”Carol Davila” University of Medicine and Pharmacy
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# **”Endothelial dysfunction in inflammatory bowel diseases”**

Abstract of the PhD Thesis

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# CONTENT

## Author scientific publications' list

1. **Filimon AM**, Negreanu L, Doca M, Ciobanu A, Preda CM, Vinereanu D. Cardiovascular involvement in inflammatory bowel disease: Dangerous liaisons **World J Gastroenterol 2015 21(33): 9688-9692 ISI 3,477** https://www.wjgnet.com/1007-9327/full/v21/i33/9688.htm I cap 1,2,3
2. Goran L, State M, **Negreanu A M**, Negreanu L. Pursuing therapeutic success in Crohn's disease: A matter of definition, tools and longterm outcomes **European Journal of Inflammation 2020 Oct;18: 1–10. IF 0,26** https://journals.sagepub.com/doi/full/10.1177/2058739220962896 I cap 1,2,3
3. **Negreanu AM**, Badiu S, Panaitescu E, Ionita M, Negreanu L .Echo Intima-Media Thickness (IMT) in the Evaluation of Early Atherosclerosis in Inflammatory Bowel Disease (IBD) Patients – a Prospective Study **Modern Medicine 2021**; 28 (3),281-290 SCOPUS, EBSCO, DOAJ, CiteFactor, Scipio of UEFISCDI, and INDEX COPERNICUS https://medicinamoderna.ro/early-atherosclerosis-and-acute-vascular-events-in-ulcerative-colitis-patients-a-case-series/ partea II
4. **Negreanu AM**, Stemate A, Spataru T, Negreanu L. Early Atherosclerosis and acute vascular events in ulcerative colitis patients – a case series. **Modern Medicine 2022**; 29 (2),123-127 SCOPUS, EBSCO, DOAJ, CiteFactor, Scipio of UEFISCDI, and INDEX COPERNICUS https://medicinamoderna.ro/echo-intima-media-thickness-imt-in-the-evaluation-of-early-atherosclerosis-in-inflammatory-bowel-disease-ibd-patients-a-prospective-study/ partea II
5. Goran L, State M, **Negreanu AM**, Negreanu L.Quality of Care in Inflammatory Bowel Disease: the Role of Steroid Assessment Tool (SAT)-a Review **Modern Medicine 2020** 27 (3), 172 SCOPUS, EBSCO, DOAJ, CiteFactor, Scipio of UEFISCDI, and INDEX COPERNICUS https://medicinamoderna.ro/quality-of-care-in-inflammatory-bowel-disease-the-role-of-steroid-assessment-tool-sat-a-review/ partea II
6. Goran L, Negreanu L, **Negreanu AM** Role of new endoscopic techniques in inflammatory bowel disease management: Has the change come? **World J Gastroenterol. 2017** Jun 28;23(24):4324-4329. doi: 10.3748/wjg.v23.i24.4324. **IF 3,455** https://www.wjgnet.com/1007-9327/full/v23/i24/4324.htm I cap 4,5
7. Goran L, **Negreanu AM**, Stemate A, Negreanu L Capsule endoscopy: Current status and role in Crohn's disease. **World J Gastrointest Endosc. 2018** Sep 16;10(9):184-192 Thomson Reuters master journal list Emerging Sources Citation Index https://www.wjgnet.com/1948-5190/full/v10/i9/184.htm I cap 4,5

**Introduction and aim**

Early atherosclerosis appears to be a frequent finding among patients with immune-mediated diseases[1]. First mentions of a correlation between autoimmune pathology and increased cardiovascular mortality come from rheumatology clinical trials, a connection particularly observed in patients with rheumatoid arthritis[2].

The hypothesis that inflammatory bowel diseases are also accompanied by an early and accelerated atherosclerotic process has been probed by previous studies, with conflicting results being obtained [3,4].

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. Previous studies showed that IMT is a reliable marker for early atherosclerosis in this group of patients [5]. 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, conventional vs. biological therapy.

The PhD thesis consists in two sections.

In the general part, I presented the main data on inflammatory bowel diseases epidemiology, pathogenesis and monitoring. A special chapter is dedicated to endothelial dysfunction with a focus on digestive inflammation and the correlations with cardiovascular morbidity. I conducted an extensive search of the literature on the subject in order to clarify my objectives and to prepare a tailored designed personal study.

In the special part, I conducted a prospective case controlled study on 61 patients with inflammatory bowel disease (41 on biological therapy, 20 on conventional therapy) and 19 healthy individuals.

## Methods

The diagnosis of IBD was based on clinical, endoscopic, and histological parameters, criteria used being in line with those of current guidelines. The minimum disease duration was 12 months. The inclusion criteria for the IBD group were age ≥18 years and the evaluation of the carotid arteries by ultrasonography. Exclusion criteria were pregnancy or breastfeeding, previous history of cardiovascular disease, arterial hypertension (TA ≥ 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 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 in our study and signed a written informed consent document. They were evaluated through a questionnaire interview, clinical and biological examination. The demographic characteristics of the control group matched those of patients with inflammatory disease included in the study. Subjects with traditional cardiovascular risk factors such as obesity (BMI ≥ 28), diabetes, arterial hypertension were excluded from both groups, while smokers and *dyslipidemic* 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. They had their blood pressure measured and ECG was performed on each subject. Disease duration, surgical history, past and current medication were all noted down.

Disease extension was assessed by Montreal classification in patients with Chron s disease and by lesion extension in the ulcerative colitis group ( proctitis, proctosigmoiditis, left colitis and pancolitis). Disease activity was evaluated using the CDAI score for subjects with Chron s disease and the Mayo score for those with ulcerative colitis.The endoscopic activity was assessed using the Mayo endoscopic score in the ulcerative colitis group and the SES CD score for the Chron s disease group. An endoscopic investigation, no older than 3 months was required for each subject.

**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 Organization.

**Biological evaluation**

All patients with inflammatory bowel disease were evaluated taking into account recommendations from the CALM study, in the sense that regular, algorithmic assessments, 3 to 6 months apart were performed, heading towards a proactive approach. Fasting glucose, C reactive protein, cholesterol (HDL, LDL), triglycerides, iron, ferritin and albumin levels, as well as coagulation tests were particularly evaluated in our study.

**Doppler ultrasonography of the carotid arteries (CIMT)**

Doppler ultrasonography was performed, in a fully equipped ultrasound room, on subjects in the 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, equipped with a 7.0-MHz linear ultrasonic transducer and an image recording system. The examiner performed the ultrasounds without knowing if the subjects belonged to the control or IBD group. 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 atherosclerosis.

**Statistics**

Data are presented as mean ± standard deviation and median and quartile 1- 25% and quartile 3 -75%. Comparisons were made between controls and IBD patients, as well as inside the IBD population, for exploratory reasons. Student's t-test, Mann Whitney test, and ANOVA test were used for the comparison of continuous variables. Fisher's exact test and chi-square test were performed for the comparison of proportions. 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 significant. The statistical analyses and sample size calculation were performed using INSERT PROGRAM.

**Results**

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 ones [ 49.50 (37.75-56) vs 40.00 (31.00-48) vs 37.00 (29.00-49.00), p=0.064197 ], 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 biotherapies 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 greater in the biological therapy group of subjects, reaching statistical significance. The 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**

| | Biologic treatment (N=41) | Conventional (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] | **0.064197** (Kruskal-Wallis) |
| **sex**<br>F<br>M | <br>11/41 (26.8%)<br>30/41 (73.2%) | <br>8/20 (40.0%)<br>12/20 (60.0%) | <br>7/19 (36.8%)<br>12/19 (63.2%) | 0.528054 (Pearson Chi-Square) |
| **disease**<br>CD<br>CU | <br>25/41 (61.0%)<br>16/41 (39.0%) | <br>11/20 (55.0%)<br>9/20 (45.0%) | - | 0.655969 (Pearson Chi-Square) |
| **surgery**=1 | 16/41 (39.0%) | 3/20 (15.0%) | - | **0.057164** (Pearson Chi-Square) |
| **steroids**=1 | 16/41 (39.0%) | 7/20 (35.0%) | - | 0.760785 (Pearson Chi-Square) |
| **CRP mg/dL** | 1.74 [0.30, 7.84] | 1.01 [0.17, 3.77] | - | 0.418558 (Mann-Whitney) |
| **Hb** | 13.20 [12.00, 14.70] | 13.75 [12.87, 14.82] | - | 0.142104 (Mann-Whitney) |
| **INR** | 1.03 [1.02, 1.21] | 1.03 [1.00, 1.11] | - | 0.412658 (Mann-Whitney) |
| **Col** | 171.63±49.3187 | 167.26±31.8919 | 191.79±43.5387 | 0.178615 (ANOVA) |
| **TG** | 92.00 [66.00, 134.50] | 92.50 [70.75, 135.50] | 77.00 [63.00, 155.00] | 0.980198 (Kruskal-Wallis) |
| **Ferritin** | 71.00 [24.78, 124.50] | 67.30 [50.40, 125.25] | - | 0.574552 (Mann-Whitney) |
| **Iron** | 51.00 [27.00, 78.50] | 63.00 [42.00, 93.50] | - | 0.128164 (Mann-Whitney) |
| **CDAI** | 155.0 [116.5, 197.0] | 142.0 [132.0, 231.0] | - | 0.877138 (Mann-Whitney) |
| **Mayo** | 5.00 [3.00, 8.00] | 3.00 [3.00, 4.00] | - | **0.087769** (Mann-Whitney) |
| **SESCD** | 5.00 [3.00, 8.00] | 3.00 [2.00, 5.00] | - | 0.133396 (Mann-Whitney) |
| **Mayo E** | 2.00 [1.25, 3.00] | 1.00 [1.00, 1.50] | - | **0.004877** (Mann-Whitney) |
| **BMI** | 23.10 [21.80, 25.05] | 23.90 [22.02, 25.25] | - | 0.470057 (Mann-Whitney) |
| **Smoking status**=1 | 11/41 (26.8%) | 4/20 (20.0%) | 7/19 (36.8%) | 0.495289 (Pearson Chi-Square) |
| **Disease duration** | 5.00 [3.00, 7.00] | 5.00 [2.25, 9.75] | - | 0.564158 (Mann-Whitney) |
| **eco IMT** | 0.78 [0.71, 0.88] | 0.84 [0.74, 0.89] | 0.78 [0.72, 0.81] | 0.557390 (Kruskal-Wallis) |
| **Plaque=1** | 7/41 (17.1%) | 3/20 (15.0%) | 4/19 (21.1%) | 0.881000 (Likelihood Ratio) |

Univariate logistic regression analysis was conducted in order to verify which factors influence the presence of atherosclerotic plaques in patients with IBD. As presented in **table 2**, age is an independent predictor, reaching statistical significance (OR=1.11; 95% CI:1.05,1.18; p=0.000153). CRP mg/dL ( p= 0.054007) and Hb (p=0.051756) values, both either important severity criteria or

indicators of an inflammatory bowel disease flare, show a tendency toward statistical significance. 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:15.64, 26654.6;p=0.000653). Echo-IMT ( OR=2.480X10 $^{10}$; 95% CI:96370, 6.383X10 $^{15}$;p=0.000166) is an independent predictor of the atherosclerotic process, reaching statistical significance, with values considerably higher among patients with atherosclerotic plaques, as expected.

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.

**Table2**

| | Plaque=0 (N=66) N=51 | Plaque=1 (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]** | **0.000263** (Mann-Whitney) | **0.000153,** 1.11 (1.05, 1.18) |
| **sex**<br>F<br>M | <br>20/66 (30.3%)<br>46/66 (69.7%) | <br>6/14 (42.9%)<br>8/14 (57.1%) | 0.365808 (Fisher's Exact Test) | 0.365760 |
| **Disease**<br>CD<br>CU | <br>30/51 (58.8%)<br>21/51 (41.2%) | <br>6/10 (60.0%)<br>4/10 (40.0%) | 1.000000 (Fisher's Exact Test) | 0.944859 |
| Biologic (1)<br>Conventional(ref)<br>Control (2) | <br>34/66 (51.5%)<br>17/66 (25.8%)<br>14/66 (22.7%) | <br>7/14 (50.0%)<br>3/14 (21.4%)<br>4/14 (28.6%) | 0.881000 (Likelihood Ratio) | <br>0.837429<br>0.879710<br>0.623877 |
| **Trat01=1 (Bio+Conv)** | 51/66 (77.3%) | 10/14 (71.4%) | 0.731371 (Fisher's Exact Test) | 0.641555 |
| **surgery**=1 | 15/51 (29.4%) | 4/10 (40.0%) | 0.710104 (Fisher's Exact Test) | 0.510910 |
| **steroids**=1 | 19/51 (37.3%) | 4/10 (40.0%) | 1.000000 (Fisher's Exact Test) | 0.869951 |
| **CRP mg/dL** | 1.74 [0.30, 4.80] | 0.71 [0.25, 36.36] | 0.791967 (Mann-Whitney) | **0.054007** |
| **Hb** | 13.70 [12.30, 14.70] | 12.55 [10.47, 14.15] | 0.123602 (Mann-Whitney) | **0.051756** |
| **INR** | **1.03 [1.02, 1.10]** | **1.53 [1.15, 1.84]** | **0.000468** (Mann- | **0.000653**, 645.6 (15.64, 26654.6) |

| | | | | |
|---|---|---|---|---|
| | | | Whitney) | |
| **Col** | 175.76±42.8604 | 173.77±55.6898 | 0.884860 (Indepdendent Sample T Test) | 0.883034 |
| **TG** | 89.50 [62.50, 133.00] | 125.00 [85.00, 152.00] | 0.190590 (Mann-Whitney) | 0.296510 |
| **Feritin** | 67.60 [40.30, 119.50] | 72.15 [16.15, 228.20] | 1.000000 (Mann-Whitney) | 0.454232 |
| **Iron** | 56.00 [37.00, 80.00] | 61.50 [23.00, 90.00] | 0.937872 (Mann-Whitney) | 0.993760 |
| **CDAI** | 142.0 [119.7, 192.0] | 153.5 [128.2, 327.0] | 0.432111 (Mann-Whitney) | 0.272206 |
| **Mayo** | 4.00 [3.00, 7.00] | 3.00 [2.00, 7.75] | **0.343543 (Mann-Whitney)** | **0.567755** |
| **SESCD** | 4.50 [2.75, 6.25] | 3.00 [2.50, 7.75] | 0.762658 (Mann-Whitney) | 0.752814 |
| **Mayo E** | 2.00 [1.00, 3.00] | 1.00 [1.00, 1250] | 0.410275 (Mann-Whitney) | 0.471055 |
| **BMI** | 23.10 [22.10, 24.50] | 24.60 [20.45, 26.22] | 0.755172 (Mann-Whitney) | 0.864750 |
| **Smoking status**=1 | 16/66 (24.2%) | 6/14 (42.9%) | 0.191878 (Fisher's Exact Test) | 0.163785 |
| **Disease duration** | 5.00 [3.00, 7.00] | 5.00 [2.75, 11.00] | 0.637040 (Mann-Whitney) | 0.348296 |
| **eco IMT** | 0.76 [0.71, 0.81] | 1.05 [0.92, 1.10] | **0.000000** (Mann-Whitney) | **0.000166**, $2.480x10^{10}$ (96370, $6.383x10^{15}$) |

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, 387.713x$10^{18}$; 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 $R^2$ =48.61%, Nagelkerke $R^2$ =82.34%).

The second model created differs from the first one in the sense that the echo-IMT variable was

excluded. 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 $R^2$ =46.60%, Nagelkerke $R^2$ =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 atherosclerotic 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 (β= 0.006059, 95%CI 0.004490,0.007627,p=0.000000), INR ( β= 0.2895, 95%CI 0.1789,0.4000,p=0.000002) and CRP mg/dL (β= 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 (β= 0.008343, 95%CI -0.001265,0.01795,p=0.087576) and endoscopic severity (β= -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**

| Toate datele (Risk variables) | Coefficient correlation | Regresion Coefficient β (95%CI) | p_value (simple linear regression) |
|---|---|---|---|
| **Age** | 0.6567 | 0.006059 (0.004490 to 0.007627) | **0.000000** |
| **CRP mg/dL** | 0.2748 | 0.001494 (0.0001321 to 0.002855) | **0.032104** |
| **Hb** | -0.1945 | -0.01556 (-0.03601 to 0.004882) | 0.133071 |
| **INR** | 0.5636 | 0.2895 (0.1789 to 0.4000) | **0.000002** |
| **Col** | -0.04778 | -0.0001315 (-0.0007555 to 0.0004924) | 0.675819 |
| **TG** | 0.1319 | 0.0002090 (-0.0001498 to 0.0005678) | 0.249682 |
| **Feritin** | -0.009019 | -0.00001183 (-0.0004924 to 0.0004687) | 0.960269 |
| **Iron** | -0.09695 | -0.0003405 (-0.001251 to 0.0005701) | 0.457313 |
| **CDAI** | 0.1686 | 0.0002763 (-0.0002868 to 0.0008394) | 0.325763 |
| **Mayo** | -0.2464 | -0.01416 (-0.03819 to 0.009870) | 0.235183 |
| **SESCD** | 0.03309 | 0.001540 (-0.01467 to 0.01775) | 0.848053 |
| **Mayo E** | -0.3431 | -0.05230 (-0.1141 to 0.009459) | **0.093130** |
| **BMI** | 0.1041 | 0.004803 (-0.007152 to 0.01676) | 0.424692 |
| **Disease duration** | 0.2206 | 0.008343 (-0.001267 to 0.01795) | **0.087576** |

A multiple linear regression model was built taking into account 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 (β=0.2016 p=0.0006 ) and age (β=0.005264 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 ( β= 0.006357, 95%CI 0.0003516,0.008979, p=0.000017), INR value ( β=0.2778, 95%CI 0.1294,0.4263, p=0.000516), CRP mg/dL level ( β=0.001695,95%CI

0.0003516,0.003039,p=0.01472) and Hb level ( β= - 0.02267,95%CI -0.04470, -0.0006463,p=0.01472p=0.043947) are independent predictors of an increased echo-IMT, reaching statistical significance **(table 4)** .

**Table 4**

| **Biologic treatment** (Risk variables) | Coefficient correlation | Regresion Coefficient β (95%CI) | p_value (simple linear regression) |
|---|---|---|---|
| **Age** | 0.6176 | 0.006357 (0.003735 to 0.008979) | **0.000017** |
| **CRP mg/dL** | 0.3783 | 0.001695 (0.0003516 to 0.003039) | **0.014742** |
| **Hb** | -0.3163 | -0.02267 (-0.04470 to -0.0006463) | **0.043947** |
| **INR** | 0.5184 | 0.2778 (0.1294 to 0.4263) | **0.000516** |
| **Col** | -0.1548 | -0.0004186 (-0.001284 to 0.0004466) | 0.333791 |
| **TG** | 0.0153 | 0.00002702 -0.0005447 to 0.0005987) | 0.924329 |
| **Ferritin** | -0.3048 | -0.0003973 -0.0009935 to 0.0001988) | 0.179118 |
| **Iron** | -0.2223 | -0.0007869 (-0.001905 to 0.0003311) | 0.162473 |
| **CDAI** | 0.2422 | 0.0003634 (-0.0002643 to 0.0009912) | 0.243260 |
| **Mayo** | 0.0010 | 0.00005000 (-0.02869 to 0.02879) | 0.997075 |
| **SESCD** | 0.0324 | 0.001417 (-0.01745 to 0.02028) | 0.877911 |
| **Mayo E** | -0.1115 | -0.01731 (-0.1057 to 0.07109) | 0.680944 |
| **BMI** | 0.0897 | 0.003671 (-0.009533 to 0.01687) | 0.577093 |
| **Duration of disease** | 0.0997 | 0.004204 (-0.009386 to 0.01779) | 0.535160 |

In the conventional treatment group, age ( β=0.007436,95%CI 0.005051,0.009820,p=0.000004) and INR value ( β=0.3134,95%CI 0.1374,0.4894,p=0.001494) were the only variables significantly impacting echo-IMT **(table 5).**The differences in variables influencing echo-IMT can be explained by differences between the conventional and biological treatment groups themselves.

| **Conventional treatment** (Risk variables) | Coefficient correlation | Regresion Coefficient β (95%CI) | p_value (simple linear regression) |
|---|---|---|---|
| **Age** | 0.8394 | 0.007436 (0.005051 to 0.009820) | **0.000004** |
| **CRP mg/dL** | -0.0284 | -0.0005036 (-0.009283 to 0.008276) | 0.905403 |
| **Hb** | 0.0408 | 0.004875 (-0.05425 to 0.06400) | 0.864397 |
| **INR** | 0.6614 | 0.3134 (0.1374 to 0.4894) | **0.001494** |
| **Col** | -0.1557 | -0.0006476 (-0.002750 to | 0.524321 |

| | | | |
|---|---|---|---|
| | | 0.001454) | |
| **TG** | 0.0200 | 0.00005199 (-0.001326 to 0.001430) | 0.937234 |
| **Ferritin** | 0.4238 | 0.0005655 (-0.0002860 to 0.001417) | 0.169750 |
| **Iron** | 0.0747 | 0.0002668 (-0.001496 to 0.002030) | 0.754171 |
| **CDAI** | -0.1644 | -0.0003912 (-0.002161 to 0.001379) | 0.629038 |
| **Mayo** | -0.5251 | -0.04274 (-0.1046 to 0.01916) | 0.146565 |
| **SESCD** | -0.1432 | -0.01204 (-0.07478 to 0.05070) | 0.674412 |
| **Mayo E** | -0.3674 | -0.08538 (-0.2785 to 0.1078) | 0.330647 |
| **BMI** | 0.1497 | 0.009465 (-0.02149 to 0.04042) | 0.528640 |
| **Duration of disease** | 0.3827 | 0.01233 (-0.002409 to 0.02707) | **0.095817** |

As mentioned before, patients receiving biological therapy included in our study have a more severe disease, CRP and Hb being criteria used to assess severity/ activity. Cholesterol level (β=0.001286,95%CI 0.0004952,0.002076,p=0.003180) and triglycerides level (β=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 ( β=0.005645, $p < 0.0001$), INR value ( β=0.1728 , p=0.0089) and Iron value ( β = -0.0008147, p=0.0476) remain strong predictors of an increased echo-IMT, maintaining statistical significance. In the conventional therapy group, age ( β=0.006658, p=0.0001) 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**

A novel approach, using algorithmic tight-control scenarios has recently been introduced in order

to improve inflammatory bowel disease patient's prognosis and quality of life [6]. The accepted therapeutic goal is the mucosal healing although more ambitious voices argue that histologic healing might be a better step[7,8] and that in Crohn's disease transmural healing will be advisable[9]. There is a consensus towards that an active approach with periodic evaluations, 3 to 6 months apart, with CRP and fecal calprotectin assessments are recommended[6,10], endoscopy being also used in a proactive manner[11]. This allows to permanently adjust the therapy according to results obtained.

In this study, we evaluated a series of patients with inflammatory bowel disease, both on conventional or biological therapy. In order to evaluate early atherosclerosis, we used IMT, a marker with a high predictive value for subclinical atherosclerosis [12,13].

We demonstrated the presence of ultrasonographic markers for subclinical atherosclerosis in IBD patients. Indeed, common carotid IMT, a highly predictive marker of early atherosclerosis, was greater in IBD patients than in matched healthy controls although not significantly. These findings can be explained by the fact we included in the study patients with a limited duration of the disease, early treated and the most importantly efficiently treated(the majority of cases being in remission).

In the IBD group, age was correlated with increased eco IMT values. Also severity of the disease and its duration were predictors of an increased IMT. We also found that the disease activity (measured by the endoscopic scores and CRP) correlates with higher eco IMT. Also anemia was correlated with increased eco IMT in the biologic treatment group with statistical significance [12,13]. These findings are superposable with the data from multiple other studies largely presented in the general part of the thesis.

As shown by previous studies, an efficient treatment, started early in the course of the disease, prevents intestinal destruction, lowers the need for hospitalization and surgery and it is ultimately able to change the natural course of the disease[14]. Therefore, this proactive approach can significantly change the natural history of the disease, as well as the patient' s prognosis[6].

The major complications of atherosclerosis represent an important cause of morbidity and

mortality and it is to be considered if a "treat to target" proactive approach should be extended to the cardiovascular prevention as well. We consider that although in this small study we did not reach statistical significance of eco IMT between IBD patients and control, there was a trend of the eco IMT values which proved that a uncontrolled bowel disease correlates with endothelial dysfunction. A particular finding is the correlation between eco IMT and increased INR values which is an original contribution of our study and proves in our opinion the complex mechanisms and imbalances involved in intestinal inflammation and endothelial dysfunction. It is our goal to further explore this dangerous liaison in further studies.

Ultrasound evaluation of IMT might serve as an 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[17,18] a scenario where treatment can be tailored with a step up/add on approach of their biologic treatment for example but also adding statin therapy or other therapeutic measures targeting cardiovascular risk.

Probably a better control of the disease might result also in a decrease of the cardiovascular risk. We must however acknowledge that in our group of patients their disease was controlled by the biologic therapy in the majority of cases and their activity indexes were rather low.

**Conclusions**

Carotid ultrasound with eco IMT should be considered in high-risk groups for cardiovascular disease such as in patients with 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. This approach can lead to early intervention, top down therapeutic approach or escalation of therapy, thereby contributing to reduced incidence of unfavourable outcomes related to endothelial dysfunction and atherosclerosis in these patients.

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