PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(38):6038-6042
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Hyperhomocysteinemia in ulcerative colitis is related to folate levels

Petros Zezos, Georgia Papaioannou, Nikolaos Nikolaidis, Themistoclis Vasiliadis, Olga Giouleme, Nikolaos Evgenidis

Petros Zezos, Nikolaos Nikolaidis, Themistoclis Vasiliadis, Olga Giouleme, Nikolaos Evgenidis, Division of Gastroenterology, 2nd Propaedeutic Department of Internal Medicine, Hippokration General Hospital, Aristotle University of Thessaloniki, 49 Konstantinoupoleos Str., Thessaloniki 54642, Greece
Georgia Papaioannou, Department of Haematology, Papageorgiou General Hospital of Thessaloniki, Ring Road Nea Efkarpia, Thessaloniki 56403, Greece
Correspondence to: Associate Professor Nikolaos Evgenidis, Division of Gastroenterology, 2nd Propaedeutic Department of Internal Medicine, Aristotle University of Thessaloniki, Hippokration General Hospital, 49 Konstantinoupoleos Str., Thessaloniki 54642, Greece. zezosp@hol.gr
Telephone: +30-2310-892073 Fax: +30-2310-848354
Received: 2004-12-17 Accepted: 2005-03-21

Abstract

AIM: To study the prevalence and clinical significance of hyperhomocysteinemia (hHcys), an independent factor for arterial and venous thrombosis, in a group of patients with ulcerative colitis (UC).

METHODS: Fasting homocysteine (Hcys), folate, and vitamin B_{12} serum levels were measured in 40 UC patients and 50 healthy controls. Clinical data regarding UC were gathered.

RESULTS: Median serum Hcys levels in UC patients were similar to those in controls (12.26 μmol/L *vs* 12.32 μmol/L), but the prevalence of hHcys was higher in UC patients than in controls (30% *vs* 10%, $P = 0.028$). UC significantly increased the risk of hHcys (adjusted odds ratio: 4.125; 95%CI: 1.26-13.44). Multivariate regression analysis showed that male sex, folate and vitamin B_{12} deficiency or lower serum values were significant independent predictors of higher Hcys levels in UC patients ($r^2 = 0.4$; $P<0.001$).

CONCLUSION: hHcys is common in UC patients and it is related to folate and vitamin B_{12} deficiency or lower serum values. It would be reasonable for patients with UC to receive folate and vitamin B complex supplements as a prophylactic measure.

Key words: Folate; Homocysteine; Hyperhomocysteinemia; Thrombosis; Ulcerative colitis; Vitamin B_{12}

Zezos P, Papaioannou G, Nikolaidis N, Vasiliadis T, Giouleme O, Evgenidis N. Hyperhomocysteinemia in ulcerative colitis is related to folate levels. *World J Gastroenterol* 2005; 11 (38): 6038-6042
http://www.wjgnet.com/1007-9327/11/6038.asp

INTRODUCTION

The risk for thromboembolic complications is increased in patients with inflammatory bowel disease (IBD). The incidence of arterial and venous thromboembolic disease in patients with ulcerative colitis (UC) and Crohn's disease (CD) has been reported between 1% and 8%[1,2], rising to an incidence of 39% in some autopsy studies[3]. Several studies have shown that a hypercoagulable state involving all components of clotting system exists in IBD[4-6]. This hypercoagulable state may be related to an increased tendency for thromboembolic events and may be linked to the disease pathogenesis through promoting microthrombi formation in intestinal microcirculation[7,8]. The etiology and pathogenesis of the hypercoagulable state in IBD have not been fully elucidated but may be induced through a procoagulant effect of proinflammatory cytokines[9-13] in combination with acquired or genetic defects of clotting factors (protein S, protein C, antithrombin, factor V Leiden, prothrombin mutation 20210A, and antiphospholipid antibodies)[14-16]. Recently, the factor V Leiden has been implicated in the increased risk of venous thrombosis in IBD patients[17-19].

Homocysteine (Hcys) is a non-essential, sulfur-containing amino acid formed during the metabolism of methionine. Mild hyperhomocysteinemia (hHcys), which occurs in approximately 5-7% of the general population, has been proved to be thrombogenic and an independent risk factor for coronary artery disease[20], arterial and venous thrombosis[21-26]. Elevated levels of Hcys may result from abnormalities in metabolism pathways due to inherited abnormalities of the enzymes involved or nutrient deficiencies such as insufficiency of folate and vitamins B_2, B_6, and B_{12}[27,28].

The mechanism by which hHcys promotes thrombosis is uncertain, but it may be related to a hypercoagulatory state due to endothelial dysfunction[28-30].

Vitamin B_{12} and folate deficiency are relatively common conditions in IBD (especially in active disease) through malnutrition, malabsorption or antifolate drugs such as methotrexate and sulfasalazine (SASP). Deficiencies of key nutrients/cofactors in Hcys metabolism pathways (B_2, B_6, B_{12}, and folate) might lead to raised Hcys levels in IBD. The association between IBD and hHcys has been shown

in some recent studies, reporting an increased prevalence of hHcys in IBD (both UC and CD)[31-36].

The aim of this study was to evaluate whether Hcys levels were elevated compared to a group of healthy controls and whether Hcys levels were related to vitamin B_{12} and folate serum concentrations, disease activity, disease extent or history of thrombotic complications.

MATERIALS AND METHODS

Patients and control population

Forty patients with UC (20 females and 20 males, mean age 41.65±15.21 years, range 17-71 years) were consecutively recruited from our outpatient clinic between February 1999 and February 2000. Diagnosis of UC was based on standard clinical, endoscopic and histological criteria. A detailed clinical history was taken from each patient regarding current symptoms, activity and duration of disease, extraintestinal manifestations, present medication, smoking status, and thrombotic complications. Patients with significant liver or kidney diseases were excluded. Endoscopy was performed in all patients in order to evaluate endoscopic activity, disease extension and histological confirmation and grading. Blood samples from fasting UC patients were collected. Serum Hcys, folate and vitamin B_{12} levels were determined.

Serum Hcys levels were also measured in blood samples from 50 healthy control subjects (HC) with similar age and gender (25 females and 25 males, age 39.96±14.33 years, range 17-72 years), under similar conditions and in the same laboratory. Healthy control subjects were visitors in the outpatient clinic of Hematology Department and had no known diseases, or any clinical or laboratory evidence of metabolic, neoplastic or inflammatory disease. They also had no history of thromboembolic disease. Patients and healthy controls were from the same geographical area (Northern Greece) and had Greek ancestry. Patients and controls reported that they had no daily alcohol intake above 35 g, and no use of drugs affecting Hcys status (phenytoin, theophylline, and vitamin supplements).

Measurements

Total serum Hcys concentrations were measured by the IMx homocysteine assay, which is a fluorescence polarization immunoassay (FPIA, Abbott Laboratories). Reference serum Hcys concentrations for both men and women were <15 μmol/L. Vitamin B_{12} and folate serum levels were measured by enzymatic immunoassays (ELISA). Reference ranges for vitamin B_{12} and serum folate were >223 pg/mL and >2.8 ng/mL respectively.

Statistical analysis

Descriptive statistics for continuous variables (including means and medians) were calculated. Categorical variables were described using proportions. Odds ratios and the 95%CI for the risk of hHcys in UC patients, as compared to healthy controls, were calculated. Multivariate logistic regression was used to adjust these crude odds for confounding differences in sex. Comparisons of continuous variables between the two groups were made by Student's *t*-test for normally distributed data or by Mann-Whitney *U*-test when data were not normally distributed. Fisher's exact test was used for the comparison of proportions. Pearson's correlation coefficient was calculated to describe the relationship between variables. Multiple linear regression analysis was performed in order to study the influence of individual factors on Hcys levels (treated as continuous variable). Since the concentrations of Hcys, folate and B_{12} were not normally distributed, correlation and regression analysis were performed with log-transformed data. Statistical analysis was performed using the SPSS for Windows package (version 11.0, SPSS, Chicago, IL, USA).

RESULTS

Demographics

The baseline characteristics of the patients and controls are shown in Table 1. There were no significant differences in age between healthy controls and patients, or between sexes or between study groups. There were also no significant differences in duration of disease, disease activity, endoscopic score, disease extent, smoking, and use of medication between sexes in UC group.

Table 1 Epidemiological and clinical data of study subjects

	UC patients (95%CI)	Healthy controls (95%CI)
Subjects (*n*)	40	50
Gender (female/male)	20/20	25/25
Mean age (yr±SD)(range)	41.65±15.21	39.96±14.33
	[36.78–46.52]	[35.89–44.03]
	(17–71)	(17–72)
Mean disease duration (mo±SD)	56.65±63.18	
(range)	[36.44–76.86]	
	(3–216)	
Current smoking (%)	9 (22.5)	
Extent of UC (%)		
Rectum/sigmoid	9 (22.5)	
Left colitis	22 (55.5)	
Pancolitis	9 (22.5)	
Activity of UC (%)		
Active	28 (70)	
Inactive	12 (30)	
Extraintestinal complications (%)	6 (15)	
Thrombotic complications (%)	2 (5)	
Medical treatment		
None	1 (2.5)	
5-ASA	35 (87.5)	
SASP	1 (2.5)	
Steroids	17 (42.5)	
Immunosuppressors (AZA/6-MP)	11 (27.5)	

Homocysteine determination and associations

The median serum levels of Hcys in UC patients were similar to those in controls (12.26 μmol/L [range 7.15-35.8 μmol/L] *vs* 12.32 μmol/L [range 5.97-22.06 μmol/L], $P = 0.518$), but hHcys was more prevalent in UC patients (30% [12/40] *vs* 10% [5/50], $P = 0.028$) than in controls. Male sex had higher median serum levels of Hcys both in HC and in UC groups (Table 2). Logistic regression analysis showed an odds ratio of 3.857 [95%CI: 1.22-12.12] for hHcys in the UC group as compared to healthy controls. Advanced age and

male sex were associated with higher Hcys levels, and there was not any difference in age and sex, but males had significant higher Hcys values in both study groups and we believe that sex might be a significant confounder. The adjusted odds ratio for the sex difference was 4.125 (95%CI: 1.26-13.44).

Table 2 Serum Hcys levels in male and female subjects in study groups

	Hcys (μmol/L)		*P*
	Male	Female	
Controls	13.7 (8.17–22.06)	11.94 (5.97–16.99)	0.005
UC	14 (9.13–35.8)	11.07 (7.15–26.6)	0.011
Total	13.72 (8.17–35.8)	11.87 (5.97–26.6)	0.0003

Values are expressed as medians (range). Comparisons were performed using Mann–Whitney's test.

Serum homocysteine, folate and vitamin B_{12} levels in ulcerative colitis patients

In UC patients, the median serum levels of folate and vitamin B_{12} were 7.2 ng/mL (range 2.4-13.4 ng/mL) and 431 pg/mL (range 195-1 430 pg/mL) respectively. One patient had serum folate below the lower limit (2.4 ng/mL) and two others had normal folate near the lower limit (2.9 and 3.4 ng/mL respectively). All three had high levels of Hcys (the three highest values). One patient had vitamin B_{12} below the lower limit (195 pg/mL) and two others had normal B_{12} near the lower limit (226 and 237 pg/mL, respectively). The patient with low B_{12} and one of the two others had high levels of Hcys. These patients had no overlapping low values for both folate and B_{12} levels. The disease duration, activity, extent, endoscopic severity, medical treatment, and smoking status did not significantly influence Hcys, folate, and vitamin B_{12} levels. There were no significant differences in levels of folate and vitamin B_{12} between sexes.

Predictors of hyperhomocysteinemia in ulcerative colitis

Because the concentrations of Hcys, folate and B_{12} were not normally distributed, correlation and regression analysis were performed with log-transformed data. To directly assess the effect of age, folic acid and B_{12} on Hcys levels, the Pearson correlation coefficients between these variables were determined, as shown in Table 3. A relatively strong inverse correlation was observed between log-transformed serum levels of Hcys and log-transformed serum concentrations of folate (r = -0.466, P = 0.002). To further define the role of folic acid and B_{12} in determining Hcys levels, a multiple regression analysis was performed where log-Hcys was the dependent variable whereas age, gender, log-B_{12}, log-folic acid, smoking and history of thrombosis were the independent variables. Multiple linear regressions revealed the following variables to be significant independent predictors of Hcys levels in UC patients: male sex, folate, and B_{12} deficiency or lower serum values (r^2 = 0.4; P<0.001).

Table 3 Pearson coefficient analysis of variables within UC group

	log-Hcys	Age (yr)	log-folic acid	log-B_{12}
log-Hcys				
Coefficient	1.000	–0.016	–0.466	–0.163
P	0.000	0.920	0.002	0.314
Age (yr)				
Coefficient	–0.016	1.000	0.189	–0.039
P	0.920	0.000	0.244	0.811
log-folic acid				
Coefficient	–0.466	0.189	1.000	–0.193
P	0.002	0.244	0.000	0.234
log-B_{12}				
Coefficient	–0.163	–0.039	–0.193	1.000
P	0.314	0.811	0.234	0.000

Homocysteine metabolism in ulcerative colitis patients with previous thrombotic events

Two patients had a history of previous thrombotic events. hHcys was found in one of them. This patient had folate deficiency and the highest value of Hcys. The other one had vitamin B_{12} close to lower normal limit without hHcys.

DISCUSSION

This study showed that although Hcys levels were similar in UC patients and healthy control subjects, hHcys (serum Hcys ¡Ý15 μmol/L) was more common in UC patients than in healthy controls (adjusted odds ratio, 4.125; prevalence 30% *vs* 10%). Increased Hcys levels and high prevalence of hHcys in IBD patients have been reported in previous studies[31-36]. In our study, similar findings were observed in this cohort of UC patients with a slightly higher prevalence of hHcys (30%) than in other studies (10-26%)[31,32,35,36].

Our analysis showed that male sex and low serum levels of folic acid and vitamin B_{12} were correlated with high Hcys levels. Multiple regression analysis and Pearson's correlation coefficient showed that serum folic acid was the most significant predictor of Hcys levels. We did not find significant correlation between age, disease activity, medication, smoking status, and Hcys levels in this UC cohort. Chowers *et al*.[33], have observed similar findings in a group of patients with CD.

We did not study the frequency of MTHFR C677T variant in our study groups. In a previous study, Mahmud *et al*.[31], reported that the frequency of the homozygous C677T mutation, which results in slower synthesis of 5-methyltetrahydrofolate, is increased from 7.3% in controls to 17.5% in patients with UC and is related with high Hcys levels especially in folate deficiency status. However, in that study, Hcys levels were also elevated in patients with IBD with no mutation to MTHFR enzyme. The levels of Hcys decreased after folate supplementation, regardless of the fact that the genotype of the mutation was detected or not. It has been suggested that folate status should be addressed in all IBD patients and prophylactic folate supplementation has been recommended to all patients with IBD. In our study, the prevalence of hHcys in UC patients was much higher than the frequency of homozygous mutation of MTHFR as detected by Mahmud *et al*.[31]. The differences between prevalences of hHcys and MTHFR mutant can be explained by the existence of additional factors affecting Hcys levels. Vitamin deficiencies can be a significant

contributing factor as it has been supported by our study and previous reports. The suboptimal vitamin status in IBD patients can be due to a combination of several factors. Folate levels may be low due to either inadequate dietary intake, or increased utilization, or drug effects, mainly SASP[33]. In our study, one patient was receiving SASP and had low folate levels and hHcys. Other studies have conflicting conclusions about the significance of SASP antifolate effects[31,32]. Disease activity may contribute to increased demand for folate due to inflammation, but like Chowers *et al*.[33], we did not find any correlation between disease activity and folate or Hcys levels. Furthermore, Chowers *et al*.[33], found no change in Hcys levels, despite a significant improvement in the disease activity in a group of CD patients. These data point out that an inadequate intake of folate may be the most significant factor affecting folate levels in IBD patients.

The prevalence of thromboembolic complications in IBD patients is higher than that in the normal population, in large retrospective studies 1.3-8% of the patients develop these complications[1,2]. The pathogenesis of thromboembolism in IBD is unknown, but it seems to be multifactorial[37]. In our study we found a high prevalence (2/40; 5%) of thromboembolic events in this group of UC patients. hHcys was found in one of them. There are case reports that hHcys was noted in the test results in IBD patients with thrombosis[38,39]. In our study a correlation between hHcys and history of thrombosis was noted, but the number of patients is too small to supply safe conclusions. Nevertheless high Hcys levels may predispose IBD patients to thrombotic complications in combination with other existing circumstantial or permanent risk factors[37].

In conclusion, UC patients have a higher prevalence of hHcys than healthy controls. hHcys in these patients is related to low folate and B_{12} status but not to disease activity. All UC patients, irrespective of disease activity, are at risk for vitamin deficiencies. It is recommended that all patients should have an assessment of the nutritional status in order for the vitamin deficiencies to be detected and that they should also receive folate and vitamin B complex supplements for protection from complications of HHcys.

ACKNOWLEDGMENTS

The authors thank Associate Professor Rigas AG, Department of Electrical and Computer Engineering, School of Engineering of Democritus University of Thrace, and his associates Ms. Kotti VK and Mr. Vassiliadis VG, for their support in the detailed statistical analysis of data.

REFERENCES

1 **Talbot RW,** Heppell J, Dozois RR, Beart RW Jr. Vascular complications of inflammatory bowel disease. *Mayo Clin Proc* 1986; **61**: 140-145
2 **Vecchi M,** Cattaneo M, de Francis R, Mannucci PM. Risk of thromboembolic complications in patients with inflammatory bowel disease. Study of hemostasis measurements. *Int J Clin Lab Res* 1991; **21**: 165-170
3 **Graef V,** Baggenstoss AH, Sauer WG. Venous thrombosis occurring in non-specific ulcerative colitis. *Arch Intern Med* 1965; **117**: 377-382
4 **Hudson M,** Hutton RA, Wakefield AJ, Sawyerr AM, Pouder RE. Evidence for activation of coagulation in Crohn's disease. *Blood Coagul Fibrinolysis* 1992; **3**: 773-778
5 **Souto JC,** Martinez E, Roca M, Mateo J, Pujol J, Gonzalez D, Fontcuberta J. Prothrombotic state and signs of endothelial lesion in plasma of patients with inflammatory bowel disease. *Dig Dis Sci* 1995; **40**: 1883-1889
6 **Collins CE,** Cahill MR, Newland AC, Rampton DS. Platelets circulate in an activated state in inflammatory bowel disease. *Gastroenterology* 1994; **106**: 840-845
7 **Dhillon AP,** Anthony A, Sim R, Wakefield AJ, Sankey EA, Hudson M, Allison MC, Pounder RE. Mucosal capillary thrombi in rectal biopsies. *Histopathology* 1992; **21**: 127-133
8 **Wakefield AJ,** Sawyerr AM, Dhillon AP, Pittilo RM, Rowles PM, Lewis AA, Pounder RE. Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction. *Lancet* 1989; **2**: 1057-1062
9 **Van Deventer SJ.** Tumor necrosis factor and Crohn's disease. *Gut* 1997; **40**: 443-448
10 **Nassif A,** Longo WE, Mazuski JE, Vernava AM, Kaminski DL. Role of cytokines and platelet-activating factor in inflammatory bowel disease. Implications for therapy. *Dis Colon Rectum* 1996; **39**: 217–223
11 **Bevilacqua MP,** Pober JS, Majeau GR, Fiers W, Cotran RS, Gimbrone MA Jr. Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin 1. *Proc Natl Acad Sci USA* 1986; **83**: 4533–4537
12 **Nawroth PP,** Stern DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. *J Exp Med* 1986; **163**: 740–745
13 **Dosquet C,** Weill D, Wautier JL. Cytokines and thrombosis. *J Cardiovasc Pharmacol* 1995; **25**(Suppl 2): S13-19
14 **Aadland E,** Odegaard OR, Roseth A, Try K. Free protein S deficiency in patients with chronic inflammatory bowel disease. *Scand J Gastroenterol* 1992; **27**: 957-960
15 **Aadland E,** Odegaard OR, Roseth A, Try K. Free protein S deficiency in patients with Crohn's disease. *Scand J Gastroenterol* 1994; **29**: 333-335
16 **Heneghan MA,** Cleary B, Murray M, O'Gorman TA, McCatrhy CF. Activated protein C resistance, thrombophilia, and inflammatory bowel disease. *Dig Dis Sci* 1998; **43**: 1356-1361
17 **Liebman HA,** Kashani N, Sutherland D, McGehee W, Kam AL. The factor V Leiden mutation increases the risk of venous thrombosis in patients with inflammatory bowel disease. *Gastroenterology* 1998; **115**: 830-834
18 **Koutroubakis IE,** Sfiridaki A, Mouzas IA, Maladaki A, Kapsoritakis A, Roussomoustakaki M, Kouroumalis EA, Manousos ON. Resistance to activated protein C and low levels of free protein S in Greek patients with inflammatory bowel disease. *Am J Gastroenterol* 2000; **95**: 190-194
19 **Novacek G,** Miehsler W, Kapiotis S, Katzenschlager R, Speiser W, Wogelsang H. Thromboembolism and resistance to activated protein C in patients with inflammatory bowel disease. *Am J Gastroenterol* 1999; **94**: 685-690
20 **Gallagher PM,** Meleady R, Shields DC, Tan KS, McMaster D, Rozen R, Evans A, Graham IM, Whitehead AS. Homocysteine and risk of premature coronary disease: Evidence for a common gene mutation. *Circulation* 1996; **94**: 2154-2158
21 **Ridker PM,** Hennekens CH, Selhub J, Miletich JP, Malinow MR, Stampfer MJ. Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risk of future venous thromboembolism. *Circulation* 1997; **95**: 1777-1782
22 **Cantu C,** Alonso E, Jara A, Martinez L, Rios C, Fernandez Mde L, Garcia I, Barinagarrementeria F. Hyperhomocysteinemia, low folate and vitamin B12 concentrations, and methylene tetrahydrofolate reductase mutation in cerebral venous thrombosis. *Stroke* 2004; **35**: 1790-1794
23 **den Heijer M,** Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, Vandenbroucke JP, Rosendaal FR. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med* 1996; **334**: 759-762
24 **Ray JG.** Meta-analysis of hyperhomocysteinemia as a risk

factor for venous thromboembolic disease. *Arch Intern Med* 1998; **158**: 2101-2106
25 **Langman LJ,** Ray JG, Evrovski J, Yeo E, Cole DE. Hyperhomocyst(e)inemia and the increased risk of venous thromboembolism: more evidence from a case-control study. *Arch Intern Med* 2000; **160**: 961-964
26 **McCully KS.** Homocysteine and vascular disease. *Nat Med* 1996; **2**: 386-389
27 **Seshadri N,** Robinson K. Homocysteine, B vitamins and coronary artery disease. *Med Clin North Am* 2000; **84**: 215-237
28 **Welch GN,** Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998; **338**: 1042-1050
29 **McCully KS.** Homocysteine, folate, vitamin B6, and cardiovascular disease. *JAMA* 1998; **279**: 392-393
30 **Loscalzo J.** The oxidant stress of hyperhomocyst(e)inemia. *J Clin Invest* 1996; **98**: 5-7
31 **Mahmud N,** Molloy A, McPartlin J, Corbally R, Whitehead AS, Scott JM, Weir DG. Increased prevalence of methylenetetrahydrofolate reductase C677T variant in patients with inflammatory bowel disease, and its clinical implications. *Gut* 1999; **45**: 389-394
32 **Oldenburg B,** Fijnheer R, van der Griend R, vanBerge-Henegouwen GP, Koningsberger JC. Homocysteine in inflammatory bowel disease: a risk factor for thromboembolic complications? *Am J Gastroenterol* 2000; **95**: 2825-2830
33 **Chowers Y,** Sela BA, Holland R, Fidder H, Simoni FB, Bar-Mier S. Increased levels of homocysteine in patients with Crohn's disease are related to folate levels. *Am J Gastroenterol* 2000; **95**: 3498-3502
34 **Koutroubakis IE,** Dilaveraki E, Vlachonikolis IG, Vardas E, Vrentzos G, Ganotakis E, Mouzas IA, Gravanis A, Emmanouel D, Kouroumalis EA. Hyperhomocysteinemia in Greek patients with inflammatory bowel disease. *Dig Dis Sci* 2000; **45**: 2347-2351
35 **Romagnuolo J,** Fedorak RN, Dias VC, Bamforth F, Teltscher M. Hyperhomocysteinemia and inflammatory bowel disease: prevalence and predictors in a cross-sectional study. *Am J Gastroenterol* 2001; **96**: 2143-2149
36 **Papa A,** De Stefano V, Danese S, Chiusolo P, Persichilli S, Casorelli I, Zappacosta B, Giardina B, Gasbarrini A, Leone G, Gasbarrini G. Hyperhomocysteinemia and prevalence of polymorphisms of homocysteine metabolism-related enzymes in patients with inflammatory bowel disease. *Am J Gastroenterol* 2001; **96**: 2677-26782
37 **Koutroubakis IE.** Unraveling the mechanisms of thrombosis in inflammatory bowel disease. *Am J Gastroenterol* 2001; **96**: 1325-1327
38 **Slot WB,** van Kasteel V, Coerkamp EG, Seelen PJ, van der Werf SD. Severe thrombotic complications in a postpartum patient with active Crohn's disease resulting in ischemic spinal cord injury. *Dig Dis Sci* 1996; **40**: 1395-1399
39 **Gonera RK,** Timmerhuis TP, Leyten AC, van der Heul C. Two thrombotic complications in a patient with active ulcerative colitis. *Neth J Med* 1997; **50**: 88-91

Science Editor Wang XL and Guo SY **Language Editor** Elsevier HK