



# Abdominal aortic calcification in patients with inflammatory bowel disease: does anti-tumor necrosis factor $\alpha$ use protect from chronic inflammation-induced atherosclerosis?

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**Background/Aims:** Abdominal aortic calcium (AAC) deposition has been suggested as a marker of early atherosclerosis. There is no published data on the evaluation of AAC in inflammatory bowel disease (IBD). **Methods:** AAC was quantified by computed tomography or enterography scans performed in 98 IBD patients and 1:1 age and sex matched controls. AAC deposition was correlated with IBD characteristics, disease activity or severity parameters, laboratory tests and cardiovascular disease (CVD) risk factors. **Results:** Moderate-severe grade of AAC was found in 35.7% of IBD patients compared to 30.6% of controls ( $P=0.544$ ). IBD with CVD and ulcerative colitis patients had significantly higher rates of more severe atherosclerotic lesions ( $P=0.001$  and  $P=0.01$ , respectively). AAC deposition was similarly distributed in age groups ( $<45$ ,  $45-64$ , and  $\geq 65$  years) among patients and controls. Multivariate analysis after excluding CVD risk confounders for non-CVD patients found extensive disease ( $P=0.019$ ) and lifetime steroids ( $P=0.04$ ) as independent risk factors for AAC. Anti-tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) use was negatively associated with AAC deposition in non-CVD IBD patients (odds ratio, 0.023; 95% confidence interval, 0.001–0.594;  $P=0.023$ ). **Conclusions:** More than one-third of IBD patients have moderate to severe AAC. Better control of inflammation with anti-TNF- $\alpha$  agents seems to protect IBD patients from AAC deposition and subsequent atherosclerosis. (Intest Res 2022;20:495-505)

**Key Words:** Abdominal aortic calcium; Atherosclerosis; Tumor necrosis factor-alpha; Crohn disease; Colitis, ulcerative

## INTRODUCTION

Inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) is characterized by a chronic intermittent inflammation of the gastrointestinal tract accompanied with various extraintestinal manifestations (EIMs). Cardiovascular diseases (CVD) have been considered as an EIM in IBD and indices of subclinical atherosclerosis such as

carotid intima-media thickness, aortic stiffness and percentage of atherosclerotic plaque in the carotid have been found to increase in IBD patients.<sup>1-3</sup> CVD risk among IBD patients is modestly elevated, although the prevalence of traditional cardiovascular risk factors among patients with IBD is not higher than in healthy controls.<sup>3,4</sup>

The role of chronic systemic inflammation in accelerating atherogenesis and establishing early CVD has been extensively investigated in rheumatological diseases.<sup>5</sup> Inflammatory burden and immune dysregulation play a fundamental role in arterial stiffening, plaque formation subsequently leading to CVD.<sup>6-8</sup> Endothelial dysfunction, oxidative stress, macrophage

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accumulation, and pro-inflammatory cytokine production are common pathways implicated in both chronic inflammatory and atherogenic process.<sup>9-11</sup> In IBD the interplay between genetic and environmental factors cause imbalance of gut bacteria flora and consequent disruption of the intestinal barrier that leads to release of inflammatory cytokines into the systemic circulation resulting to systemic inflammation and accelerating atherosclerotic process.<sup>12</sup>

The risk for coronary artery disease and stroke have been found to increase in IBD patients especially in female and young patients.<sup>13-15</sup> A recent meta-analysis of cohort studies found that the disease activity is an independent risk factor for acute arterial events.<sup>16</sup>

Abdominal aortic calcium (AAC) deposition has been recently proposed as a marker of early atherosclerosis, taking place earlier than coronary atherosclerosis.<sup>17,18</sup> A study with participants from the Framingham cohorts showed that baseline measurement of AAC using serial multidetector computed tomography scans is an independent risk factor for coronary aortic calcium (CAC) progression.<sup>18</sup> In the same study incident AAC and absolute progression of AAC were detected more frequently than CAC pointing out a predicting role of AAC for asymptomatic CVD.

As far as we are aware there is no published data on AAC in patients with IBD. Taking into consideration that abdominal computed tomography (CT) or computed tomography enterography (CTE) imaging is performed routinely in clinical practice to map or monitor disease especially in patients with CD, we aimed for the first time at comparing AAC grading among IBD patients and controls and investigating any possible association between AAC grading and markers of IBD activity or severity.

## METHODS

### 1. Study Population

Consecutive patients that are regularly followed at the outpatient IBD clinic of University Hospital of Heraklion with complete data and who had a CT or CTE performed for evaluation of disease activity, disease extent and possible complications the last 8 years (March 2013 to March 2021) were included.

Inclusion criteria for IBD patients were (1) established IBD diagnosis for more than 6 months before CT scan was performed, (2) available CT or CTE scan that was performed during the study period, and (3) complete follow up clinical data available.

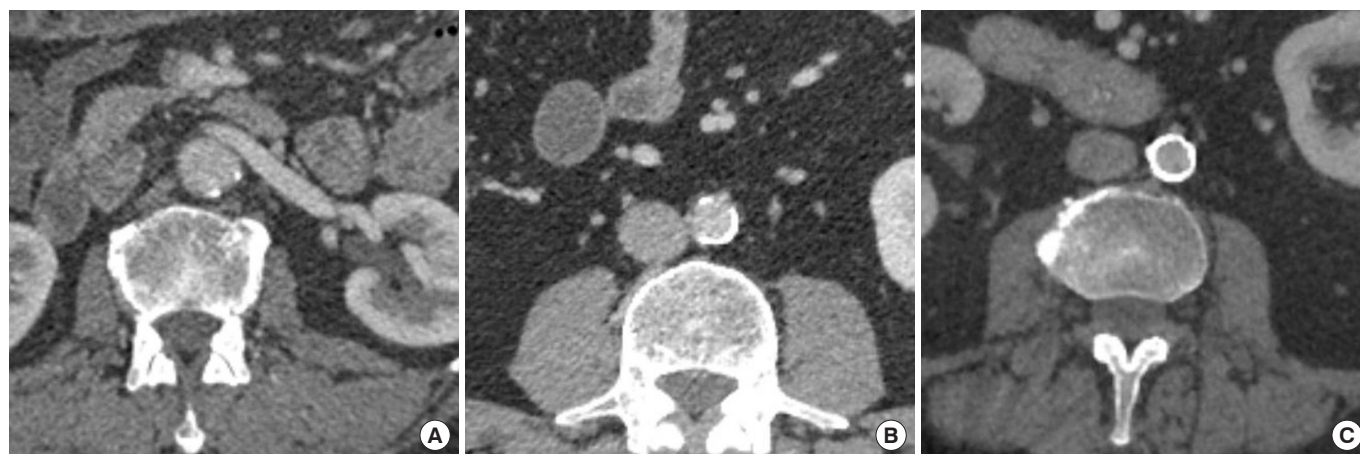
Exclusion criteria for IBD patients were chronic renal disease, presence of other chronic inflammatory disease and history of malignancy.

As controls were selected non-IBD patients among those that had a CT scan at the outpatient clinic during the same period of our study and were matched 1:1 by age  $\pm 5$  years, sex and CVD diagnosis. History of IBD, rheumatic disease, malignancy or chronic renal disease were among exclusion criteria for the control group. Furthermore, IBD patients without CVD were matched 1:1 with controls according to their history of any of the classical CVD risk factors including hypertension, dyslipidemia or diabetes. This information was confirmed by International Classification of Diseases, 10th Revision (ICD-10) codes used in the national health system electronic database of prescribed medicines (e-prescription).

### 2. Data Collection

We retrospectively collected data for 98 IBD participants concerning demographics, age at the time of CT or CTE, Montreal classification, disease duration, traditional risk factors for atherosclerosis including smoking, body mass index (BMI), hypertension, dyslipidemia and diabetes mellitus, history of CVD (myocardial infarction, coronary insufficiency, angina, ischemic stroke, hemorrhagic stroke, transient ischemic attack, peripheral artery disease, and heart failure), IBD treatment until the time CT or CTE scan was performed (steroids at least once used lifetime for an IBD flare, immunomodulators, anti-tumor necrosis factor  $\alpha$  [TNF- $\alpha$ ]), history of IBD related surgery, history of IBD related hospitalization and EIMs of the musculoskeletal system, i.e., peripheral and axial spondylarthritis (sacroiliitis, ankylosing spondylarthritis) all derived from our IBD electronic registry.

Analysis of calcium deposits on abdominal aorta from CT or CTE scans was carried out by the same radiologist. AAC quantification was performed using a previously described semi-quantitatively grading system, included grade 0: no calcifications, grade 1: minimal non-circumferential non-contiguous scattered arterial calcifications, grade 2: mild non-circumferential non-contiguous arterial calcifications involving numerous segments with  $< 50\%$  vessel wall calcification, grade 3: moderate non-circumferential non-contiguous calcifications of multiple arterial segments with  $> 50\%$  vessel wall calcification of multiple segments, but without any completely concentric calcifications, grade 4: moderate calcifications involving multiple segments of arteries with most areas having  $> 50\%$  involvement with calcification.<sup>19,20</sup> Isolated completely con-



**Fig. 1.** Computed tomography of 3 inflammatory bowel disease patients with different grades of abdominal aortic calcium deposition. (A) Grade 1, minimal non-circumferential non-contiguous scattered arterial calcifications. (B) Grade 3, moderate non-circumferential non-contiguous calcifications of multiple arterial segments with >50% vessel wall calcification but without any completely concentric calcifications. (C) Grade 5, diffuse calcifications in multiple levels of completely concentric calcifications.

centric calcifications may be also present, grade 5, diffuse calcifications with multiple levels of completely concentric calcifications (Fig. 1). AAC grades 0–2 was coded as none to mild atherosclerotic lesions and grades 3–5 was coded as moderate-severe atherosclerotic lesions.

Other information derived from CTE were (1) features of active (including mucosal hyperenhancement, wall thickening (>3 mm), mural stratification with a prominent vasa recta [comb sign], and mesenteric fat stranding) or inactive inflammatory disease (none of the above),<sup>21</sup> (2) presence of stricture, and (3) the extend of CD, classified as limited disease in case of less than 40 cm ileal involvement and as extended disease if the affected intestinal area was over 40 cm.<sup>22</sup>

For UC, proctitis or left-sided colitis according to Montreal classification were also coded as limited disease. In cases that endoscopy was available close to the time of the CT scan (until 1 month before or after), large deep ulcers on the mucosa for CD or Mayo score III for UC, were coded as severe endoscopic lesions in contrast to mild-moderate endoscopic lesions for both CD (none, aphthous ulcers, superficial small ulcers) and UC (Mayo 0-II).

Among laboratory tests hemoglobin, C-reactive protein, albumin, glucose, total cholesterol and triglycerides, all assessed the same day or up to 14 days before or after the CT scan, were recorded.

### 3. Ethical Considerations

All participants provided written informed consent prior to enrollment and the research protocol was approved by the Uni-

versity Hospital of Heraklion Institutional Review Board (protocol No. 28-2-2019/3075).

### 4. Statistical Analysis

Comparisons were made between all IBD patients and age and sex matched controls, IBD patients without CVD and age and sex matched non-IBD patient group without CVD and IBD patients with CVD and non-IBD patients with CVD. Student *t*-test was used for continuous variables and Fisher exact probability test or the chi-square test for the analysis of categorical variables. All variables found to be significant in the univariate analyses for IBD patients were entered into the multivariate analyses using a forward step-wise logistic regression model (0.05 for entry and 0.10 for removal probability).

All tests were performed at a 95% confidence level. A *P*-value of <0.05 was considered statistically significant. Statistical analyses were performed, using the SPSS software package version 24 (IBM Corp., Armonk, NY, USA).

## RESULTS

Demographics, clinical characteristics, and treatment data of the 98 IBD patients who were included in the study are shown in Table 1. The 24 IBD patients with CVD had significant differences in age, BMI, smoking status, gender, as well as clinical IBD characteristics compared with the 74 non-CVD IBD patients (Tables 1 and 2). Furthermore, the mean age at UC diagnosis was higher compared to CD ( $48.73 \pm 14.57$  years vs.  $43.03 \pm 15.65$  years,  $P=0.093$ ) and even higher among non-CVD pa-

**Table 1.** Demographics and Clinical Characteristics of IBD Patients Included in the Study

Characteristics	IBD patients (n = 98)	IBD with CVD (n = 24)	IBD without CVD (n = 74)	P-value	OR (95% CI)
Age at diagnosis (yr)	44.78 ± 15.48	54.42 ± 12.65	41.65 ± 15.08	< 0.001	3.42 (5.99–19.55)
Age at study entry (yr)	58.75 ± 14.45	68.17 ± 10.39	55.40 ± 14.20	< 0.001	3.14 (6.53–19.01)
IBD duration at CTE/CT (yr)	11.67 ± 10.13	11.12 ± 10.56	11.85 ± 10.05	NS	
IBD duration (yr)	14.04 ± 10.40	13.66 ± 10.60	14.16 ± 10.41	NS	
Female sex	29 (29.6)	4 (13.8)	25 (86.2)	< 0.001	
Body mass index (kg/m <sup>2</sup> )	27.25 ± 4.93	31.62 ± 27.41	29.17 ± 25.44	0.006	1.22 (1.02–5.89)
Smoking status (n = 95)				< 0.001	
Ever smokers	75 (79.0)	20 (87.0)	55 (76.4)		
Never smokers	20 (21.1)	3 (13.0)	17 (23.6)		
Diagnosis				< 0.001	
UC	30 (30.6)	11 (45.8)	19 (25.7)		
CD	68 (69.4)	13 (54.2)	55 (74.3)		
CD Montreal classification age				< 0.001	
A1	1 (1.0)	1 (4.2)	0		
A2	36 (36.7)	4 (16.7)	32 (43.2)		
A3	61 (62.3)	19 (79.2)	42 (56.8)		
Location (n = 68)				< 0.001	
L1	30 (44.1)	6 (46.1)	24 (43.6)		
L2	12 (17.7)	3 (23.1)	9 (16.4)		
L3	26 (38.2)	4 (30.8)	22 (40.0)		
Behavior (n = 68)				< 0.001	
B1	32 (47.1)	8 (61.5)	24 (43.6)		
B2	29 (42.6)	3 (23.1)	26 (47.3)		
B3	7 (10.3)	2 (15.4)	5 (9.0)		
Perianal disease (n = 69) <sup>a</sup>				< 0.001	
Perianal disease	15 (21.7)	5 (35.7)	10 (18.2)		
No perianal disease	54 (78.3)	9 (64.3)	45 (81.8)		
UC Montreal classification extent (n = 30)				< 0.001	
E1	2 (66.7)	2 (18.2)	0		
E2	13 (43.3)	6 (54.5)	7 (36.8)		
E3	15 (50.0)	3 (27.3)	12 (63.2)		
Musculoskeletal extraintestinal manifestations	35 (35.7)	12 (50.0)	23 (31.1)	< 0.001	
IBD related surgery	18 (18.4)	3 (12.5)	15 (20.3)	< 0.001	
Endoscopic lesions: severe lesions (n = 97)	28 (28.9)	3 (12.5)	25 (34.3)	< 0.001	
CTE/CT findings					
Disease extend: extensive disease (n = 96)	41 (42.7)	7 (29.2)	34 (47.2)	< 0.001	
Inflammatory disease: active (n = 68)	56 (57.1)	10 (41.7)	46 (61.6)	< 0.001	
Strictureing disease <sup>b</sup>	28 (28.6)	3 (12.5)	25 (33.8)	< 0.001	

Values are presented as mean ± standard deviation or number (%).

<sup>a</sup>One patient with UC and perianal disease was included.

<sup>b</sup>Patients with structuring disease according to CT/CTE findings are 28, one less than B2 Montreal classification because of a right colectomy history.

IBD, inflammatory bowel disease; CVD, cardiovascular disease; OR, odds ratio; CI, confidence interval; CTE, computed tomography enterography; CT, computed tomography; UC, ulcerative colitis; CD, Crohn's disease; NS, not significant.

**Table 2.** Cardiovascular Risk Factors, Inflammation Modulating Factors and Blood Tests at Study Entry

Variable	IBD patients (n = 98)	IBD with CVD (n = 24)	IBD without CVD (n = 74)	P-value
Hypertension	33 (33.7)	21 (87.5)	12 (16.8)	<0.001
Diabetes mellitus	12 (12.2)	8 (33.3)	4 (5.4)	<0.001
Dyslipidemia	28 (28.6)	17 (70.8)	11 (14.9)	<0.001
Anti-TNF- $\alpha$ agents	57 (58.2)	9 (37.5)	48 (64.9)	<0.001
Lifetime steroids	78 (79.6)	18 (75.0)	60 (81.1)	<0.001
Immunomodulator	65 (66.3)	14 (58.3)	51 (68.9)	<0.001
Hemoglobin (g/dL)	13.30 $\pm$ 1.50	13.22 $\pm$ 1.82	13.36 $\pm$ 1.41	0.710
C-reactive protein (mg/dL)	1.72 $\pm$ 0.40	1.42 $\pm$ 0.57	1.83 $\pm$ 0.52	0.673
Glucose (mg/dL)	105.70 $\pm$ 44.00	118.70 $\pm$ 74.80	102.08 $\pm$ 30.42	0.135
Cholesterol (mg/dL)	179.50 $\pm$ 59.00	165.10 $\pm$ 26.10	184.50 $\pm$ 62.60	0.195
Albumin (mg/dL)	4.00 $\pm$ 0.60	3.99 $\pm$ 0.55	4.04 $\pm$ 0.55	0.723

Values are presented as number (%) or mean  $\pm$  standard deviation.

IBD, inflammatory bowel disease; CVD, cardiovascular disease; TNF, tumor necrosis factor.

tients ( $47.74 \pm 16.11$  years for UC patients vs.  $39.55 \pm 14.27$  years for CD patients,  $P = 0.04$ ). Regarding other classical risk factors for CVD, the percentage of male sex, hypertension, diabetes mellitus and dyslipidemia was higher among UC patients than CD patients (86.7% vs. 63.2%,  $P < 0.001$ ; 50% vs. 26.5%,  $P < 0.001$ ; 16.7% vs. 10.3%,  $P < 0.001$ ; and 46.7% vs. 20.6%,  $P < 0.001$ , respectively).

Regarding IBD treatment, anti-TNF, steroids, and immunomodulators were used more frequently among CD patients compared to UC (76% vs. 30%,  $P < 0.001$ ; 80.9% vs. 23%,  $P < 0.001$ ; and 83.8% vs. 26.7%,  $P < 0.001$ , respectively).

Moderate-severe grade of AAC deposition was found in 35.7% of IBD patients compared to 30.6% of control group ( $P = 0.448$ ). IBD patients with CVD and UC patients had significantly higher rates of more severe atherosclerotic lesions compared to IBD patients without CVD and CD patients respectively (66.7% vs. 25.7%,  $P = 0.001$  and 56.7% vs. 26.5%,  $P = 0.01$ ) (Table 3). CD patients without CVD had lower rates of severe AAC deposition compared to non-CVD UC patients (18.2% vs. 47.4%,  $P = 0.025$ ). Mild degree of AAC deposition was associated with more frequent use of biologic agents ( $P = 0.016$ ) and lifetime steroids ( $P = 0.001$ ) and reversely associated with endoscopic ( $P = 0.004$ ) or radiological activity ( $P = 0.026$ ) (Table 3).

The distribution of AAC deposition was similar among all IBD patients and controls (grade 0: 27.6% vs. 30.6%, grade 1: 15.3% vs. 17.3%, grade 2: 21.4% vs. 21.4%, grade 3: 19.4% vs. 20.4%, grade 4: 13.3% vs. 9.2%, and grade 5: 3.1% vs. 1%, respectively,  $P = 0.844$ ), IBD patients without CVD and their matched controls (grade 0: 33.8% vs. 40.5%, grade 1: 18.9% vs. 16.2%, grade

2: 21.6% vs. 23%, grade 3: 13.5% vs. 14.9%, grade 4: 9.5% vs. 5.4%, and grade 5: 2.7% vs. 0%, respectively,  $P = 0.623$ ) or IBD patients with CVD and their matched controls with CVD (grade 0: 8.3% vs. 0%, grade 1: 4.2% vs. 20.8%, grade 2: 20.5% vs. 16.7%, grade 3: 37.5% vs. 37.5%, grade 4: 25% vs. 20.8%, and grade 5: 4.2% vs. 4.2%, respectively,  $P = 0.432$ ). Furthermore, no association of AAC with gender was found among IBD patients with ( $P = 0.053$ ) or without CVD (32% of female vs. 22.4% of male with moderate to severe AAC: odds ratio [OR], 0.615; 95% confidence interval [CI], 0.210–1.803;  $P = 0.408$ ).

No statistically significant difference was found in the degree of AAC among age groups (<45, 45–64, and  $\geq 65$  years) of IBD patients and controls ( $P = 0.85$  for mild and  $P = 0.634$  for more severe AAC lesions).

Among 55 non-CVD patients with CD, ileal involvement >40 cm was found in 21 patients. Moderate-severe AAC deposition was found in 2 patients with extensive CD compared to 19 patients with mild AAC deposition and extensive CD ( $P = 0.229$ ). No association was also found among 12 UC patients with extensive disease and the degree of AAC deposition. Moderate-severe AAC deposition was found in 4 UC patients with extensive disease compared to 8 UC patients with mild AAC deposition and extensive disease ( $P = 0.089$ ).

Multivariate regression analysis after adjustment with traditional risk factors for CVD (including sex, smoking, BMI, hypertension, diabetes, and dyslipidemia) revealed a positive association of the degree of AAC deposition among all IBD patients with extensive disease according to radiological findings (OR, 22.711; 95% CI, 1.469–208.897;  $P = 0.006$ ), age at IBD di-



**Table 3.** Univariate Analysis between Study Variables and Extent of AAC

Variable	None-mild atherosclerotic lesions (AAC)	Moderate-severe atherosclerotic lesions (AAC)	P-value
IBD patient (n = 98)	63 (64.3)	35 (35.7)	0.544
Control (n = 98)	68 (69.4)	30 (30.6)	
IBD with CVD (n = 24)	8 (33.3)	16 (66.7)	1.000
Control with CVD (n = 24)	9 (37.5)	15 (62.5)	
IBD without CVD (n = 74)	55 (74.3)	19 (25.7)	0.558
Control without CVD (n = 74)	59 (79.7)	15 (20.3)	
IBD with CVD (n = 24)	8 (33.3)	16 (66.7)	0.001
IBD without CVD (n = 74)	55 (74.3)	19 (25.7)	
UC	13 (43.3)	17 (56.7)	0.010
CD	50 (73.5)	18 (26.5)	
Age at diagnosis (yr)	40.25 ± 14.52	52.91 ± 13.89	<0.001
IBD duration at CTE/CT (yr)	10.68 ± 9.21	13.47 ± 11.52	0.193
Sex			
Female, IBD	20 (69.0)	9 (31.0)	0.773
Female, control	21 (72.4)	8 (27.6)	
Male, IBD	43 (62.3)	26 (37.7)	0.475
Male, control	47 (68.1)	22 (31.9)	
CD Montreal location (n = 68)			0.370
L1	21 (70.0)	9 (30.0)	
L2	8 (66.7)	4 (33.3)	
L3	21 (80.8)	5 (19.2)	
CD behavior (n = 68)			0.396
B1	22 (68.7)	10 (31.3)	
B2	22 (75.9)	7 (24.1)	
B3	6 (85.7)	1 (14.3)	
Perianal disease (n = 69) <sup>a</sup>	38 (70.4)	16 (29.6)	0.366
IBD surgery	15 (85.3)	3 (16.7)	0.123
Anti-TNF- $\alpha$ agents	43 (75.4)	14 (24.6)	0.016
Lifetime steroids	57 (73.1)	21 (26.9)	0.001
Immunomodulator	44 (67.7)	21 (32.3)	0.452
Endoscopic lesions: severe lesions (n = 97)	25 (89.3)	3 (10.7)	0.004
CTE/CT findings			
Disease extend: extensive disease (n = 96)	31 (75.6)	10 (24.4)	0.060
Inflammatory disease (n = 68)	40 (81.6)	9 (18.4)	0.026
Strictureing disease	22 (78.6)	6 (21.4)	0.123
Musculoskeletal extraintestinal manifestations	20 (57.1)	15 (42.9)	0.401
Body mass index (kg/m <sup>2</sup> )	27.47 ± 4.80	26.82 ± 5.25	0.554
Smoking history (n = 95)			0.097
Ever smokers	45 (60.0)	30 (40.0)	
Never smokers	17 (85.0)	3 (15.0)	

(Continued to the next page)

**Table 3.** Continued

Variable	None-mild atherosclerotic lesions (AAC)	Moderate-severe atherosclerotic lesions (AAC)	P-value
Hypertension	11 (33.3)	22 (66.7)	<0.001
Diabetes mellitus	5 (41.7)	7 (58.3)	0.155
Dyslipidemia	10 (35.7)	18 (64.3)	0.001
Hemoglobin (g/dL)	13.27 ± 1.67	13.43 ± 1.44	0.657
C-reactive protein (mg/dL)	2.26 ± 4.76	0.82 ± 1.40	0.100
Glucose (mg/dL)	105.33 ± 51.82	106.45 ± 22.52	0.909
Cholesterol (mg/dL)	187.04 ± 64.75	166.44 ± 33.44	0.129
Albumin (mg/dL)	3.98 ± 0.59	4.16 ± 0.45	0.182

Values are presented as number (%) or mean ± standard deviation.

<sup>a</sup>Plus 1 UC patient with perianal disease.

AAC, abdominal aortic calcium; IBD, inflammatory bowel disease; CVD, cardiovascular disease; UC, ulcerative colitis; CD, Crohn's disease; CTE, computed tomography enterography; CT, computed tomography; TNF, tumor necrosis factor.

**Table 4.** Multivariate Regression Analysis for Identifying Parameters Associated with Abdominal Aortic Calcium Severity among IBD Patients

Variable	aOR (95% CI)	P-value
Age at IBD diagnosis	1.142 (1.038–1.257)	0.006
IBD duration	1.179 (1.037–1.342)	0.012
Extensive IBD (CTE/CT)	22.711 (1.469–208.897)	0.006
Hypertension	13.118 (1.810–95.079)	0.011
Steroids	0.038 (0.003–0.521)	0.014
Immunomodulators	101.379 (3.745–2,744.33)	0.006

Variables entered: age IBD diagnosis, IBD duration, IBD diagnosis, Montreal behavior, sex, smoking, body mass index, CTE findings (disease extent), anti-tumor necrosis factor  $\alpha$  use, lifetime steroids, immunomodulators, hypertension, dyslipidemia, diabetes.

IBD, inflammatory bowel disease; aOR, adjusted odds ratio; CI, confidence interval; CTE, computed tomography enterography; CT, computed tomography.

agnosis (OR, 1.142; 95% CI, 1.038–1.257;  $P=0.006$ ), IBD duration (OR, 1.179; 95% CI, 1.037–1.342,  $P=0.012$ ), lifetime steroids (OR, 0.038; 95% CI, 0.003–0.521,  $P=0.014$ ), immunomodulators' use (OR, 101.379; 95% CI, 3.745–2,744.33;  $P=0.006$ ), and hypertension (OR, 13.118; 95% CI, 1.810–95.079;  $P=0.011$ ) (Table 4). Subgroup analysis including IBD patients without CVD found extensive disease (OR, 25.661; 95% CI, 1.714–384.181;  $P=0.019$ ) and lifetime steroids (OR, 18.287; 95% CI, 1.141–292.983;  $P=0.040$ ) as independent risk factors for AAC and subsequent CVD (Table 5). Anti-TNF- $\alpha$  use had a reverse association with the degree of ACC in non-CVD IBD patients (OR, 0.023; 95% CI, 0.001–0.594;  $P=0.023$ ) (Table 5).

**Table 5.** Multivariate Regression Analysis for Identifying Parameters Associated with Abdominal Aortic Calcium Severity among IBD Patients without Cardiovascular Disease

Variable	aOR (95% CI)	P-value
Extensive IBD	25.661 (1.714–384.181)	0.019
Anti-TNF- $\alpha$ use	0.023 (0.001–0.594)	0.023
Steroids lifetime	18.287 (1.141–292.983)	0.040

Variables: age at IBD diagnosis, Montreal behavior (B2 vs. B1, B3), computed tomography enterography findings (IBD extent), anti-TNF- $\alpha$ , steroids, sex, smoking, body mass index, hypertension, diabetes, dyslipidemia. IBD, inflammatory bowel disease; aOR, adjusted odds ratio; CI, confidence interval; TNF, tumor necrosis factor.

## DISCUSSION

This is the first study evaluating the association between the inflammatory burden of IBD and AAC, a marker that has been positively correlated with CAC and CVD risk.

More than one-third of the IBD participants with a longstanding disease course had moderate or severe AAC deposits based on their CT/CTE findings.

However, the distribution of AAC was found similar among all IBD patients and the control group, even in the subgroup with concurrent established CVD. There is possibly a dual explanation for these results; either the inflammatory burden in IBD plays a less important role in the chronic atherogenic process, or that in the era of biologic agents and anti-TNF- $\alpha$  drugs a better control of inflammation is achieved in combination with their anti-fibrotic properties. The latter is probably suggested by our study, whereas a protective role of anti-TNF- $\alpha$  in

AAC among non-CVD IBD patients was found after excluding all CVD risk confounders. Interestingly, this subgroup included patients with lower age at diagnosis and study entry, but more severe inflammatory burden (more extensive disease, severely active disease, stricturing disease, and history of previous bowel surgery) (Tables 1 and 2) with almost two-thirds of the patients (64.9%) anti-TNF exposed. The protective role is also supported by the findings of a nationwide cohort French study that showed a decreased risk for acute arterial events among anti-TNF exposed IBD patients.<sup>23</sup> Additionally, in the group of IBD patients with CVD, antihypertensives, lipid-lowering and anti-diabetic medicines may contribute to both decelerate the atherogenic process and IBD course.<sup>24,25</sup>

In other autoimmune diseases such as rheumatoid arthritis (RA) accelerated atherosclerosis has been attributed to TNF- $\alpha$  modifying effect on endothelium, insulin and lipids metabolism and hemostatic balance, indicating a protective role of anti-TNF treatment on CVD.<sup>26</sup> In a recent systematic review and meta-analysis use of anti-TNF- $\alpha$  inhibitors and methotrexate was associated with a decreased risk with CVD events in RA, whereas use of corticosteroids was associated with an increased risk.<sup>27</sup>

As expected steroids have been associated with the degree of AAC in all IBD patients and in the non-CVD group, in our study. The known effect of chronic steroid use on metabolism causing hyperglycemia, hyperlipidemia and hypertension increase the risk for CVD in IBD patients as well as in the general population.<sup>28-30</sup>

In the present study moderate to severe AAC was more prevalent in IBD participants without CVD compared to controls (25.7% vs. 20.3%) but the difference was not significant. These results are in line with the 19% prevalence of high AAC in the Framingham Heart Study.<sup>31</sup> However, there were methodological differences of the 2 studies regarding the used protocol for AAC quantification and the AAC classification.

In our IBD population, the degree of AAC distribution did not differ among gender groups of both IBD patients and controls, with or without established CVD. In the Framingham Heart Study, a greater AAC in male participants without any CVD risk was reported.<sup>31</sup> Indeed, AAC >0 ranged from 15.5% in males versus 7.8% in females aged <45 years, 45.2% versus 22.6% aged 45–54 years, 81.8% versus 59.1% aged 55–64 years respectively to 100% of both sexes aged  $\geq 75$  years. Among all participants aged <45 years old with or without CVD factors, less than 1 in 6 participants had AAC >0, reaching approximately 9 in 10 at age over 65 years. In line with the above findings, our study

showed that AAC burden increased significantly with advancing age in both IBD and controls. More advanced age at diagnosis remained an independent risk factor for more severe AAC lesions.

Furthermore, subgroup analysis for IBD type showed higher mean age at UC diagnosis compared to CD ( $P=0.093$ ). This difference at mean age at diagnosis was even higher among non-CVD patients ( $P=0.04$ ) which could in part justify the increased rates of moderate-severe AAC deposition found among older UC participants. These results are in line with the known association of older age with higher rates of AAC deposition and atherosclerosis.<sup>32</sup>

Compared to CD, UC patients had also higher rates of other classical risk factors for CVD (male sex, hypertension, diabetes mellitus, and dyslipidemia). Finally, multivariate analysis after adjustment with traditional risk factors did not reveal diagnosis of UC as risk factor for higher grade of AAC deposition in our study population.

As for IBD treatment, anti-TNF ( $P<0.001$ ), steroids use ( $P<0.001$ ), and immunomodulators use ( $P<0.001$ ) use was more frequent among CD patients compared to UC and CD patients without CVD had lower rates of severe AAC deposition ( $P=0.025$ ). Further multivariate subgroup analysis including all IBD non-CVD patients showed a protective role of anti-TNF use on AAC deposition, but no association was found on IBD type, suggesting that better disease control may inhibit mechanisms of immune mediated atherogenesis. Future studies with larger sample size could better evaluate the possible relation of UC or CD chronic inflammation and atherosclerosis.

In contrast to IBD, AAC has been found more prevalent in patients with RA than in controls (71.2% vs. 54.7%,  $P=0.04$ ).<sup>32</sup> The mean AAC score was  $1.0 \pm 1.3$  in patients with RA and  $0.7 \pm 1.4$  in controls ( $P=0.02$ ). In multivariate analysis older age and erosive arthritis were found as independent determinants of abdominal aortic calcification in patients with RA.<sup>33</sup>

A key strength of the present study is that we included both radiological and endoscopic data performed at the time of the study to assess disease severity. Most studies focus on Montreal classification and/or including the mucosal lesions found in a recent endoscopy which represent a time-point evaluation and lack information on the radiological extent and total structural damage at the time of the study. In our study, increased AAC deposition was further associated with extensive disease in UC and over 40 cm ileal involvement in CD according to radiological findings implying a link between chronic inflammation-induced arterial changes leading to CVD. In line with the



above, the duration of UC or CD was also found as an independent risk factor for more severe AAC. Conversely, C-reactive protein and other biomarkers that represent a snapshot of the disease biochemical inflammatory activity did not show any association with AAC grade.

Limitations of our study are the small sample size, the retrospective study design, the lowest prevalence of female patients and UC diagnosis in our study group but this could be justified by the fact that CT and especially CTE is routinely asked mainly in patients with CD. Patients with UC usually undergo abdominal CT for other reasons rather than evaluation of UC. Therefore, selection bias may influence the results of our study. The older age in our IBD population could be attributed to several factors: (1) CTE scan is not performed regularly at young IBD patients due to radiation safety reasons, physicians order MRE instead; (2) often CTE is used late in the disease course in order to detect for complications; (3) one-fifth of the included patients had established CVD and usually these patients are more aged; (4) our patients' age at IBD diagnosis was also older. Regarding the high proportion of ever smokers of our study population, it could be related with the reported high prevalence of smoking in Greece (up to 38.2% of adults  $\geq 15$  years, over 60% among male Greeks).<sup>34,35</sup>

Another arguable weakness of the study is that there is no validation of the ACC quantification scores in the Greek population.

In conclusion, moderate-severe AAC is found in almost 35% of IBD patients and is associated with disease extend and duration in patients with IBD. It could be suggested to perform a baseline CT/CTE at diagnosis of IBD to both map IBD and quantify AAC in order to predict the group of patients at risk for CVD. In that case further research in larger IBD population could define the exact time for radiological re-evaluation and possible need for timely reference to cardiologist.

Furthermore, it seems that better control of chronic inflammation in IBD probably protects from atherogenesis and future CVD events. The exact mechanisms by which anti-TNF- $\alpha$  agents exert their protective role in CVD risk in IBD should be elucidated.

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No potential conflict of interest relevant to this article was reported.

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Not applicable.

### Author Contribution

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