calculations. Normal (Gaussian) distributions of the continuous variables were evaluated by using One-Sample Kolmogorov-Smirnov test. Categorical variables were compared with X^2 test. Comparisons between the groups were made by using Student's t-test, Mann-

Whitney U test, and ANOVA as appropriate. Correlations between the variables were determined with Pearson or Spearman correlation tests as appropriate. Differences were considered statistically significant at $p<0.05$.

A receiver-operating characteristic (ROC) curve demonstrates characteristics of a diagnostic method by plotting the false positive rate (1-specificity) on a horizontal axis and the true positive rate (sensitivity) on a vertical axis for various cut-off values. The area under the ROC curve (AUC) is a popular measure for accuracy of a diagnostic test. A diagnostic test that has a greater AUC is a better predictor of presence of a disease. To determine the accuracy and respective best cut-off values of inflammation and coagulation markers for predicting response to steroid or cyclosporine treatment in patients with active UC, ROC curves, and their corresponding AUC were used. Sensitivity and specificity were calculated for each cut-off value.

Results

There was not a significant difference in gender and age between the UC patients and the controls ($p> 0.05$). The patients with active disease and those at remission did not differ significantly in terms of gender, age, duration of disease, and extent of disease ($p> 0.05$). Baseline demographic, clinical and laboratory data about the patients and the healthy controls are shown in Table I. The active UC patients had significantly higher scores for clinical, endoscopic, and histological indices than the UC patients at remission ($p< 0.001$) (Table I).

Hematological, Biochemical, Inflammatory, Coagulation Parameters and Effects of Treatment on these Parameters

The hematological, and biochemical variables creatinine, AST, ALT, PT, platelet count, and PT were significantly higher in active UC than UC in remission ($p<0.05$) (Table II). However, total protein, albumin, triglyceride, hemoglobin and MPV levels were lower in active UC than in UC in remission ($p< 0.05$).

As inflammatory and coagulation variables, PV, hs-CRP, ESR, fibrinogen, D-Dimer, and WBC on admission were higher in patients with active UC than in those with UC in remission and the control group (Table III). A comparison of the same parameters across different measurement times is shown in Table IV. In the patients with active UC, all inflammatory and coagulation parameters were significantly decreased on the 3rd day of treatment except for WBC. In the 3rd month of treatment, all inflammatory and coagulation parameters were

Table I - *Demographic, and clinical data about ulcerative colitis and controls*

	Ulcerative Colitis (n = 105)				
	Active (n = 59)	Remission (n = 46)	Control (n = 42)	P value (Active vs Remission)	P value (Active vs Control)
Age (Range, year)	41.9±16.5 (18 – 74)	48.3±16.6 (22 – 80)	42.2±12.9 (18 – 71)	NS	NS
Gender (M/F)	24/35	26/20	23/19	NS	NS
Mean duration of disease, months (range)	14 (1 – 92)	23 (3 – 102)		NS	
Extent of disease					
Proctitis	20	21			
Left-sided colitis	20	13		NS	
Extensive colitis	19	12			
SCCAI score	9.6 (5 – 15)	0 (1 – 2)		<0.001	
EGS score	10.9 (4 – 17)	0.8 (0 – 3)		<0.001	
HDA score	8.2 (4 – 12)	0 (0 – 3)		<0.001	
Medications					
5-ASA compounds	59	45			
iv steroids	21	0			
Topical steroids	16	0			
Oral steroids	40	3			
Azathioprine	9	20			
Infliximab	0	3			
Adalimumab	0	1			

All results are expressed in mean±standard deviation
M – Male; F – Female; SCCAI – Simple Clinical Colitis Activity Index; EGS – Endoscopic Grading Scale; HAD – Histologic Disease Activity

TABLE II - *Hematological, and Biochemical Characteristics of Patients with Ulcerative Colitis and Controls*

	Ulcerative Colitis (n = 105)				
	Active (n = 59)	Remission (n = 46)	Control (n = 28)	P value (Active vs Remission)	P value (Active vs Control)
FBG (mg/dL)	93.6 ± 30.1	94.7 ± 27.3	89.2±14.3	NS	NS
BUN (mg/dL)	16.9±7.0	18.1±3.9	18.0±4.6	NS	NS
Creatinine (mg/dL)	0.9±0.1	0.9±0.1	0.8±0.1	0.031	0.021
Total Protein (g/dL)	6.3±0.6	6.8±0.2	6.8±0.1	<0.001	<0.001
Albumin (g/dL)	3.5±0.5	3.9±0.2	3.8±0.1	<0.001	<0.001
AST (IU/L)	36.1±10.0	31.9±7.7	34.5±7.8	0.012	NS
ALT (IU/L)	35.0±8.4	31.2±5.4	30.4±8.0	0.029	0.016
Total Bilirubin (mg/dL)	1.0±0.1	0.9±0.0	0.9±0.1	NS	0.007
Total Cholesterol (mg/dL)	167.4±21.5	171.4±24.1	180.5±23.7	NS	0.007
Triglyceride (mg/dL)	150.0±23.6	166.1±32.5	175.1±36.9	0.018	<0.001
PT (s)	12.7±1.2	11.6±0.7	12.05±0.5	<0.001	0.012
Hemoglobin g/dL	10.3±1.7	12.1±0.7	13.1±0.8	<0.001	<0.001
Platelet count /μL	389353.5±150921.9	261287.1±59301.0	262261.8±79429.8	<0.001	<0.001
MPV (fL)	6.7±0.8	9.1±0.9	9.2±0.9	<0.001	<0.001

All results are expressed in mean±standard deviation
FBG – Fasting Blood Glucose; BUN – Blood urine nitrogen; AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; PT – Prothrombin time; MPV – Mean Platelet Volume NS – Non significant; P value: study groups in comparison with controls and patients

TABLE III - *Inflammatory and Coagulation Parameters*

	Ulcerative Colitis (n = 105)						
	Active (n = 59)						
	Admission	3rd day	3rd month	Remission (n = 46)	Control (n = 28)	P value 1	P value 2
PV (mPa-s)	1.63±0.03	1.40±0.03	1.19±0.02	1.15±0.01	1.13±0.00	<0.001	<0.001
hs-CRP (mg/L)	27.5±2.89	16.20±2.17	3.93±0.45	4.64±2.11	1.95±0.14	<0.001	<0.001
ESR (mm/h)	33.40±1.96	23.61±1.21	19.79±0.80	21.95±0.62	18.33±0.50	<0.001	<0.001
Fibrinogen (mg/dL)	332.74±17.44	274.84±8.67	260.59±6.01	234.78±7.02	234.80±6.35	<0.001	<0.001
D-Dimer (ng/mL)	383.52±23.84	317.01±17.34	301.67±11.33	267.21±10.05		<0.001	<0.001
WBC count 109/L	11001.12±466.42	13519.49±223657	7820.61±208.04	7721.52±208.32	7106.24±200.81	<0.001	<0.001

All results are expressed as mean±standard deviation
PV – Plasma Viscosity; hs-CRP – High-sensitive C-reactive protein; ESR – Erythrocyte Sedimentation Rate; WBC- White Blood Cell
P value 1: values for Active Ulcerative Colitis in comparison with Ulcerative Colitis in Remission on admission
P value 2: Values for patients with Active Ulcerative Colitis in comparison to Control Group on Admission

TABLE IV - Comparison of repeated parameters of inflammatory and coagulation across measurement times

	Active Ulcerative Colitis				
	Admission vs 3rd day (p Value)	3rd day vs 3rd month (p Value)	Admission vs 3rd month (p Value)	3rd month vs Remission (p Value)	3rd month vs Control (p Value)
PV (mPa-s)	< 0.001	< 0.001	< 0.001	NS	NS
hs-CRP (mg/L)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
ESR (mm/h)	< 0.001	< 0.001	< 0.001	0.015	NS
Fibrinogen (mg/dL)	< 0.001	NS	< 0.001	0.002	0.006
D-Dimer (ng/mL)	< 0.001	NS	0.002	NS	NS
WBC count 109/L	NS	0.015	< 0.001	NS	0.017

PV – Plasma Viscosity; hs-CRP – High-sensitive C-reactive protein; ESR – Erythrocyte Sedimentation Rate; WBC- White Blood Cell

TABLE V - *Inflammatory and coagulation variables According to Response to Medical Treatment*

	Steroid Treatment Group			Cyclosporine Treatment Group		
	Refractory (n=15)	Responsive (n=44)	P Value	Refractory (n= 5)	Responsive (n= 10)	P Value
Age (year)	50.8±17.0	38.9±15.4	0.032	55.8±12.9	46.4±19.1	NS
PV (mPa-s)	1.85±0.08	1.55±0.2	<0.001	1.93±0.03	1.78±0.14	0.003
hs-CRP (mg/L)	46.53±16.47	21.12±10.2	<0.001	60.80±9.12	36.63±16.67	0.005
ESR (mm/h)	46.86±16.74	28.81±11.4	<0.001	52.00±10.09	42.09±19.63	NS
Fibrinogen (mg/dL)	492.06±139.19	278.43±77.71	<0.001	583.20±172.11	428.63±112.06	NS
D-Dimer (ng/mL)	605.00±173.73	308.02±103.54	<0.001	766.00±114.14	504.09±45.01	0.009
WBC count 109/L	14837.07±1666.38	9693.41±3086.45	<0.001	15351.20±1879.97	13800.00±2994.66	NS

All results are expressed in mean±standard deviation
PV – Plasma Viscosity; hs-CRP – High-sensitivity C-reactive protein; ESR – erythrocyte sedimentation Rate; WBC- White Blood Cell

significantly decreased in comparison with those at admission excluding fibrinogen. It was significantly higher in active UC than in UC in remission in the 3rd month. Moreover, in the 3rd month, hs-CRP and WBC were significantly higher in active UC than in the control group.

Of 59 active UC patients, 44 responded to steroid treatment. The remaining 15 were switched to cyclosporine treatment. Of 15 patients, 5 required colectomy after 3 months. The mean age,PV, hs-CRP, ESR, fibrinogen, D-Dimer, and WBC were significantly higher in steroid refractory active UC than in steroid responder active UC (Table V). Similarly, PV, hs-CRP, and D-Dimer were significantly higher in the cyclosporine refractory active UC patients than inthe cyclosporine responder active UC patients. The mean age and fibrinogen, D-Dimer and WBC were higher in the cyclosporine refractory active UC patients than in the cyclosporine responder active UC patients, though not significantly.

Activity Indices of Ulcerative Colitis

As PV increased sodid SCCAI scores [remission (n= 46): 1.15±0.10 mPa-s; mild (n= 8): 1.25±0.16 mPa-s; moderate (n= 37): 1.61±0.21 mPa-s; severe (n= 14): 1.90±0.05 mPa-s], indicating that PV changes in UC patients depending on disease activity (one-way ANOVA, p< 0.001) (Fig. 1). There was not a significant difference in PV between remission and mild activity groupsbased on SCCAI scores (p= 0.433).

A similar relation was detected between the EGS score and PV (p< 0.001). As PV increased so did EGS scores in patients with UC [remission (n= 46): 1.15±0.10 mPa-s; mild (n= 10): 1.27±0.16 mPa-s; moderate (n= 26): 1.57±0.21 mPa-s; severe (n= 23): 1.85±0.08 mPa-s] (one-way ANOVA, p< 0.001) (Fig. 1).

In addition, as PV increased so did HDA scores in patients with UC [remission (n= 45): 1.15±0.10 mPa-s; mild (n= 11): 1.29±0.22 mPa-s; moderate (n= 26): 1.60±0.22 mPa-s; severe (n= 22): 1.83±0.10 mPa-s] (one-way ANOVA, p< 0.001) (Fig. 1).

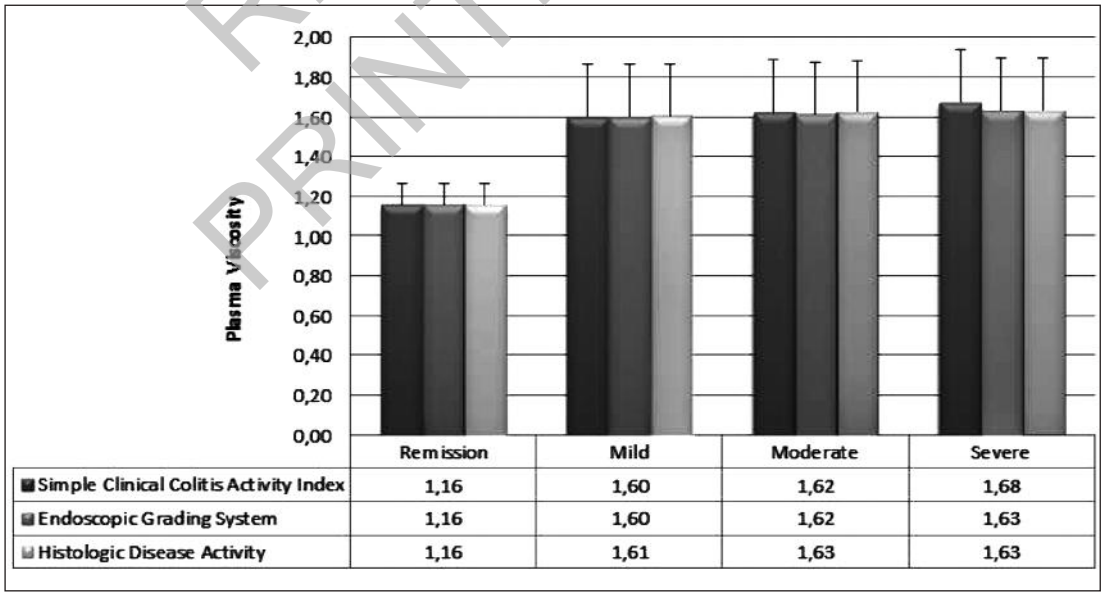

Fig. 1: Plasma Viscosity According to Various Activity Indices of UC.

Correlations Analysis of Inflammation Indices, Markers of Coagulation and Fibrinolysis Activation with Various Activity Indices of UC

In a total of 105 UC patients, clinical, endoscopic, and histological scores were strongly correlated with laboratory parameters. Table VI shows correlation coefficients between these three activity indices of UC and laboratory variables. The highest positive correlation coefficient was obtained between PV and either of three activity indices (r= 0.890 for EGS; r= 0.883 for SCCAI; r= 0.874 for HDA). Although MPV had the second highest correlation coefficient of the laboratory parameters, the relation between MPV and UC activity indices was negative. In the patients with UC, PV had a significant positive correlation with defecation count, hs-CRP, ESR, fibrinogen, D-Dimer, body temperature, heart rate, PT, PLT, AST, ALT, bilirubin and creatinine (Table VII) However, there was a negative correlation between PV and Hgb, MPV, total protein, albumin, and triglyceride. The highest correlation coefficient was obtained between PV and MPV (r= -0.750; p<0.001). However, similar relationships were not found in the control group. In fact, there was a significant negative correlation between PV and PT (r= -0.325; p= 0.036), PLT (r= -0.318; p= 0.043), and AST (r= -0.344; p= 0.028).

Gender was not significantly correlated with PV in the patient group and the control group. The mean PV was 1.59±0.31 and 1.66±0.23 (p= 0.369) in the male active UC patients and the female active UC patients respectively. It was 1.14±0.08 and 1.17±0.12 in the male UC patients at remission and the female UC patients at remission respectively (p= 0.368). It was 1.14±0.05 and 1.12±0.04 in the male healthy controls and the female healthy controls respectively (0.337).

Table VI - *Pearson coefficient analysis of laboratory variables and various activity indices of ulcerative colitis*

		SCCAI	EGS	HDA
PV	r	0.883	0.890	0.874
	p	<0.001	<0.001	<0.001
hs-CRP	r	0.686	0.686	0.650
	p	<0.001	<0.001	<0.001
ESR	r	0.645	0.647	0.586
	p	<0.001	<0.001	<0.001
Fibrinogen	r	0.648	0.665	0.614
	p	<0.001	<0.001	<0.001
D-Dimer	r	0.635	0.618	0.590
	p	<0.001	<0.001	<0.001
MPV	r	- 0.825	- 0.815	- 0. 839
	p	<0.001	<0.001	<0.001

PV – Plasma Viscosity; SCCAI – Simple Clinical Colitis Activity Index; EGS – Endoscopic Grading Scale; HAD – Histologic Disease Activity; ESR – erythrocyte sedimentation Rate; hs-CRP – High-sensitivity C-reactive protein; MPV – Mean platelet volume

Table VII - *Pearson coefficient analysis of clinical and laboratory variables in the ulcerative colitis patient group (n=105).*

Plasma viscosity	Defecation count	hs-CRP	ESR	Fibrinogen	D-Dimer	WBC count	SCCAI	EGS	HDA	Body Temperature	Heart Rate
r	0.635	0.679	0.706	0.655	0.626	0.744	0.883	0.890	0.874	0.648	0.646
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	PT	HGB	PLT	MPV	Total Protein	Albumin	AST	ALT	Bilirubin	Triglyceride	Creatinine
r	0.665	-0.772	0.735	-0.750	-0.560	-0.698	0.356	0.326	0.369	-0.200	0.352
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.043	<0.001

WBC – White blood cell; SCCAI – Simple Clinical Colitis Activity Index; EGS – Endoscopic Grading Scale; HAD – Histologic Disease Activity; ESR – erythrocyte sedimentation Rate; hs-CRP – High-sensitivity C-reactive protein; PT – Prothrombin time; HGB – Hemoglobin; PLT – Platelet count; MPV – Mean platelet volume; AST – Aspartate aminotransferase; ALT – Alanine aminotransferase

Colonoscopy Results and Extent of Disease

The distribution of the UC patients by extent of the disease was as follows (%; active vs remission): of all the patients with proctitis, 33.9% had active UC and 45.7% had UC in remission. Of all the patients with left-sided colitis, 33.9% had active UC and 28.3% had UC in remission. Of all the patients with extensive colitis, 32.2% had active UC and 26.1% had UC in remission. There was not a significant difference in extent of the disease between patients with active and those with inactive disease (p= 0.272) (Table I). There was a significant gradual increase in the mean values for inflammation and coagulation markers in active UC patients (Table VIII). The patients with extensive colitis had the highest values of inflammation and coagulation markers except for MPV. They had the lowest mean value of MPV.

Predictive Performance of Inflammation and Coagulation Markers

ROC curve analyses were conducted to determine whether a cut-off point for marker indices could be reliably used to predict responses to steroids. The AUC was 0.948 (SE: 0.026) for D-Dimer, 0.944 (SE: 0.028) for fibrinogen, 0.895 (SE: 0.040) for WBC, 0.869 (SE: 0.046) for PV, 0.823 (SE: 0.058) for MPV, 0.817 (SE: 0.057) for hs-CRP, and 0.814 (SE: 0.066) for ESR (highest to lowest) (Fig. 2A). The best cut-off values were 390 ng/mL for D-Dimer, 362.5 mg/dL for fibrinogen, 13650 10^9/L for WBC, 1.79 mPa-s for PV, 6.75 fL for MPV, 26 mg/L for hs-CRP, and 43 mm/h for ESR.

ROC curve analyses were also made to predict responses to cyclosporine. The AUC was 0.945 (SE: 0.059) for PV, 0.927 (SE: 0.073) for hs-CRP, 0.900 (SE: 0.079) for D-Dimer, 0.800 (SE: 0.129) for fibrinogen, 0.691 (SE: 0.138) for WBC, 0.655 (SE: 0.138) for ESR, and 0.564 (SE: 0.167) for MPV (from the highest to the lowest) (Fig. 2B). The best cut-off values were1.92 mPa-s for PV, 52 mg/L for hs-CRP, 687.5 ng/mL for D-Dimer, 563 mg/dL for fibrinogen, 13950 10^9/L for WBC, 45 mm/h for ESR, and 5.95 fL for MPV. The sensitivity of PV and fibrinogen was 60%. The sensitivity of hs-CRP, ESR, D-dimer, and WBC was 80%.

In addition to these, the AUC was calculated for stool frequency, PV, hs-CRP, fibrinogen, D-dimer and WBC on admission, on the 3rd day and in the 3rd month

Table VIII - *Inflammation and coagulation markers in UC patients with active disease according to the extent of bowel inflammation*

	Proctitis	Left-Sided Colitis	Extensive Colitis	p
PV (mPa-s)	1.40±0.25	1.65±0.20	1.84±0.10	<0.001
hs-CRP (mg/L)	11.67±10.42	24.75±20.33	47.31±18.38	<0.001
ESR (mm/h)	25.30±10.38	31.55±14.28	43.89±14.49	<0.001
Fibrinogen (mg/dL)	246.30±49.92	310.05±94.61	447.63±151.57	<0.001
D-Dimer (ng/mL)	273.00±81.93	320.35±83.97	566.3684±190.07	<0.001
WBC count 109/L	7718.00±1900.34	11106.50±3028.75	14346.11±2016.14	<0.001
PT (s)	11.7650±0.64	12.43±0.78	14.1105±0.78	<0.001
Platelet count /µL	256200.00±79554.41	390583.00±126838.62	528221.05±98359.18	<0.001
MPV (fL)	7.33±1.11	6.42±0.50	6.32±0.53	<0.001

PV – Plasma Viscosity; ESR – erythrocyte sedimentation Rate; hs-CRP – High-sensitivity C-reactive protein; PT – Prothrombin time; HGB – Hemoglobin; PLT – Platelet count; MPV – Mean platelet volume; MPV – Mean platelet volume

Table IX - *Receiver–operating characteristic curve of Inflammation and coagulation markers for response to steroid treatment in patients with active UC*

	PV	hs-CRP	ESR	Fibrinogen	D-Dimer	WBC	MPV
Cut off	1.79	26	43	362.5	390	13650	6.75
Sensitivity	80%	93%	73%	80%	93%	80%	93%
Specificity	81%	70%	86%	90%	86%	80%	66%
PPV	60%	52	55	60	60	57	48
NPV	92%	97%	95%	93%	97%	92%	97%
AUC	0.869	0.817	0.814	0.944	0.948	0.895	0.823
Accuracy	81%	76%	83%	88%	88%	80%	73%

PV – Plasma Viscosity; PPV – positive predictive value; NPV – negative predictive value; AUC – area under curve; hs-CRP – High-sensitivity C-reactive protein; ESR – erythrocyte sedimentation Rate; WBC- White Blood Cell

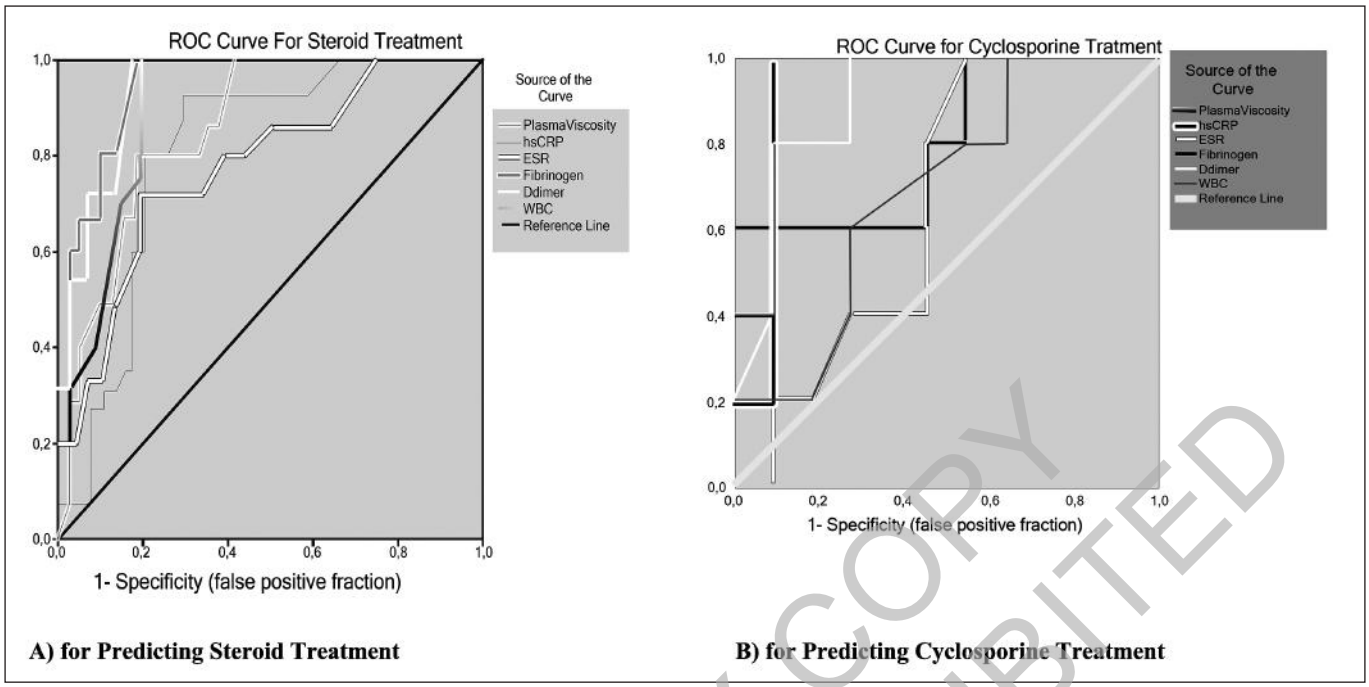

Fig. 2: Receiver–operating characteristic (ROC) curve for Inflammation and coagulation markers.

A) for Predicting Steroid Treatment
B) for Predicting Cyclosporine Treatment

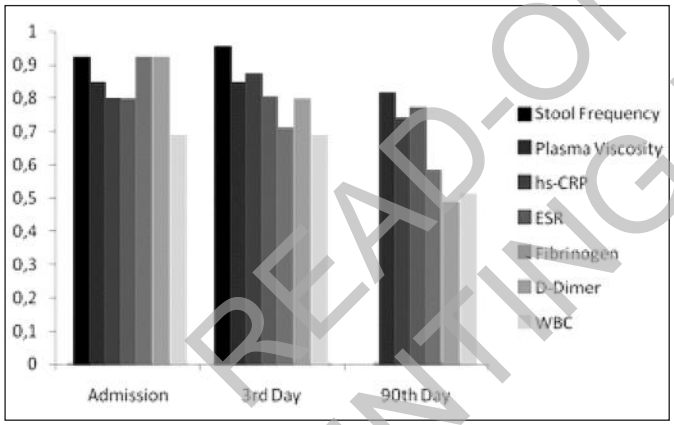

Fig. 3: The area under the curve for predicting responsiveness to steroid treatment.

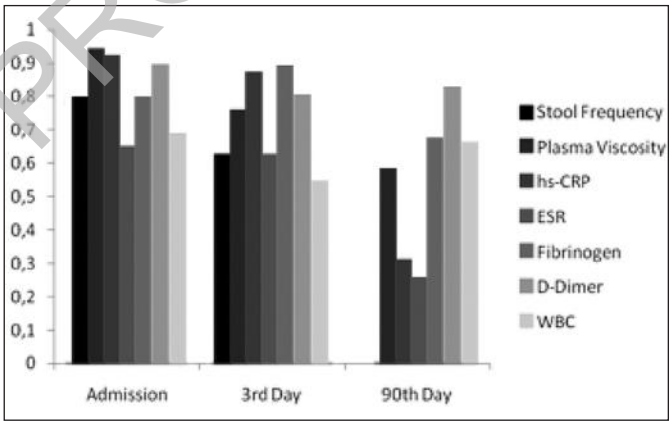

Fig. 4: The area under the curve for predicting responsiveness to cyclosporine treatment.

separately to predict response to steroid and cyclosporine treatment (Figs. 3, 4).

Discussion

Both activity and courses of UC are generally considered as unpredictable. During the course of the disease, nearly 15% of patients with UC suffer from an acute severe flare [16]. In nearly 60% of these acute attacks, steroid treatment is successful [3,17]. In patients with UC refractory to steroid treatment, cyclosporine may be efficient with initial response rates ranging from 60% to 85% [18,19]. Approximately 50% of patients with UC will respond to cyclosporine but nearly half of these will have a relapse when treatment is discontinued. The responsiveness to these medical treatments is also poorly predictable. It is quite important to know that administration of steroids or cyclosporine influence success of colectomy. Therefore, this study was directed towards describing the laboratory factors that predict responsiveness to medical treatment on admission and the 3rd day of treatment.

PV is a major indicative of capillary blood stream

through the microcirculation; an elevation in blood viscosity decreases bloodstream in the microcirculation, thus reducing tissue perfusion, which, if impaired, could lead to potentially important clinical states [20]. It is superior to ESR in that it is practical and independent of packed cell volume, gender, and age [21]. PV measurement is exactly harmless, very simple, cheap, and noninvasive. It has been indicated that PV may be less useful in clinical conditions associated with anemia and hypoalbuminemia; however, UC is associated with both conditions [12].

In organic diseases, PV is an index of changes in plasma proteins, principally a change in fibrinogen. Fibrinogen strongly affects both blood coagulation and rheology, and platelet aggregation. Clinical cohort investigations also suggest that fibrinogen is a risk factor for cardiovascular disease. Half-life of fibrinogen is three to six days whereas plasma half-life of CRP is about 19 hours and it is a nonspecific systemic marker of inflammation. In addition,it has been reported that CRP levels significantly differ between patients with UC and those with Crohn's disease (CD). It has been noted that serum CRP levels are clearly elevated in patients with CD, but are not elevated or slightly elevated in patients with UC [22,23]. There are two explanations for this difference. First,patients with CD have significantly higher serum IL-6 levels than those with UC and healthy controls. Also, inflammation is limited to the mucosa in UC, but transmural in CD [24].

ESR is another traditional inflammation marker and nonspecific like CRP. It is still widely used, but the usefulness of this test has decreased. ESR varies not only with plasma protein levels but also hematocrit, size, shape, and number of RBC, age, gender, anemia, blood dyscrasias and pregnancy. During the first 24 hrs. of acute-phase responses, CRP is a better indicator of the acute phase. Compared with CRP, ESR reaches the highest point less quickly, decreases more slowly and has a lesser degree of change [25].

MPV has been considered as a potential predictive marker of clinical disease activity, being inversely proportional to ESR and plasma CRP levels. The reason for a decrease in MPV in clinically active UC is unknown, but it may be a direct result of thrombopoiesis disorder often observed in the early phases of systemic inflammatory progression [26].

There is also a relation between platelets and the increased incidence of thromboembolic phenomena in both CD and UC. Several investigations identified that spontaneous platelet aggregation is observed in more than 30% of IBD patients [27].

Early treatment can improve management of patients with UC at high risk of relapses. Bitton et al. reported that ESR, CRP, IL-1b, IL-6, and IL-15 were not predictive of clinical recurrences in UC [28]. In light of this evidence from the literature and results of this study, PV can be a better predictive marker in order to determine whether UC responds or is refractory on the 3rd day of medical treatment. In the present study, PV as a surrogate marker of disease activity in UC was evaluated. The results showed that it was a sensitive and reliable indicator of active disease in patients with UC. it was also correlated with the degree of clinical activity as well as disease severity on colonoscopy. itwas the only parameter associated with more extensive involvement in UC.

There has been only one study on PV in patients with IBD [CD (n= 60) and UC (n= 71). However, it did not have a control group absent] and was performed in 1992 [10]. The researchers reported that PV was higher in patients with active CD than those with active UC. They also reported that PV was correlated with CRP, but not with any other laboratory measurements in patients with UC. In the same study, they did not find a difference between active UC and UC in remission in terms of PV or a correlation between PV and UC disease severity. Unlike their study, the present study included a larger sample with a UC group and a healthy control group and revealeda higher PV level inthe active UC patients than in the patients with UC in remission and the control group. In addition, UC activity indices, EGS, SCCAI, and HAD had the highest correlation with PV. This means PV has the most prognostic significance. According to the correlation analysis, PV was correlated with not only hs-CRP but also with ESR, fibrinogen, D-Dimer, body temperature, heart rate, PT, PLT, AST, ALT, bilirubin, and creatinine. MPV was the parameter having the highest correlation with PV. In addition, this is the first study to elucidate PV levels in active UC patients in terms of responsiveness to medical treatment and extent of bowel inflammation. In the present study, ROC curves were constructed to predict responsiveness to steroid and cyclosporine treatment for PV, fibrinogen, major determiner of PV, and D-dimer fibrinogen/fibrin degradation products.

In the present study, it can be emphasized that PV, D-dimer, fibrinogen, platelet count and PT should be investigated together because of their relation with both pathogenesis and complications of UC. The vascular theory encourages the notion that intestinal vascular injury is related to UC pathogenesis [29,30]. There is evidence that abnormalities within the microcirculation of the gastrointestinal tract play a pivotal role in development of UC [30-32]. Prothrombotic abnormalities within the coagulation system [33-35], the presence of microthrombi within the intestinal mucosa [30-32] and the increased risk of thromboembolic complications in IBD patients [36-38] strongly suggest that a hypercoagulable state may be an important contributory factor in the pathogenesis of UC[39]. Moreover, the incidence of thrombotic complications in UC patients has been found to be from 1.3% to 8% in clinical investigations [36,37]. The results of the present study are consistent with those of the previous studies. Zezos et al. found that both active and inactive UC

patients had significantly higher proportions of coagulation markers than healthy controls [40]. They also showed a strong correlation between ESR, fibrinogen, and D-dimers, which were correlated with the severity and extent of ulcerative colitis.

A number of investigations have been carried out in order to predict responsiveness of UC to steroid treatment based on laboratory and clinical parameters. Laboratory parameters include increased levels of CRP and ESR and decreased levels of hemoglobin, prealbumin, albumin, orosomucoid, and haptoglobin. Clinical parameters include tachycardia and temperature >101°F, stool frequency greater than eight per day, longer duration of flare, polypoid mucosal tags, and pancolitis [41]. In addition, predictive factors of resistance to steroid treatment identified are CRP >45 mg/L, severe endoscopic lesions, reduced albumin, persistence of Truelove and Witts criteria, toxic megacolon, and long duration of current attack on the 3rd day of treatment [41-43]. To our knowledge, up to now, PV, fibrinogen and D-dimer have not been investigated to predict responsiveness of UC to steroid treatment. The present study demonstrates that sensitivity for differentiation of responsiveness to steroid treatment from resistance to steroid treatment was the highest (93%) for hs-CRP, D-dimer, and MPV. However, the highest accuracy (88%) was obtained for D-dimer and fibrinogen. According to ROC curve analyses, the first highest three AUCs belonged to D-Dimer, fibrinogen, and PV in order. The results of the present study can support replacement of ESR by D-dimer for assessment of UC to steroid treatment. hs-CRP, D-Dimer, and MPV were more sensitive at detecting steroid responsiveness, but fibrinogen was the most specific. In addition, on admission, stool frequency was another clinical parameter, but on the 3rd month, the greatest AUC belonged to PV.

The second aim of this study was to identify predictive factors of responsiveness to cyclosporine in steroid-refractory UC. There have been five retrospective studies on the specific response to cyclosporine therapy [44,45]. The first two of them were conducted by Carbonnel et al [46] (n=32 patients with UC) and Travis et al. [41] (n= 14 patients with UC). They failed to define predictive markers. The third study was performed by Rowe et al. [44] on 36 patients with UC. They identified three predictive factors of resistance to cyclosporine: tachycardia, a high percentage of band neutrophils, and low albumin. The fourth study was performed by Cacheux et al. on 135 patients with steroid-refractory UC. They identified three predictive factors of resistance to cyclosporine: body temperature >37.5 C, heart rate >90 bpm, and CRP >45 mg/L [45]. Rowe et al. explained that lower bands, higher albumin, and lower pulse on admission were associated with patients'initial response to cyclosporine [44]. A major finding in the present study was that the greatest AUC belonged to PV on admission, belonged to hs-CRP on the 3rd day, and belonged to D-dimer on the 3rd month.

Conclusions

In spite of the fact that PV, a neglected variable, is a useful marker in predicting medical treatment and evaluation of clinical and colonoscopic activity in UC, it is not the best. It can be used as an adjunct to traditional markers such as hs-CRP and ESR in clinical practice. It seems thatthe best predictor of responsiveness to medical treatment is only a possible cut-off value of a combination of factors, namely, hs-CRP, PV, fibrinogen, and D-dimer. The markers predictive of a response to medical treatment would be useful in selecting treatment options and success of colectomy. The ability to predict the activity of UC and responsiveness to steroid or cyclosporine treatment would greatly help both physicians and patients. Prospective studies are needed to evaluate whether this new combined cut-off value is useful to predict responsiveness to steroid or cyclosporine.

Riassunto

La colite ulcerosa (UC) è una delle maggiori forme di colonpatia infiammatoria cronica recidivante. La capacità di identificare tipo, gravità e sensibilità alla terapia su parametri di laboratorio è da tempo oggetto di ricerca degli studi clinici. Lo scopo di questo studio è quello di accertare la relazione tra la viscosità del plasma (PV), l'attività della malattia e la risposta al trattamento medico.

Lo studio si è svolto su 105 pazienti con UC e 42 volontari sani. I prelievi di sangue sono stati utilizzati per valutare la viscosità del plasma (PV), la velocità di eritrosedimentazione (ESR), la proteina C-reattiva ultrasensibile (hs-CRP), il D-dimero e il fibrinogeno.

I pazienti con UC sono stati raggruppati in relazione al grado di attività flogistica (cioè 59 attivi e 46 in remissione). La PV è risultata più elevata in quelli con UC attiva rispetto a quella dei soggetti in remissione o nei soggetti sani. È risultata significativamente più elevata sia nei casi di UC refrattaria al trattamento steroideo rispetto a quelli sensibili ($p< 0.001$), e nelle UC rafrattarie alla ciclosrporina rispetto a quelli sensibili ($p= 0.003$).

Con l'aumento della PV nei pazienti affetti da UC sono risultati significativamente associati il punteggio del SCCAI (Increased Simple Clinical Colitis Activity Index), dell'EGS (Endoscopic Grading Scale) e del HAD (Histological Disease Activity (HAD).

Si conclude che la PV è un marker utile per la previsione di sensibilità al trattamento sterorideo e con ciclosporina nei pazienti con UC attiva. Potrebbe essere rimpiazzata dalla ESR e dalla hs-CRP come misura della risposta in fase acuta in quanto sufficientemente sensibile. Questi risultati possono essere utili per l'identificazione dei pazienti con UC attiva da destinare alla colectomia.

References

1. Meuwis MA FM, Geurts P, de Seny D, Lutteri L, Chapelle JP, Bours V, Wehenkel L, Belaiche J, Malaise M, Louis E, Merville MP: *Biomarker discovery for inflammatory bowel disease, using proteomic serum profiling*. Biochem Pharmacol, 2007; 73:1422-433.

2. Cellier C ST, Froguel E, Adenis A, Belaiche J, Bretagne JF, Florent C, Bouvry M, Mary JY, Modigliani R: *Correlations between clinical activity, endoscopic severity, and biological parameters in colonic or ileocolonic Crohn's disease. A prospective multicentre study of 121 cases. The Groupe d'Etudes Thérapeutiques des Affections Inflammatoires Digestives.* Gut, 1994; 35:231-35.

3. Cioffi M DRA, Serao R, Picone I, Vietri MT: *Laboratory markers in ulcerative colitis: Current insights and future advances.* World J Gastrointest Pathophysiol. 2015; 6(1):13-22.

4. Reumaux D SB, Poulain D, Duthilleul P, Dewit O, Colombel JF:*Serological markers in inflammatory bowel diseases*. Best Pract Res Clin Gastroenterol, 2003;17:19-35.

5. Kornbluth A LS, Present D, Hanauer S. *Long term results of oral cyclosporin in patients with severe ulcerative colitis: A double blind randomized, multi-center trial*, 1994.

6. Actis GC OA, Pera A, Barletti C, Ponti V, Pinna-Pintor M, Verme G: *Continuously infused cyclosporin at low dose is sufficient to avoid emergency colectomy in acute attacks of ulcerative colitis without the need for high dose steroids.* J Clin Gastroenterol, 1993; 17:10-3.

7. Lichtiger S PD, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S: *Cyclosporin in severe ulcerative colitis refractory to steroid therapy*. N Engl J Med, 1994; 330:1841-584

8. Rosenson RS SS, Katz R: *Elevated blood viscosity in systemic lupus erythematosus*. Semin Arthritis Rheum, 2001; 31:52-57.

9. Miller AK WR: *Plasma viscosity in pulmonary tuberculosis*. Lancet, 1942; 5:10-11.

10. Lobo AJ JS, Juby LD, Axon ATR: *Plasma viscosity in inflammatory bowel* disease. J Clin Pathol, 1992; 45:54-57.

11. Stuart J LS: *Monitoring the acute phase response.* Br Med J, 1988; 297(1):143-44.

12. Rheology ICfSoHEPoB: *Guidelines on the selection of laboratory tests for monitoring the acute phase response*. J Clin Pathol, 1988; 41:1203-212.

13. Walmsley RS AR, Pounder RE, Allan RN: *A simple clinical colitis activity index.* Gut, 1998; 42:29-32.

14. Vrij AA JJ, Schoon EJ, de Bruïne A, Hemker HC, Stockbrügger RW: *Low molecular weight heparin treatment in steroid refractory ulcerative colitis: Clinical outcome and influence on mucosal capillary thrombi.* Scand J Gastroenterol, Suppl 2001; 234:41-47.

15. Riley SA MV, Goodman MJ, Herd ME, Dutt S, Turnberg LA: *Comparison of delayed-release 5-aminosalicylic acid (mesalazine) and sulfasalazine as maintenance treatment for patients with ulcerative colitis*. Gastroenterology, 1988; 94:1383-389.

16. Edwards FC TS: *The course and prognosis of ulcerative colitis*. Gut, 1963; 4:299-315.

17. Gokturk HS DM, Akcaer Ozturk N, Kanat Unler G, Kulaksizoglu S, Kozanoglu I, Serin E, Yilmaz U: *Plasma Viscosity changes in patients with liver cirrhosis.* Southern Medical Journal, 2009; 102(10):1013-18.

18. Campbell S TS, Jewell D: *Ciclosporin use in acute ulcerative colitis: A long-term experienc*e. Eur J Gastroenterol Hepatology, 2005; 17:79-84 .

19. Stack WA LR, Hawkey CJ: *Short- and long-term outcome of patients treated with cyclosporin for severe acute ulcerative colitis.* Aliment Pharmacol Ther, 1998; 12:973-78.

20. Barnes A WED, in Chien S, Dormandy J: *Clinical Hemorheology*. Dordrecht, The Netherlands: Martinus Nijhoff Publishers, 1987.

21. Jung F RH, Ringelstein EB, Leipnitz G, Schneider R: *Effect of sex age, body weight, and smoking on plasma viscosity*. Klin Wochenschr, 1986; 64:1076-81.

22. Pepys MB DM, Klass HJ, Dash AC, Mirjah DD, Petrie A: *Immunological studies in inflammatory bowel disease*. Ciba Found Symp, 1977; 46:283-304.

23. Saverymuttu SH HH, Chadwick VS, Pepys MB: *Differing acute phase responses in Crohn's disease and ulcerative colitis*. Gut 1986; 27:809-13.

24. Gross V AT, Caesar I, Roth M, Schölmerich J: *Evidence for continuous stimulation of interleukin-6 production in Crohn's disease.* Gastroenterology, 1992; 102:514-19.

25. Mendoza JL AM: *Biological markers in inflammatory bowel disease: practical consideration for clinicians*. Gastroenterol Clin Biol, 2009; 33:S158-S73.

26. Kapsoritakis AN KM, Sfiridaki A, Potamianos SP, Kosmadaki MG, Koutroubakis IE, Kouroumalis EA: *Mean platelet volume: A useful marker of inflammatory bowel disease activity*. Am J Gastroenterol, 2001; 96:776-81.

27. Webberley MJ HM, Melikian V: *Thromboembolism in inflammatory bowel disease: Role of platelets*. Gut, 1993; 34:247-51.

28. Bitton A PM, Antonioli DA, Niles JL, Shah S, Bousvaros A, Ransil B, Wild G, Cohen A, Edwardes MD, Stevens AC: *Clinical, biological, and histologic parameters as predictors of relapse in ulcerative colitis*. Gastroenterology, 2001; 120:13-20.

29. Hamilton MDR, Crawford L: *Is proximal demarcation of ulcerative colitis determined by the territory of the inferior mesenteric artery?* Lancet, 1995; 345:688-90.

30. Wakefield AJ SA, Dhillon AP, Pittilo RM, Rowles PM, Lewis AA, Pounder RE: *Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction.* Lancet, 1989; 2:1057-62.

31. Juhlin L KU, Shelley WB: *Endotoxin-induced microclots in ulcerative colitis and Crohn's disease.* Scand J Gastroenterol, 1980; 15:311-14.

32. Dhillon AP AA, Sim R, Wakefield AJ, Sankey EA, Hudson M, Allison MC, Pounder REz: *Mucosal capillary thrombi in rectal biopsies*. Histopathology, 1992; 21:127-33.

33. Lake AM SJ, Stuart MJ: *Hemostatic alterations in inflammatory bowel disease: Response to therapy*. Am J Dig Dis, 1978; 23:897-902.

34. Hudson M CA, Hutton RA, Smith MS, Pounder RE, Wakefiel AJ: *Thrombotic vascular risk factors in inflammatory bowel disease.* Gut, 1996; 38:733-3.7

35. Chiarantini E VR, Liotta AA, CellaiAP, Fedi S, Ilari I, Prisco D, Tonelli F: *Hemostatic abnormalities in inflammatory bowel disease.* Thromb Res, 1996; 82:137-46.

36. Talbot RW HJ, Dozois RR, Beart RW Jr: *Vascular complications of inflammatory bowel disease*. Mayo Clin Proc, 1986; 61:140-45.

37. Jackson LM OGP, O'Connell J, Cronin CC, Cotter KP, Shanahan F: *Thrombosis in inflammatory bowel disease: clinical setting, procoagulant profile and factor V Leiden*. QJM, 1997; 90:183-88.

38. Bernstein CN BJ, Houston DS, Wajda A: *The incidence of deep venous thrombosis and pulmonary embolism among patients with inflammatory bowel disease: A population-based cohort* study. Thromb Haemost, 2001; 85:430-34.

39. AA VV: *Hemostasis in infl ammatory bowel diseases: Clinical relevance*. Scand J Gastroenterology, 2003; 38:51-62.

40. Zezos P PG, Nikolaidis N, Patsiaoura K, Vassiliadis T, Mpoumponaris A, Giouleme O, Evgenidis N: *Elevated markers of thrombin generation and fibrinolysis in patients with active and quiescent ulcerative colitis*. Med Sci Monit, 2009; 15(11):563-72.

41. Travis SPL FJ, Ricketts C, Nolan DJ, Mortensen NM, Kettlewell MGW: *Predicting outcome in severe ulcerative colitis.* Gut, 1996; 38(905-10).

42. Carbonnel F GD, Lemann M, Beaugerie L, Cattan S, Cosnes J, Gendre JP: *Predictive factors of outcome of intensive intravenous treatment for attacks of ulcerative colitis*. Aliment Pharmacol Ther, 2000; 14:273-79.

43. Ho GT MC, Goddard CJ, Fennell JM, ShahNB, Prescott RJ, Satsangi J: *Predicting the outcome of severe ulcerative colitis: Development of a novel risk score to aid early selection of patients for secondline medical therapy or surgery*. Aliment Pharmacol Ther, 2004; 19:1079-87.

44. Rowe FA WJ, Karp LC, Vasiliauskas EA, Plevy SE, Targan SR: *Factors predictive of response to cyclosporin treatment for severe, steroid-resistant ulcerative colitis*. The American journal of Gastroenterology, 2000; 95(8):2000-8.

45. Cacheux W SP, Lemann M, Marteau P,Nion-Larmurier I, Afchain P, Daniel F, Beaugerie L, Cosnes J: *Predictive Factors of Response to Cyclosporine in Steroid-Refractory Ulcerative Colitis.* The American journal of Gastroenterology, 2008; 2:637-42.

46. Carbonnel F BA, Duclos B: *Intravenous Cyclosporine in attacks of ulcerative colitis: short-term and long-term responses*. Digestive Diseases and Sciences, 1996; 41(12):2471-476.