ORIGINAL PAPER

Echo Intima-Media Thickness (IMT) in the Evaluation of Early Atherosclerosis in Inflammatory Bowel Disease (IBD) Patients – a Prospective Study

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Abstract

Introduction: Inflammatory bowel diseases (IBD) are accompanied by an early and accelerated atherosclerotic process. Previous studies showed that Doppler ultrasonography and intima-media thickness (IMT) is a reliable marker for early atherosclerosis diagnosis. The aim of our study was to evaluate the presence of early atherosclerosis in a group of subjects with inflammatory bowel diseases, using Doppler ultrasonography and intima-media thickness (IMT) as a marker of subclinical atherosclerosis. **Methods:** A case-control study was conducted on 61 patients with inflammatory bowel disease and 19 healthy individuals. The included patients had an established IBD diagnosis, based on clinical, endoscopic, and histological criteria for at least 12 months. **Results:** In our study echo IMT was not significantly higher in IBD patients than in matched healthy controls. We found no difference between Crohn's disease and ulcerative colitis patients or between conventional or biologic treatment regarding the echo IMT. Severity of the disease and its duration were predictors of an increased IMT. We found that disease activity (measured by the endoscopic scores and CRP) correlates with higher values of echo IMT. Also the presence of anemia reached statistical significance and was correlated with increased echo IMT in the biologic treatment group. **Conclusions:** In our study we found an increased echo IMT in IBD patients versus control, but the values did not reach statistical significance. We found no difference between Crohn's disease and UC, conventional or biologic treatment. Age and duration of disease were correlated with increased IMT. Disease that was not controlled (high endoscopic, clinical and biological markers of activity) was correlated with increased echo IMT. The limitations of our study are that in our patients therapy was started early in the course of the disease and that the majority of the patients were in remission when echography was made. Further studies are necessary to evaluate factors correlated with early atherosclerosis in IBD and how echo IMT can be used as a tool in the proactive evaluation of these patients.

Keywords: inflammatory bowel diseases, inflammation, atherosclerosis, carotid ultrasound, intima media thickness.

Rezumat

Introducere: Bolile inflamatorii intestinale (IBD) sunt însoțite de un proces aterosclerotic precoce și accelerat. Studiile anterioare au arătat că evaluarea grosimii intima-media (echo-IMT) prin ultrasonografia Doppler este un marker fiabil pentru diagnosticul precoce al aterosclerozei. Scopul studiului nostru a fost de a evalua prezența aterosclerozei timpurii la un grup de subiecți cu boli inflamatorii intestinale, utilizând ultrasonografia Doppler și grosimea intima-media (echo-IMT) ca marker al aterosclerozei subclinice. **Metode:** Un studiu caz-control a fost efectuat la 61 de pacienți cu boală inflamatorie intestinală și 19 persoane sănătoase. Pacienții incluși au avut un

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diagnostic de IBD stabilit, bazat pe criterii clinice, endoscopice și histologice și o vechime a bolii de cel puțin 12 luni.

Rezultate: În studiul nostru eco-IMT nu a fost semnificativ mai mare la pacienții cu IBD decât la grupul control. Nu am găsit nicio diferență între pacienții cu boala Crohn și cu colită ulcerativă sau între tratamentul convențional sau biologic în ceea ce privește eco-IMT. Severitatea bolii și durata acesteia au fost predictorii ai unei IMT crescute. Am constatat că activitatea bolii (măsurată prin scorurile endoscopice și CRP) se corelează cu valori mai mari ale eco-IMT. De asemenea, prezența anemiei a atins semnificație statistică și a fost corelată cu creșterea eco-IMT în grupul de tratament biologic. **Concluzii:** În studiul nostru valorile eco IMT au fost mai crescute la pacienții cu IBD versus lotul control, dar valorile nu au atins semnificația statistică. Nu am găsit nicio diferență între boala Crohn și colita ulcerativă respectiv tratamentul convențional sau biologic. Vârsta și durata bolii au fost corelate cu eco-IMT crescut. Boala necontrolată (markeri de activitate endoscopici, clinici și biologici ridicați) s-a corelat cu creșterea eco-IMT. Limitările studiului nostru sunt că la pacienții noștri terapia biologică a fost administrată precoce după diagnostic și că majoritatea pacienților erau în remisiune atunci când a fost făcută ecografia. Sunt necesare studii suplimentare pentru a evalua factorii corelați cu ateroscleroza timpurie în IBD și modul în care eco-IMT poate fi utilizat ca instrument în evaluarea pro activă a acestor pacienți.

Cuvinte cheie: boli inflamatorii intestinale, inflamație, ateroscleroză, ecografie Doppler, indicele intima medie.

INTRODUCTION

Early atherosclerosis appears to be a frequent finding among patients with immune-mediated diseases¹. First correlations between autoimmune pathology and an increased cardiovascular mortality were observed in patients with rheumatoid arthritis².

The hypothesis that inflammatory bowel diseases (IBD) are also accompanied by an early and accelerated atherosclerotic process has been shown in previous studies but sometimes results were conflicting^{3,4}.

The aim of our study was to evaluate the presence of early atherosclerosis in a group of subjects with inflammatory bowel disease, using Doppler ultrasonography and intima-media thickness (IMT) as a marker of sub-clinical atherosclerosis. Previous studies showed that IMT is a reliable marker for early atherosclerosis in this group of patients⁵. A control group of healthy subjects was included in the study in order to evaluate to what degree does the atherosclerotic process differs in the IBD patients compared to the general population. Differences in cardiovascular risk assessed by the IMT were also studied inside the IBD population, depending on the type of inflammatory disease, ulcerative colitis vs. Crohn's disease, and treatment options, mainly conventional vs. biological therapy. Also, different predictors of early atherosclerosis were identified using complex statistical analysis.

MATERIALS AND METHOD

Study design and population

A case-control study was conducted on 61 patients with inflammatory bowel disease and 19 healthy individuals. The patients included in the study had an established IBD diagnosis, based on clinical, endoscopic, and histological criteria, according to the current guidelines. The minimum disease duration accepted for inclusion in this study was 12 months.

The inclusion criteria for the IBD group were age ≥ 18 years and patients willing to undergo evaluation of the carotid arteries by ultrasonography.

Exclusion criteria were gestation or breastfeeding, previous history of cardiovascular disease, arterial hypertension (TA $\geq 140/90$ mmHg), dyslipidemia, pre-existing renal or hepatic disease and impossibility of carotid artery evaluation due to technical difficulty related to the ultrasonography exam. The presence of rheumatological signs and symptoms, considered as extraintestinal manifestations of the inflammatory bowel disease was accepted, but we excluded subjects who fulfilled the criteria for immune-mediated rheumatological diseases.

The control group was composed of 19 healthy subjects, healthcare workers, who were invited to participate and signed a written informed consent document. They were evaluated through a questionnaire interview, clinical and biological examination. The demographic char-

acteristics of the control group matched those of patients with inflammatory disease included in the study.

Subjects with traditional cardiovascular risk factors such as obesity ($\text{BMI} \geq 28$), diabetes, arterial hypertension were excluded from both groups, while smokers subjects were not.

Ethics

The study was approved by the local research ethics committee at the Bucharest Emergency University Hospital. All subjects signed a written informed consent document, after being extensively informed about the objectives of the clinical trial.

Clinical evaluation

Demographic data, comorbidities, risk factors and current medication were assessed at the moment of the medical visit. Blood pressure measurement and ECG were performed on each subject. Additionally, we collected data regarding disease duration, surgical history, past and current medication.

Disease extension was assessed by Montreal classification in patients with Crohn's disease and by anatomic extension of the inflammation in the ulcerative colitis group (proctitis, proctosigmoiditis, left colitis and pa-colitis⁶).

Disease activity was evaluated using the Crohn's disease Activity Index (CDAI) score for subjects with Crohn's disease and the partial Mayo score for those with ulcerative colitis⁶.

The endoscopic activity was assessed using the Mayo endoscopic score in the ulcerative colitis group and the Simple Endoscopic Score in Crohn's disease (SES-CD) for the Crohn's disease group. An endoscopic evaluation, no older than 3 months was required for each subject⁶.

Anthropometric evaluation

Subjects were weighed in, measured and had their BMI calculated. The nutritional status was evaluated using the BMI charts endorsed by the World Health Organization⁷.

Biological evaluation

All patients with inflammatory bowel disease were evaluated taking into account recommendations from the CALM study⁶, in the sense that regular, algorithmic assessments, 3 months apart were performed, heading towards a proactive approach. Fasting glucose, C reactive protein, cholesterol (HDL, LDL), triglycerides, serum iron, ferritin and albumin levels, as well as coagulation tests were particularly evaluated in our study.

Doppler ultrasonography of the carotid arteries

The Doppler ultrasonography was performed, in a fully equipped ultrasound room, on subjects in supine position, with a slight hyperextension of the neck. Both carotid arteries were examined. The right common carotid artery was assessed in multiple longitudinal planes, proximal to the carotid bulb in order to obtain the best resolution for calculating the carotid intima-media thickness, defined as the distance from the lumen-intima interface to the media-adventitia interface of the artery wall. The measurement was made at the end of the diastole, approximately 1 cm proximal to the bifurcation.

Ultrasonography examination of the carotid arteries was performed by a trained and qualified physician using a General Electric Ultrasound Machine Vivid E90, equipped with a 7.0-MHz linear ultrasonic transducer and an image recording system. The examiner performed the ultrasound without knowing if the subjects belonged to the control or IBD group and was blinded to patient characteristics included in the IBD group (disease duration, severity or extension). The CIMT values lower than 0.9 mm were considered normal, values in between 0.9 mm and 1.2 mm were classified as a moderate thickening, while values greater than 1.2 mm were considered subclinical atherosclerosis⁸.

STATISTICS

Based on other studies in rheumatoid arthritis and HIV associated atherosclerosis we considered that a study population of 70 patients will generate statistically significant data.

Comparisons were made between controls and IBD patients, as well as inside the IBD population, for exploratory reasons. Student's t-test, Mann Whitney U test, and ANOVA test were used for comparison of continuous variables. Fisher's exact test and chi-square test were performed for comparison of proportions. Data are presented as mean $\pm$ standard deviation and median and quartile 1- 25% and quartile 3 -75%.

In order to identify the factors associated with the presence of atherosclerotic plaque as well as the relationship between them and patient or disease-related characteristics, multivariate logistic regression and multiple linear regression analyses were performed. The regression models built were adjusted for age, INR, CRP, Iron, Triglycerides, Cholesterol levels, BMI and disease duration. A p -value < 0.05 was considered sig-

nificant. The statistical analyses and sample size calculation were performed using INSERT PROGRAM. IBM SPSS version 20.0 for Windows (IBM Corp., Armonk NY, USA).

RESULTS

61 patients with inflammatory bowel disease (41 on biological therapy, 20 on conventional therapy) and 19 healthy individuals were evaluated.

No statistically significant differences were noted between the control and biological therapy groups regarding demographic data.

In the conventional therapy group, the median age was higher compared to the control and biological therapy groups [49.50 (37.75–56) vs 40.00 (31.00–48) vs. 37.00 (29.00–49.00), $p=0.064$], results potentially

explained by the fact that older patients generally tend to be on more regular, well-documented therapies, with less known side effects. Since biological therapies are usually used in more severe cases, which oftentimes require surgery, the endoscopic Mayo score [2.00 (1.25–3.00) vs 1.00 (1.00–1.50), $p=0.004877$], and surgical need (39% vs 15%, $p=0.057164$) were also higher in the biological therapy group of subjects, reaching statistical significance.

The partial Mayo score was also higher in the biological treatment group, as expected, with a tendency towards statistical significance [5.00 (3.00–8.00) vs 3.00 (3.00–4.00), $p=0.087769$]. No other statistically significant differences were observed between the two treatment groups (**Table 1**).

As shown in **Table 1**, we found no statistical difference of the Echo IMT values between the IBD group

Table 1. Patient demographic characteristics

Variable	Biologic treatment (N=41)	Conventional treatment (N=20)	Control (N=19)	p_value (group comparison)
Age	40.00 [31.00, 48.00]	49.50 [37.75, 56.00]	37.00 [29.00, 49.00]	0.06
Gender				
Female	11/41 (26.8%)	8/20 (40.0%)	7/19 (36.8%)	0.52
Male	30/41 (73.2%)	12/20 (60.0%)	12/19 (63.2%)	
CD	25/41 (61.0%)	11/20 (55.0%)	NA	0.65
UC	16/41 (39.0%)	9/20 (45.0%)		
Surgery=1	16/41 (39.0%)	3/20 (15.0%)	NA	0.05
Steroids=1	16/41 (39.0%)	7/20 (35.0%)	NA	0.76
CRP (mg/dL)	1.74 [0.30, 7.84]	1.01 [0.17, 3.77]	NA	0.41
Hb	13.20 [12.00, 14.70]	13.75 [12.87, 14.82]	NA	0.14
INR	1.03 [1.02, 1.21]	1.03 [1.00, 1.11]	NA	0.41
Cholesterol	171.63±49.3187	167.26±31.8919	191.79±43.5387	0.17
Triglycerides	92.00 [66.00, 134.50]	92.50 [70.75, 135.50]	77.00 [63.00, 155.00]	0.98
Ferritin	71.00 [24.78, 124.50]	67.30 [50.40, 125.25]	NA	0.57
Serum iron	51.00 [27.00, 78.50]	63.00 [42.00, 93.50]	NA	0.12
CDAI	155.0 [116.5, 197.0]	142.0 [132.0, 231.0]	NA	0.87
Partial Mayo	5.00 [3.00, 8.00]	3.00 [3.00, 4.00]	NA	0.08
SESCD	5.00 [3.00, 8.00]	3.00 [2.00, 5.00]	NA	0.13
Mayo E	2.00 [1.25, 3.00]	1.00 [1.00, 1.50]	NA	0.004
BMI	23.10 [21.80, 25.05]	23.90 [22.02, 25.25]	NA	0.47
Smoking status	11/41 (26.8%)	4/20 (20.0%)	7/19 (36.8%)	0.49
Disease duration	5.00 [3.00, 7.00]	5.00 [2.25, 9.75]	NA	0.56
Echo IMT	0.78 [0.71, 0.88]	0.84 [0.74, 0.89]	0.78 [0.72, 0.81]	0.55
Plaque	7/41 (17.1%)	3/20 (15.0%)	4/19 (21.1%)	0.88

CD=Crohn's disease; UC=ulcerative colitis; CRP=C reactive protein; Hb=hemoglobin; INR=international normalized ratio; Chol=Cholesterol; TG=triglycerides; CDAI=Crohn's disease Activity Index; SESCO= Simple Endoscopic Score in Crohn's disease; Mayo E= Mayo endoscopic score; BMI=body mass index; IMT= intima-media thickness

and the control group. The values seen in the conventional treated IBD patients were higher and comparable results were seen in the patients treated with biologics versus the control group.

Univariate logistic regression analysis was conducted in order to verify which factors influence the increased eco IMT and the presence of atherosclerotic plaques in patients with IBD and healthy controls.

As presented in **Table 2**, age is an independent predictor of atheromatosis, reaching statistical significance (OR=1.11; 95% CI:1.05,1.18; p=0.0001).

The CRP mg/dL (p= 0.054) and Hb (p=0.05) values, both either important severity criteria or indicators of an inflammatory bowel disease flare, show a tendency toward statistical significance.

Table 2. Factors influencing the presence of atherosclerotic plaque

Variable	Plaque=absent (N=66) N=51	Plaque=present (N=14) N=10	p_value (group comparison)	p_value, (logistic regression)	OR (95% CI)
Age	40.00 [31.00, 47.50]	63.50 [41.50, 69.25]	<0.001	<0.001	1.11 (1.05, 1.18)
Gender Female Male	20/66 (30.3%) 46/66 (69.7%)	6/14 (42.9%) 8/14 (57.1%)	0.36	0.36	
Disease CD UC	30/51 (58.8%) 21/51 (41.2%)	6/10 (60.0%) 4/10 (40.0%)	1.00	0.94	
Biologic therapy (1) Conventional therapy Control (2)	34/66 (51.5%) 17/66 (25.8%) 14/66 (22.7%)	7/14 (50.0%) 3/14 (21.4%) 4/14 (28.6%)	0.88	0.83 0.87 0.62	
Trat01=1 (Bio+Conv)	51/66 (77.3%)	10/14 (71.4%)	0.73	0.64	
History of surgery	15/51 (29.4%)	4/10 (40.0%)	0.71	0.51	
Previous/current use of steroids	19/51 (37.3%)	4/10 (40.0%)	1.00	0.86	
CRP mg/dL	1.74 [0.30, 4.80]	0.71 [0.25, 36.36]	0.79	0.05	
Hb	13.70 [12.30, 14.70]	12.55 [10.47, 14.15]	0.12	0.05	
INR	1.03 [1.02, 1.10]	1.53 [1.15, 1.84]	<0.001	<0.001	645.6 (15.64, 26654.6)
Col	175.76±42.8604	173.77±55.6898	0.88	0.88	
TG	89.50 [62.50, 133.00]	125.00 [85.00, 152.00]	0.19	0.29	
Ferritin	67.60 [40.30, 119.50]	72.15 [16.15, 228.20]	1.00	0.45	
Iron	56.00 [37.00, 80.00]	61.50 [23.00, 90.00]	0.93	0.99	
CDAI	142.0 [119.7, 192.0]	153.5 [128.2, 327.0]	0.43	0.27	
Partial Mayo	4.00 [3.00, 7.00]	3.00 [2.00, 7.75]	0.34	0.56	
SESCD	4.50 [2.75, 6.25]	3.00 [2.50, 7.75]	0.76	0.75	
Mayo E	2.00 [1.00, 3.00]	1.00 [1.00, 1250]	0.41	0.47	
BMI	23.10 [22.10, 24.50]	24.60 [20.45, 26.22]	0.75	0.86	
Smoking status=1	16/66 (24.2%)	6/14 (42.9%)	0.19	0.16	
Disease duration	5.00 [3.00, 7.00]	5.00 [2.75, 11.00]	0.63	0.34	
eco IMT	0.76 [0.71, 0.81]	1.05 [0.92, 1.10]	<0.001	<0.001	2.480x10 ¹⁰ (96370, 6.383x10 ¹⁵)

The INR value is significantly higher in the conventional treatment group, despite none of the patients receiving anticoagulant therapy and it is strongly correlated with the presence of atherosclerotic plaques, reaching statistical significance (OR=645.6; 95% CI, $p<0.001$).

Echo-IMT (OR=2.480x10⁻¹⁰; 95% CI:96370, 6.383x10⁻¹⁵, $p<0.001$) is an independent predictor of the atherosclerotic process, reaching statistical significance, with values considerably higher among patients with atherosclerotic plaques.

Multivariate logistic regression models were built, introducing variables that either reached or were close to statistical significance in the univariate logistic regression analysis, using the stepwise method.

In the first model created, when adjusted for age, INR, CRP mg/dL and Hb levels, it showed that regarding the impact on atherosclerotic plaque formation, increased echo-IMT is the only independent predictor maintaining statistical significance, each 1 unit increase in echo-IMT, multiplying the risk of plaque formation by 3.23 x 10⁻¹² (OR: 3.23x 10⁻¹²; 95% CI: 26900.1714, 387.713x10⁻¹⁸; $p=0.0024$). The model obtained had an AUC of 0.980 (95%CI: 0.907–0.999) and was able to explain 48.61% to 82.34% of the obtained data (Cox & Snell R²=48.61%, Nagelkerke R²=82.34%).

The second model created differs from the first one in the sense that the echo-IMT variable was excluded. We decided to do so since we considered that increased IMT is a proven predictor of increased risk of atheromatosis.

In relation to the presence of atherosclerotic plaques, age continues to be an independent predictor, reaching statistical significance, each 1 unit increase of this variable, multiplying the risk of plaque formation by 1.27 times (OR: 1.2766; 95% CI:1.0296,1.5830; $p=0.0261$). INR values also significantly correlate with the presence of atherosclerotic plaques, reaching statistical significance, each 1 unit increase in INR value augmenting the risk of plaque formation by 212x10⁻³ times (OR:212x10⁻³; 95% CI:5.0751,8.82x10⁻⁹; $p=0.0239$). The model obtained had an AUC of 0.973 (95%CI: 0.895–0.998) and was able to explain 46.60% to 78.95% of the obtained data (Cox & Snell R²=46.60%, Nagelkerke R²=78.95%).

Simple, as well as multiple linear regression analyses, were consecutively conducted in order to assess the relationship between echo-IMT (an independent predictor, strongly associated with the presence of ath-

erosclerotic plaque, as shown by the logistic regression model) and commonly known risk factors, both patient and disease-related. Linear regression analysis was performed on the entire group of participants, as well as on separate categories of subjects involved in the study: biological therapy group, conventional therapy group and control group.

Simple linear regression analysis of variables commonly associated with an increased cardiovascular risk and echo-IMT showed, as presented in **Table 3**, that age ($\beta=0.006059$, 95%CI 0.004490,0.007627, $p<0.001$), INR ($\beta=0.2895$, 95%CI 0.1789,0.4000, $p=0.000002$) and CRP mg/dL ($\beta=0.001494$, 95%CI 0.0001321,0.002855, $p=0.032104$) are statistically significant predictors for an increased echo-IMT in the IBD patients group. Disease duration ($\beta=0.008343$, 95%CI -0.001265,0.01795, $p=0.087576$) and endoscopic severity in ulcerative colitis patients ($\beta=-0.05230$, 95%CI -0.1141,0.009459, $p=0.093130$), assessed by the Mayo score, show a tendency toward statistical significance.

The results obtained are in line with those of previous studies and can once again highlight the possibility of an accelerated atherosclerotic process in patients with long-lasting severe inflammatory bowel disease.

Table 3. Predictors of increased echo IMT

Variables	Coefficient correlation	Regression Coefficient β (95%CI)	p_value (simple linear regression)
Age	0.6567	0.006 (0.004 to 0.007)	<0.001
CRP mg/dL	0.2748	0.001 (0.000 to 0.002)	0.03
Hb	-0.1945	-0.015 (-0.036 to 0.004882)	0.13
INR	0.5636	0.28 (0.17 to 0.40)	<0.001
Col	-0.04778	-0.0001 (-0.0007 to 0.0004)	0.67
TG	0.1319	0.0002 (-0.0001 to 0.0005)	0.24
Ferritin	-0.009019	-0.00001 (-0.0004 to 0.0004687)	0.96
Iron	-0.09695	-0.0003 (-0.001 to 0.0005)	0.45
CDAI	0.1686	0.0002 (-0.0002 to 0.0008)	0.32
Partial Mayo	-0.2464	-0.014 (-0.038 to 0.009)	0.23
SESCD	0.03309	0.001 (-0.014 to 0.017)	0.84
Mayo E	-0.3431	-0.05 (-0.11 to 0.009)	0.09
BMI	0.1041	0.004 (-0.007 to 0.01)	0.42
Disease duration	0.2206	0.008 (-0.0012 to 0.017)	0.08

A multiple linear regression model was built considering 9 variables associated with an increased echo-IMT: age, CRP mg/dL, INR, Hb, cholesterol, triglycerides, iron levels, BMI and disease duration. The stepwise method was used to analyze the data. The INR value ($\beta=0.2016$, $p<0.001$) and age ($\beta=0.005$, $p<0.0001$) were the only variables remaining statistically significant as predictors for an increased echo-IMT, with positive regression β coefficients, meaning that for each 1 unit increase of the predictor INR / age, the echo IMT increases by 0.206 times, respectively by 0.005264 times, if the age / INR remains constant. The multiple linear regression model obtained is significant, explaining 51,73% of the obtained data ($F=32,0781$, $p<0.0001$, $R^2=0.5173$).

Simple linear regression analysis performed in the biological therapy group showed that age ($\beta=0.006$, 95%CI 0.0003516, 0.008979, $p<0.001$), INR value ($\beta=0.2778$, 95%CI 0.1294, 0.4263, $p<0.001$), CRP mg/dL level ($\beta=0.001$, 95% CI 0.0003516, 0.003039, $p=0.01$) and Hb level ($\beta=-0.02$, 95%CI -0.04470, -0.0006463, $p=0.01472$, $p=0.04$) are independent predictors of an increased echo-IMT, reaching statistical significance (**Table 3**). In the conventional treatment group, age ($\beta=0.007436$, 95%CI 0.005051, 0.009820, $p<0.001$) and INR value ($\beta=0.3134$, 95%CI 0.1374, 0.4894, $p=0.001$) were the only variables significantly impacting echo-IMT (Table 3). The differences in variables influencing echo-IMT can be explained by differences between the conventional and biological treatment groups themselves.

As mentioned before, patients receiving biological therapy included in our study have had a more severe disease, CRP and Hb being criteria used to assess severity/ activity. Cholesterol level ($\beta=0.001286$, 95%CI 0.0004952, 0.002076, $p=0.003$) and triglycerides level ($\beta=0.0005285$, 95%CI 0.0001570, 0.0009000, $p=0.008029$) reached statistical significance in the control group, being strong predictors of atherosclerosis in non-IBD subjects. It is to be noted that more traditional risk factors, such as dyslipidemia, were found among patients without IBD, highlighting once again the possible role of the inflammatory activity itself in the accelerated atherosclerotic process in patients with IBD.

Multiple regression models were built, adjusting for age, INR, CRP mg/dL, Hb, Cholesterol, Triglycerides, Iron levels, BMI and disease duration.

In the biological therapy group, age ($\beta=0.005645$, $p<0.0001$), INR value ($\beta=0.1728$, $p=0.0089$) and Iron

value ($\beta=-0.0008147$, $p=0.04$) remain strong predictors of an increased echo-IMT, maintaining statistical significance. In the conventional therapy group, age ($\beta=0.006658$, $p<0.001$) was the only variable maintaining statistical significance. It can be observed that the results obtained through regression analysis in the conventional therapy, respectively biological therapy subgroups, are similar to those obtained when analyzing the entire group of subjects.

DISCUSSION

The major complications of atherosclerosis represent an important cause of morbidity and mortality. In the last decade the association between IBD and increased risk of cardiovascular events is supported by evidences from large epidemiological studies and several meta analyses⁹.

In this study, we evaluated a series of patients with inflammatory bowel disease, both on conventional or biological therapy and compared them to a control group.

In order to evaluate early atherosclerosis, we used IMT, a marker with a high predictive value for subclinical atherosclerosis⁸. The common carotid artery IMT is one of the most commonly used and best validated ultrasound measure of the early stages of vascular disease and has been extensively studied in multiple categories of high risk patients (diabetes, renal failure, various inflammatory conditions such as rheumatoid arthritis, psoriasis or infectious diseases such as AIDS)¹⁰.

The presence of carotid plaque is more strongly associated with coronary artery disease and is a better predictor of cardiovascular events when compared with the carotid intima-media thickness¹¹.

Although the Echo IMT values were not significantly higher in IBD patients than in matched healthy controls in our study group, we managed to identify and validate several parameters influencing the risk of early atheromatosis.

We found no differences between Crohn's disease and the ulcerative colitis patients regarding the increased echo IMT. The type of treatment conventional or biologic also had no impact on echo IMT. In some studies biologic treatment had a „protective” effect regarding the atheromatous process with lower echo IMT values in the biologic treated IBD which we did not observed in our group; however our study included patients in remission and we did not performed seriate measure-

ments prospectively for long time enough to observe such a protective effect³. Also, a beneficial effect of treatment can be speculated in our group of biologic treated patients since their IMT values are comparable with those of the control group.

Age was an independent predictor of increased echo IMT in our study group. In the IBD group, severity of the disease and its duration were predictors of an increased IMT. We found that disease activity (measured by the endoscopic score in UC and CRP in both groups) correlates with higher echo IMT. Also, the presence of anemia reached statistical significance and was correlated with increased echo IMT in the biologic treatment group.

We were surprised by the results since we expected a more definitive difference of echo IMT values between IBD versus control. In a case control study that enrolled 23 patients with IBD and 20 controls the mean echo IMT values were not statistically significant between patients and controls (3). In a smaller study of 52 patients with IBD versus 20 controls, the results showed statistical significant higher values of the echo IMT in the IBD group and mainly in UC patients and a correlation of echo IMT with patients' age and homocysteine levels¹². We believe that although the number of patients is comparable in their study, the duration of disease was at least 7,2-8,3 years versus a median of 5 years in our group.

Increased echo IMT was naturally correlated with the presence of atherosclerotic plaque in our patients. Also only age and increased INR were statistically significant factors for presence of atherosclerotic plaque. Increased age naturally correlates with atheromatosis but we had no definitive explanation for the increased INR correlating with increased values of echo IMT. Active inflammation can shift the hemostatic balance to favor the activation of coagulation which, in turn, can also promote and sustain inflammation creating a vicious circle between chronic inflammation and thrombosis¹³. A pro-thrombotic condition may result from a decrease in natural anticoagulant factors in active IBD although data is conflicting^{13,14}. Probably there is a complex interaction between endothelium, coagulation cascade and fibrinolysis, platelet activity and inflammation^{13,14}. The significant correlation of increased echo IMT with the increased INR values found in our study might be partly explained by these complex interactions.

Overall disease that was not controlled (high endoscopic, clinical and biological markers of activity) was

correlated with increased values of echo IMT. We can assume that a better therapeutic disease control might result in a decrease of the cardiovascular risk, but further studies are necessary to support this conclusion.

Our results did not definitively confirm that IBD patients present a higher risk of developing atherosclerotic plaque in the carotid arteries and, consequently, a higher risk of cardiovascular diseases when compared with the matched control group. However, several factors reflecting a poor disease control were correlated with increased echo IMT and we can speculate that we did not reach statistical significance because our patients with IBD were treated early and in a rapid *step up* manner.

Other limitations of our results are the small sample size and cross-sectional design and inclusion of patients which were „controlled” by the biologic therapy, with rather low disease activity indexes (patients in remission) and with a rather short disease duration in the majority of cases included. We believe that prospective and longitudinal studies are necessary, particularly to clarify the impact of active intestinal inflammation on the development of cardiovascular disease and also the long-term impact of biologic therapy on atherosclerosis and cardiovascular risk.

Ultrasound evaluation of IMT might serve as a useful tool in predicting cardiovascular risk and adjusting therapy in IBD patients although data regarding the effect of therapy is conflicting^{15,16}.

With the new biologic therapies that can alter the course of the disease^{7,15,16} a strategy where treatment can be tailored not only with a step up/add on approach of their biologic treatment but also by adding statin therapy or other therapeutic measures targeting cardiovascular risk might be implemented using echo IMT.

CONCLUSION

Carotid ultrasound with measurement of intima media thickness should be considered in high-risk groups for cardiovascular disease such as in patients with poorly controlled IBD. It is a non-invasive, reproducible, and relatively inexpensive method that easily provides quantitative measurements of structural changes in the arterial wall permitting the diagnosis of atherosclerosis in its subclinical phase. Further studies are necessary to evaluate the risk of early atherosclerosis in IBD patients and how echo IMT measurement can be used to further clarify this association.

Compliance with ethics requirements: The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the

Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

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