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Increased arterial stiffness in inflammatory bowel diseases is dependent upon inflammation and reduced by immunomodulatory drugs

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ABSTRACT

Background: Inflammatory bowel diseases (IBD) are associated with an increased cardiovascular risk that is not fully explained by traditional cardiovascular risk factors but may be due to inflammation and mediated by an increased arterial stiffness. Aims: Study 1, to investigate the relationship between inflammation and arterial stiffening; Study 2, to look whether aortic stiffening is reduced by immunomodulatory therapy in IBD.

Methods: Study 1 (Cross-sectional study): pulse wave velocity (PWV) was measured in 74 IBD subjects (40 ulcerative colitis and 34 Crohn's disease) and 80 matched controls. Study 2 (Longitudinal study): the effect of therapy on PWV was measured at baseline and 3.4 ± 0.5 years later in 14 IBD subjects treated only with salicylates, 11 subjects treated with steroids and azathioprine, 7 subjects treated with anti TNF-alpha and 30 matched controls.

Results: Study 1: All parameters were comparable between subjects with ulcerative colitis and Crohn's disease. Compared to controls, subjects with ulcerative colitis and those with Crohn's disease have both higher carotid-femoral PWV (7.0 ± 1.1, 7.8 ± 1.7 and 8.0 ± 1.6 m/s, respectively; $P < 0.001$) and carotid-radial PWV (7.2 ± 0.9, 8.8 ± 1.4 and 8.8 ± 1.3 m/s, respectively; $P < 0.001$). In fully adjusted models carotid-femoral PWV was positively associated with disease duration whereas carotid-radial PWV was associated with C-reactive protein and history of relapse. Study 2: in fully adjusted model carotid-femoral PWV increased significantly at follow-up in IBD subjects treated with salicylates but not in those treated with steroids and azathioprine or anti TNF-alpha.

Conclusion: Increased arterial stiffness in IBD is dependent upon inflammation and reduced by immunomodulatory drugs.

1. Introduction

In inflammatory bowel diseases (IBD), ulcerative colitis and Crohn's disease, intestinal microvascular endothelial cells are damaged by an abnormal immune response, resulting in chronic low-grade inflammation followed by episodes of acute inflammation during the periodic reactivation of the disease (relapse). Recent studies have reported that, despite the prevalence of traditional cardiovascular risk factors is lower than in the general population [1–3], the risk of cardiovascular events is increased in IBD [4,5], suggesting that additional mechanisms, such as inflammation, could be responsible for the excess cardiovascular risk observed in IBD. In these subjects, the low cardiovascular risk associated with the low prevalence of traditional cardiovascular risk factors may be at least partly counterbalanced by the increased cardiovascular risk associated with chronic inflammation. In this regard, arterial stiffening may represent a link between inflammation and

Abbreviations: AIx_{HR}, augmentation index corrected for heart rate; IBD, inflammatory bowel disease; PWV, pulse wave velocity.

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cardiovascular risk. In a small study, we previously reported that arterial stiffness is increased in IBD subjects [6]. However, it is presently unknown whether arterial stiffness is increased in both ulcerative colitis and Crohn's disease and independently associated with current or chronic inflammation. Furthermore, there are no studies in IBD reporting the effect of immunomodulatory therapy on both aortic and muscular artery stiffness whereas in other models of chronic inflammation aortic but not muscular artery stiffness was reduced by a short-term treatment with anti TNF-alpha [7–9]. Finally, none of the existing studies have examined the long-term effects of the treatment with immunomodulatory therapy on aortic stiffening.

Consequently, in Study 1 we aimed to test whether elastic and muscular artery stiffness was increased in both ulcerative colitis and Crohn's disease and associated with chronic or acute inflammation. In Study 2 we aimed to test whether arterial stiffening can be reduced by three years of immunomodulatory drugs (steroids, azathioprine or anti TNF-alpha) in IBD subjects.

2. Materials and methods

2.1. Study population

2.1.1. Study 1

Cross-sectional study. A total of 74 IBD subjects (40 subjects with ulcerative colitis and 34 subjects with Crohn's disease) and 80 age-, gender- and body mass index-matched controls were enrolled. The diagnosis of IBD was based on established criteria of clinical, radiological, endoscopic, and histological findings. Individuals with cardiovascular disease (coronary heart disease, congestive heart failure, stroke, transient ischemic attack, or intermittent claudication), diabetes, chronic kidney disease and dyslipidaemia were excluded, as were subjects treated for hypertension and current smokers. The protocol was approved by the local ethics committee, in accordance with the Declaration of Helsinki, and all participants gave written informed consent.

2.1.2. Study 2

Observational prospective study. All subjects enrolled in Study 1 were considered eligible for inclusion in Study 2. A second non-invasive hemodynamic and clinical examination, planned in IBD subjects and controls was performed at least 2.5 years after the first examination (average 3.4 ± 0.5 years, range 2.5–4.5 years). A total of 32 IBD subjects without cardiovascular risk factors were enrolled, categorized in three groups according with the treatment for IBD (14 subjects treated only with salicylates, 11 subjects treated with steroids and azathioprine, 7 subjects treated with anti TNF-alpha, from baseline to the end of follow-up) and matched for age and baseline carotid-femoral PWV among the treatment groups and with a control group of 30 subjects without cardiovascular risk factors. Anti TNF-alpha was used in IBD subjects that have not responded at other therapies. Therapy was always prescribed before the baseline examination by a physician unfamiliar with the study to avoid selection bias.

2.2. Study design

Standard laboratory and C-reactive protein were measured in IBD subjects 1–7 days before the hemodynamic study in a centralized laboratory. All participants were studied in a quiet room with a controlled temperature of $22 \pm 1\,^{\circ}C$. In each subject, the non-invasive hemodynamic study was performed by an expert operator blinded to the clinical information, including therapy. A second operator, blinded to the hemodynamic examination, collected the clinical data using a standardized questionnaire.

2.3. Non-invasive hemodynamic data acquisition

Both in Study 1 and 2, the non-invasive investigation was performed after 15 min of recumbent rest. Brachial blood pressure measurements were taken using an oscillometric device (Dinamap ProCare 100; GE Healthcare, Milwaukee, USA). Central pressures were recorded noninvasively by applanation tonometry (SphygmoCor; AtCor Medical, Sydney, Australia) [10] after calibration with brachial cuff measurements of the diastolic and mean blood pressure in the contralateral arm [11].

Carotid-femoral and carotid-radial pulse wave velocity (PWV) were measured by a SphygmoCor device (AtCor Medical, Sydney, Australia) using the foot-to-foot velocity method, the intersecting tangent algorithm and the direct distance between the measurement sites [12]: PWV (m/s) $= 0.8 \times$ [direct distance (m)/Δt].

The augmentation index was calculated as previously described [6] and corrected for heart rate using a linear regression model (AIx_{HR}).

2.4. Clinical characteristics

Active disease was defined by the presence of rectal bleeding with an increase in stool frequency (>3/day) or abnormal mucosa at endoscopy in subjects with ulcerative colitis and Harvey-Bradshaw Index ≥ 8 [13] in subjects with Crohn's disease. Relapse was defined by detection of active disease in a subject with IBD who was previously in remission. Extensive disease was defined by ileo-colic lesions in subjects with Crohn's disease or lesions extended proximal to the splenic flexure, including pancolitis, in subjects with ulcerative colitis at endoscopy.

2.5. Statistical analysis

Statistical analyses were performed using NCSS 2007 and PASS 2005 software (Gerry Hintze, Kaysville, UT, USA). The sample size was estimated to demonstrate that IBD subjects have a higher carotid-femoral PWV than controls. The group sample sizes of 74 IBD subjects and 80 controls achieved 80% power to detect a difference of −0.6 m/s, where 0.6 m/s represents the difference of carotid-femoral PWV between IBD subjects and controls previously reported by our group in young subjects without cardiovascular risk factors [6], with a significance level (alpha) of 0.05000 using a two-sided two-sample t-test.

Continuous variables are presented as means (standard deviation); categorical variables are presented as counts and percentages. Clinical and hemodynamic parameters were compared using 1-way analysis of variance (ANOVA) for continuous variables and chi-squared tests for categorical variables. In Study 2, the 1-way repeated-measures ANOVA was used to investigate the effect of treatment on arterial stiffening, where the element of time was given as discrete time points. Sphericity was confirmed with Mauchly's test. Mixed models for repeated measures adjusted for age, gender, mean arterial pressure, heart rate and duration of follow-up were used to confirm that results of 1-way repeated-measures ANOVA. When the omnibus mixed model test was significant, Bonferroni test of within-subject contrasts was performed to test the difference between baseline and end of follow-up in both controls and IBD subjects according with the treatment. Univariate and multivariate linear regression analyses were performed to study the interrelations between PWV and clinical parameters.

2.6. Ethical considerations

This is an observational study because it was considered unethical to conduct a double-blind, randomized trial of anti-TNF

alpha drug in IBD subjects that have not responded at other therapies.

3. Results

3.1. Study 1

3.1.1. Clinical characteristics of patients with IBD

Main data are presented in Table 1. There were no group differences in clinical characteristics among the groups. In IBD subjects, the lipid profile and plasma glucose levels were in the normal range, 27% ($n = 20$) IBD subjects had C-reactive protein >0.8 mg/dL.

3.1.2. Arterial parameters

Brachial and central blood pressures were similar in IBD subjects and matched controls (Table 1). Carotid-femoral PWV, carotid-radial PWV and AIx_{HR} were significantly higher in both ulcerative colitis and Crohn's disease than in controls. The relationship between age and carotid-femoral and carotid-radial PWV is reported in Supplementary Fig. 1, Panel A and B.

In a multiple regression analysis involving the entire population (Table 2), both ulcerative colitis and Crohn's disease were important determinants of carotid-femoral and carotid-radial PWV and explained 9% and 30% of its variance, respectively, even after adjustment for age, gender, mean arterial pressure and heart rate.

In all IBD subjects, a significant relationship between disease duration and carotid-femoral PWV was observed (Fig. 1, Panel A). This finding was also confirmed after adjustment for age, gender, mean arterial pressure and heart rate (Table 2). Carotid-radial PWV was independently associated with C-reactive protein and a history of relapse (Table 2). Results were confirmed even after adjustment for type of IBD (Crohn's disease or ulcerative colitis) and presence of active disease.

There were no significant group differences in arterial parameters, including arterial stiffness, or therapy between subjects with ulcerative colitis or Crohn's disease. However, the prevalence of extensive disease was slightly higher ($P = 0.11$) whereas total cholesterol was slightly lower ($P = 0.12$) in subjects with Crohn's disease than in those with ulcerative colitis (Table 1).

Table 1
Main clinical data of subjects enrolled in Study 1.

Parameters	Controls	Ulcerative colitis	Crohn's disease	*P*-value
Patients, n	*80*	*40*	*34*	
Age, years	38 (13)	38 (14)	36 (14)	0.74
Male gender, %	50	53	47	0.90
Body mass index, Kg/m^2	24.7 (4.3)	24.6 (4.9)	23.8 (5.0)	0.62
Heart rate, b/min	67 (11)	68 (10)	70 (11)	0.27
Brachial SBP, mm Hg	115 (12)	118 (12)	118 (13)	0.30
Brachial DBP, mm Hg	70 (9)	69 (10)	70 (12)	0.91
Brachial MBP, mm Hg	84 (10)	86 (10)	87 (13)	0.40
Central SBP, mm Hg	102 (12)	105 (12)	106 (16)	0.27
Central DBP, mm Hg	71 (9)	70 (10)	71 (12)	0.90
AIx_{HR}, %	13.8 (3.9)	17.1 (3.7)*	16.4 (4.0)#	<0.001
Carotid-femoral PWV, m/s	7.0 (1.1)	7.8 (1.7)#	8.0 (1.6)*	<0.001
Carotid-radial PWV, m/s	7.2 (0.9)	8.8 (1.4)#	8.8 (1.3)#	<0.001
C-reactive protein, mg/dL	–	1.12 (1.44)	0.80 (1.07)	0.29
Total cholesterol, mg/dl	–	164 (21)	156 (19)	0.12
Plasma glucose, mg/dl	–	83 (10)	84 (6)	0.50
In remission, %	–	73	71	0.86
Extensive disease, %	–	38	56	0.11
Disease duration, years	–	5.9 (5.6)	7.0 (6.2)	0.42
History of relapse, %	–	70	62	0.46
Therapy				0.92
Salicylates, %	–	38	38	
Steroids and azathioprine, %	–	45	41	
Anti TNF-alpha, %	–	17	21	

IBD, inflammatory bowel disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; PWV, pulse wave velocity; AIx_{HR}, augmentation index corrected for heart rate. *$P < 0.001$ vs. controls; #$P < 0.05$ vs. controls.

Table 2
Multivariate relationships of arterial stiffness in Study 1.

Parameters	R^2 increment	Beta coeff.	CI	*P*-value
All subjects ($n = 154$)				
Dependent variable: carotid-femoral PWV[a]				
Inflammatory bowel disease	0.09			
Ulcerative colitis		0.77	0.39–1.15	<0.001
Crohn's disease		1.01	0.61–1.42	<0.001
Dependent variable: carotid-radial PWV[a]				
Inflammatory bowel disease	0.30			
Ulcerative colitis		1.50	1.09–1.91	<0.001
Crohn's disease		1.58	1.14–2.02	<0.001
Dependent variable: AIx_{HR}[b]				
Inflammatory bowel disease	0.16			
Ulcerative colitis		3.6	2.1–5.0	<0.001
Crohn's disease		3.0	1.5–4.6	<0.001
Subjects with IBD ($n = 74$)				
Dependent variable: carotid-femoral PWV[a]				
Disease duration, 1 year	0.03	0.054	0.001–0.108	<0.05
Dependent variable: carotid-radial PWV[a]				
C-reactive protein, 1 mg/dL	0.05	0.26	0.04–0.47	0.02
History of relapse	0.08	0.84	0.24–1.45	0.01

PWV, pulse wave velocity; AIx_{HR}, augmentation index corrected for heart rate; CI, confidence interval.
[a] Adjusted for age, gender, mean arterial pressure and heart rate; Beta unit: m/s.
[b] Adjusted for age, gender and mean arterial pressure; Beta unit: %.

3.2. Study 2

The characteristics of the population at baseline and after a mean follow-up of 3.4 ± 0.5 years (range 2.5–4.5 years) are presented in Tables 3 and 4. Controls and IBD subjects, matched for age, and baseline carotid-femoral PWV, were also comparable for baseline mean blood pressure and AIx_{HR}. During follow-up, neither mean blood pressure nor AIx_{HR} was significantly modified in IBD subjects and controls. However, compared to baseline, carotid-femoral PWV increased significantly during follow-up in controls and IBD subjects treated with salicylates whereas was almost unmodified in those treated with steroids and azathioprine or anti TNF-alpha (Fig. 1, Panel B). Consequently, the annual increase of carotid-femoral PWV was significantly higher in subjects treated with salicylates than in those treated with steroids and azathioprine or anti TNF-alpha ($P = 0.004$ and $P = 0.001$, respectively; Fig. 1, Panel C). These results were also confirmed after adjustment for age, gender, mean arterial pressure, heart rate and duration of follow-up (Mixed models for repeated measures, $P = 0.003$; Table 4) as well as in a model adjusted also for type of IBD (Crohn's disease or ulcerative colitis), disease duration and history of relapse (data not shown). The carotid-radial PWV was not modified by treatment (Table 4).

4. Discussion

In the present study we reported that both aortic stiffness, an established surrogate measures of cardiovascular risk, and muscular artery stiffness are increased in subjects with ulcerative colitis and Crohn's disease and differently correlated with markers of chronic and acute inflammation. Only aortic stiffness is reduced by a long-term treatment with immunomodulating drugs.

4.1. Arterial stiffness is increased in IBD

All of the results of Study 1 are consistent with an increase in the arterial stiffness among subjects with ulcerative colitis and Crohn's

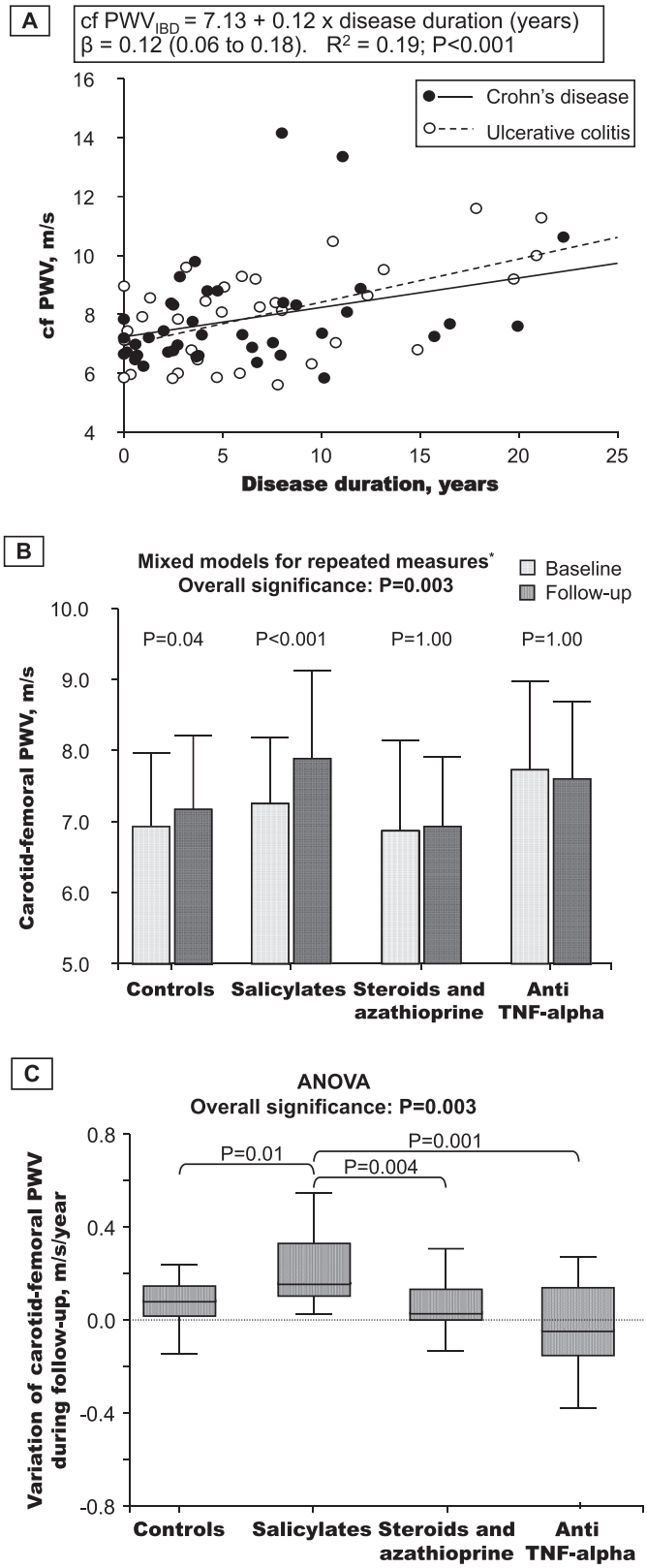

Fig. 1. Panel-A. Association between carotid-femoral pulse wave velocity and disease duration in Study 1. Cumulative analysis in subjects with inflammatory bowel diseases considered as a whole group (cf PWV_{IBD}) was reported. Regression lines of subjects with ulcerative colitis (- - -) and Crohn's disease (—) were also provided. Panel B–C. Variation of carotid-femoral pulse wave velocity (PWV) during follow-up in Study 2. *Adjusted for age, gender, mean arterial pressure, heart rate and duration of follow-up.

disease. In a previous small cross-sectional study we assessed PWV in patients with IBD without cardiovascular risk factors and showed increased arterial stiffness in IBD subjects compared with a group of healthy controls [6]. However, since we enrolled in our previous study only 32 subjects with IBD (16 with Crohn's disease and 16 with ulcerative colitis) 16–49 years old, the sample size did not allowed any conclusion on a potential difference between the effects of type of IBD (Crohn's disease or ulcerative colitis) on arterial stiffness. In contrast, in the present Study 1 the population was increased to 74 subjects in order to increase the statistical power of the study (see methods). In addition, PWV was assessed according with the latest guidelines currently available [12].

4.2. Arterial stiffness and inflammation in IBD

In Study 1, an association between arterial stiffness and several markers of acute and chronic inflammation was reported in IBD. Similar results were previously reported in healthy and hypertensive individuals [14,15] as well as in several chronic inflammatory disorders [7,16–18], in which arterial stiffening is independent from the presence of atherosclerosis and is related to disease duration [16]. Moreover, even an acute, mild, or transient inflammatory stimulus can lead to the deterioration of a large artery's elastic properties [19]. Inflammatory bowel diseases are the result of a combination of environmental, genetic and immunologic factors in which an uncontrolled immune response within the intestine leads to inflammation in genetically predisposed individuals [20,21]. Several reports have suggested that IBD are associated not only with premature atherosclerosis [22,23], a clinical feature common to several inflammatory and immunological diseases [21], but also with increased carotid intima-media thickness [24], elevated homocysteine [25], insulin resistance [26] and endothelial dysfunction [27], which is improved after the administration of a TNF-alpha antagonist [28]. At this regard, because nitric oxide is a potent regulator of arterial stiffness [29], the endothelial dysfunction may explain at least part of the effect of inflammation on arterial

Table 3
Main clinical data of subjects enrolled in Study 2.

Parameters	Controls	IBD subjects			*P*-value
		Salicylates	Steroids and azathioprine	Anti TNF-alpha	
Patients, n	*30*	*14*	*11*	*7*	
Age, years	30 (13)	31 (6)	30 (10)	33 (9)	0.65
Male gender, %	60	64	45	43	0.67
Brachial SBP, mm Hg	113 (12)	116 (11)	114 (7)	113 (12)	0.82
Brachial DBP, mm Hg	68 (9)	66 (10)	68 (9)	67 (10)	0.84
Brachial MBP, mm Hg	82 (10)	82 (9)	83 (8)	83 (11)	0.99
Carotid-femoral PWV, m/s	6.9 (1.0)	7.3 (0.9)	6.9 (1.3)	7.7 (1.2)	0.29
Carotid-radial PWV, m/s	7.2 (0.8)	8.5 (1.0)	8.5 (1.9)	9.2 (1.4)	<0.001
AIx_{HR}, %	9.6 (4.8)	11.8 (6.4)	10.7 (4.4)	11.2 (5.3)	0.57
C-reactive protein, mg/dL	–	0.74 (1.01)	0.60 (0.83)	1.40 (1.81)	0.36
Crohn's disease, %	–	36	55	43	0.64
In remission, %	–	71	91	57	0.25
Extensive disease, %	–	29	55	57	0.31
Disease duration, years	–	3.9 (4.6)	4.7 (2.7)	8.5 (7.5)	0.12
History of relapse, %	–	43	73	86	0.11

IBD, inflammatory bowel disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; PWV, pulse wave velocity; AIx_{HR}, augmentation index corrected for heart rate.

Table 4
Long-term effect of immunomodulatory drugs on inflammation and hemodynamics.

Parameters	Controls		Salicylates		Steroids and azathioprine		Anti TNF-alpha		Overall significance	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	1-way repeated-measures ANOVA	Mixed models for repeated measures
Patients, n	*30*	*30*	*14*	*14*	*11*	*11*	*7*	*7*		
Brachial MBP, mm Hg	82 (10)	83 (7)	82 (9)	82 (9)	83 (8)	81 (9)	83 (11)	83 (10)	0.59	–
C-reactive protein, mg/dL	–	–	0.74 (1.01)	1.18 (1.31)	0.60 (0.83)	0.59 (0.71)	1.40 (1.81)	0.38 (0.35)	0.04	0.04
Carotid-femoral PWV, m/s	6.9 (1.0)	7.2 (1.0)*	7.2 (0.9)	7.9 (1.2)#	6.9 (1.3)	6.9 (1.0)	7.7 (1.2)	7.6 (1.1)	0.002	0.003
Carotid-radial PWV, m/s	7.2 (0.8)	7.2 (0.7)	8.5 (1.0)	8.9 (1.1)	8.5 (1.9)	9.0 (1.8)	9.2 (1.4)	9.2 (1.5)	0.26	–
AIx_{HR}, %	9.6 (4.8)	10.1 (2.2)	11.8 (6.4)	13.6 (1.7)	10.7 (4.4)	9.0 (2.6)	11.2 (5.3)	8.7 (2.6)	0.18	–

MBP, mean blood pressure; PWV, pulse wave velocity; AIx_{HR}, augmentation index corrected for heart rate. Mixed models for repeated measures adjusted for age, gender, mean arterial pressure, heart rate and duration of follow-up. Difference between baseline and end of follow-up (Bonferroni test): $^{*}P < 0.05$; $^{\#}P < 0.001$.

stiffness in IBD subjects. Many of the above mentioned features were not directly evaluated in our study population. Therefore, their role in the pathogenesis of arterial stiffness cannot be directly inferred. Nevertheless, the prevalence of classical cardiovascular risk factors is lower in patients with IBD than in the general population, including low body mass index and lipid levels, as well as lower rates of hypertension, diabetes, and obesity [1–3]. Therefore, given the risk profile of IBD subjects, cardiovascular morbidity and mortality should be lower than in the general population. However, this seems not to be the case because the standardized mortality ratio is not reduced [30] and the risk of cardiovascular events is increased in IBD subjects [4,5]. We believe that IBD represent a useful model to study the effect of both chronic low-grade inflammation and peaks of acute inflammation in the development of cardiovascular diseases. We suggest that in IBD subjects, the low cardiovascular risk associated with the low prevalence of cardiovascular risk factors may offset the increased cardiovascular risk associated with chronic inflammation. The comprehension of these concomitant and inverse effects, mostly not considered in the cardiovascular risk stratification of IBD subjects, could explain why these subjects are associated with an increased cardiovascular risk in the absence of an increased prevalence of traditional cardiovascular risk factors. In this regard, arterial stiffening could represent a link between chronic inflammation and cardiovascular risk in IBD subjects. Further studies based on "hard clinical" endpoints are needed to confirm that the reduction of arterial stiffness is associated with a reduction of cardiovascular risk in IBD, as already reported in other clinical settings. To this regard, albeit not conclusive, the data of the changes at follow-up of C-reactive protein are supportive for a correlation between inflammation and vascular stiffness.

4.3. Arterial stiffness is reduced by immunomodulatory therapy in IBD

In Study 2, we reported that a successful long-term immunomodulatory therapy is effective to reduce aortic stiffening in IBD. This finding is in accordance with similar evidences reported in rheumatoid arthritis [7,8] showing a short-term reduction in carotid-femoral PWV, but not in carotid-radial PWV, after 12 weeks of anti-TNF-alpha therapy and suggests that the efficacy of this therapy on the reduction of arterial stiffness could be maintained in the long-term. Moreover, the absence of reduction of carotid-radial PWV during follow-up support the hypothesis that different mechanisms are involved in the stiffening of elastic and muscular arteries in response to inflammation and may help to explain why muscular artery stiffness was higher that elastic artery stiffness in IBD subjects. Our results are also in accordance with data demonstrating that successful immunomodulatory therapy improves endothelial function in IBD subjects [28] and in other models of chronic inflammation [31,32]. Finally, aortic stiffness increased significantly less in subjects treated with immunomodulatory drugs than in those treated only with salicylates, suggesting that immunomodulatory drugs could be more effective than salicylates to reduce inflammation in IBD and that subjects treated only with salicylates may still have a subclinical inflammation.

4.4. Methodological issues

The present study has several strengths. First, this is the first study to comprehensively measure the elastic and muscular artery stiffness in subjects with ulcerative colitis and Crohn's disease and to report a reduction of arterial stiffening in these subjects after a three years of immunomodulatory therapy. Second, we used the reference method for assessing arterial stiffness (SphygmoCor; AtCor Medical, Sydney, Australia) [11]. Third, the power of Study 1 was sufficient to demonstrate that IBD subjects have increased aortic stiffness and post-hoc power analysis of Study 2 revealed that the examined sample size provided adequate power (89%) to demonstrate that immunomodulatory drugs have reduced aortic stiffening. Finally, our findings show a strength of association, temporality, consistency, biological plausibility, gradient and coherence with previous studies performed in other models of chronic inflammation [7,8]. These features make it probable that the findings reflect a biological phenomenon [33].

This study may have some potential limitations. First, a larger number of subjects could be necessary to perform with sufficient power separated group analyses in subjects with Crohn's disease and ulcerative colitis as well as in those with active disease or in remission. Nevertheless, arterial stiffness was comparable between subjects with Crohn's disease and ulcerative colitis and results of multivariate analyses were almost unchanged after adjustment for type of IBD, disease duration and history of relapse. Second, this is an observational study and, therefore, the assignment of treatments to research subjects is, by definition, not randomized. This reflects the facts that it was considered unethical to conduct a double-blind, randomized trial of anti-TNF alpha drug in IBD subjects that have not responded at other therapies. A similar study design was adopted previously by others [7,17,31]. We attempted to minimize bias by using (a) a physician unfamiliar with the study for the prescription of the therapy, (b) a blinded operator for the measurement of hemodynamic parameters, (c) a second blinded operator for the collection of the clinical data using a standardized questionnaire and (d) a matching strategy. However, due to the nonrandomized design of the present study, we cannot exclude the possibility of a non-drug-related reduction in carotid-femoral PWV. Finally, because blood samples were unavailable in controls, we have not performed a direct comparison of C-reactive protein, a biomarker of acute inflammation, between IBD and healthy controls. However, our results suggest that a prolonged chronic

inflammation rather than an episode of acute inflammation could be necessary to increase aortic stiffness in IBD.

5. Conclusions

Aortic stiffness is increased in subjects with ulcerative colitis and in those with Crohn's disease and can be reversed by a long-term treatment with immunomodulatory therapy. This suggests that effective long-term control of inflammation may reduce cardiovascular risk in IBD subjects as it improves arterial stiffness, an established surrogate measures of risk.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.atherosclerosis.2014.03.023.

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