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Effects of Therapy with Maraviroc on the Carotid *Intima Media* Thickness in HIV-1/HCV Co-infected Patients

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Abstract. *Aim: To evaluate, in human immunodeficiency virus-hepatitis C virus co-infected patients, the impact of C-C chemokine receptor type 5 (CCR5) antagonist maraviroc-based antiretroviral therapy on the carotid intima media thickness and on atheromasic plaques. Patients and Methods: In this pilot prospective study, 12 HIV-HCV co-infected patients underwent color-Doppler ultrasonography before and 48 weeks after switching to a dual therapy based on maraviroc plus protease inhibitors boosted with ritonavir. Changes of intima media thickness, inflammatory and endothelial adhesion biomarkers levels, Veterans Aging Cohort Study index and Framingham risk score were evaluated. Results: At baseline 11 (91.6%) patients showed pathological ultrasonographic findings. After 48 weeks, two patients showed an amelioration of intima media thickness. Of the remaining patients with plaques, four showed a reduction of the previously diagnosed plaque; no patients worsened. Conclusion: Our data suggest that CCR5 inhibition could reduce the development of atherosclerosis especially in the non-calcific stage and could play an important role in the blockade of atheromasic plaque progression.*

HIV CCR5 co-receptor is involved in HIV viral entry, but has also a prominent role in many inflammatory pathways. CCR5 regulates the migration of monocytes, natural killer cells and T helper 1 (Th1) cells into sites of inflammation. CCR5 expression on monocytes/macrophage and T-cells and both endothelial and vascular smooth muscle cells suggests a key role in the atherosclerotic process. Several studies demonstrated that inhibition of this co-receptor produces an anti-inflammatory and anti-atherogenic effect (1-5). A body of evidence has documented that in patients with HIV, atherosclerosis is accelerated and chronic inflammatory processes are activated (6-9); furthermore, certain evidence indicates that hepatitis C (HCV) co-infection can augment the risk of cardiovascular events (10, 11). Moreover, hepatic stellate cells, that play an important role in the pathogenesis of liver fibrosis, express both HIV CCR5 and C-X-C chemokine receptor type 4 (CXCR4) co-receptors. HIV-1 glycoprotein 120 (Gp120) binding to CXCR4 and CCR5 activates hepatic stellate cells, with a pro-fibrogenic and pro-inflammatory effect (12, 13).

The aim of our pilot prospective study was to evaluate, in a group of HIV-HCV co-infected patients, the impact of a CCR5 antagonist maraviroc (MVC)-based dual antiretroviral therapy (ART) on cardiovascular risk assessed with echo color-Doppler evaluation of the epi-aortic vessels, inflammatory and adhesion cytokines dosage and risk scores Veterans Aging Cohort Study (VACS) index and Framingham risk score (FRS) in a 48-week follow-up. The effects of a CCR5 antagonist-based ART on the progression of liver fibrosis were also evaluated.

Patients and Methods

Twelve consecutive HIV-HCV co-infected outpatients attending the Institute of Infectious Diseases of the University of Bari, Italy, were enrolled in our study between January 2013 and April 2014, the follow-up was concluded in April 2015. Inclusion criteria were: stable ART and undetectable plasma HIV-1 RNA for more than 12 months and with the most recent undetectable HIV-RNA <1 month from baseline visit, R5 tropism ascertained on peripheral blood mononuclear cell DNA by V3 sequencing assessed until the previous 6 months and detectable serum HCV-RNA. Exclusion criteria were: negative HCV-RNA, positive hepatitis B surface antigen (HBsAg), detectable HIV-RNA, or alcohol abuse. All the patients were experiencing the following conditions requiring a shift from their nucleoside/nucleotide reverse transcriptase inhibitor (NRTIs) backbone therapy, as indicated by the current guidelines: nine had high cardiovascular risk (FRS more than 10% or carotid changes on echoic images), one had renal impairment (low estimated glomerular filtration, with proteinuria and hypophosphatemia) and two had bone loss (osteopenia or

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osteoporosis). All the patients shifted to MVC plus protease inhibitors boosted with ritonavir (PI/r) from their current antiretroviral therapy (ART) based on three NRTIs, or two NRTIs plus a PI/r, or two NRTIs plus a non-NRTI. Immunovirological data were monitored after 1 month and then every 3 months. During the visits, the adherence to the therapy was verified. If patients were previously treated with a PI, the same PI was maintained after switching to MVC. All drugs were prescribed at standard doses.

Patients underwent echo color-Doppler ultrasonography before and 48 weeks after antiretroviral therapy shifting. Inflammatory and endothelial adhesion biomarkers levels interleukin-6 (IL6), high-sensitivity C-reactive protein (hs-CRP), D-dimers, intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion protein 1 (VCAM1) were evaluated at the same timepoints. VACS index and FRS were also calculated.

Inflammation and enhotelial biomarkers. Plasma D-dimers were quantified by means of an enzyme-linked fluorescent assay (Vidas D-Dimer Exclusion II, Biomerieux Marcy-L'Etoile France; normal values: 0-500 ng/m) at the Central Laboratory of the University Policlinico Hospital of Bari. Hs-CRP (Biokit Quantex CRP, Netherlands; normal values: 0-300 ng/), IL-6 (Boster Biological Immunoleader, China; detection limit: 0.3 pg/ml, assay range: 4.69-300 pg/ml), ICAM1 and VCAM1 (Boster Biological Immunoleader; detection limit: 10 pg/ml, assay range: 156-10,000 pg/ml) were measured at the Infectious Diseases Laboratory of the University Policlinico Hospital of Bari by commercially available assays.

GP120 sequencing and co-receptor tropism CRT assignment. HIV-DNA was extracted and amplified from peripheral blood mononuclear cell and GP120 sequencing on proviral DNA was performed as previously described (14). CRT was inferred with the geno2pheno [co-receptor] algorithm (http://co-receptor.bioinf.mpi-inf.mpg.de/), setting the false-positive rate at 10% to classify isolates as R5.

IMT and plaque assessment. The primary outcome measure was the evaluation and description of any eventual changes of carotid *intima media* thickness (IMT). Ultrasonography of the epi-aortic vessels was performed using a power color-Doppler instrument with 7.5 mgHz probes (Esaote Biomedica, Genoa, Italy). IMT changes were studied considering the following measurement sites: common carotid: 1 cm before the carotid bifurcation, at carotid bifurcation; internal carotid: 1 cm after the carotid bifurcation. Characteristics of the *intima*, pulsation index, resistance index, minimal speed, peak speed, and mean speed, were evaluated. An IMT of more than 1 mm were considered to be pathological. Atherosclerotic plaques, if present, were described. A carotid was classified as being affected by plaque if there was a localized thickening >1.2 mm that did not uniformly involve the whole left or right common carotid bifurcation with or without flow disturbance (15, 16).

In order to better evaluate the characteristics of the plaques that showed improvements with respect to those that remained unmodified, we applied a method already described in other issues to distinguish inflammatory from atheromasic patterns (17, 18). In brief, the following ultrasound color-Doppler characteristics were examined: echogenicity of the lesion with respect to the vessel wall; presence of acoustic shadows inside the lesion; homogeneity of the lesion wall; profile of the endoluminal surface; and the presence or absence of a cleavage plain. Characterization of lesions was consistent with methods describing echogenicity and plaque surface appearance previously established by an International Consensus Conference concerning plaque morphology and risk (19).

An iso-hypoechogenic structure, a homogeneous wall, a smooth endoluminal surface, and the absence of a cleavage plain were considered as characteristics of an inflammatory pattern. In contrast, a hyperechogenic structure, the presence of shadow cones, an irregular endoluminal surface and a cleavage plain were considered characteristics of atheromasic plaques.

The ultrasonographic examination were performed by a single physician (PM). All the images were revised by FP, who was blinded to the patient's treatment history and status. Both the physicians had more than 10 year's experience in the ultrasound color-Doppler technique and had carried at least 1,000 documented epi-aortic examinations.

Liver fibrosis assessment. Liver stiffness was evaluated by a single certified operator using transient elastography (FibroScan®; Echo-Sens, Paris, France). Liver fibrosis was also determined using FIB-4 as surrogate marker. FIB-4 was calculated using Sterling's formula, as follows: age (years) × AST (IU/l)/platelet count (expressed as platelets $\times 10^9$/l]× [ALT1/2(IU/l).

Statistical methods. Descriptive statistics were produced for all variables. Chi-square or Fisher's exact test and *t*-test for unpaired samples were used for statistical analysis. A *p*-value of less than 0.05 was considered significant.

Ethic considerations. Written informed consent was obtained from all patients. The local Ethics Committee (Comitato Etico Indipendente Locale – Azienda Ospedaliera Ospedale Policlinico Consorziale Bari – Italy) approved the study, within the PREVALEAT cohort study. PREVALEAT (PREmature VAscular LEsions and Antiretroviral Therapy) is a multicenter, longitudinal cohort study aimed at evaluation of cardiovascular risk in HIV-infected patients since 2004. All procedures were in accordance with the ethical standards of the institutional and National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

All images of the echo-color Doppler were photographed and properly archived, together with all the other data in the archives of the Institute.

Results

The clinical characteristics at baseline are summarized in Table I. The median age was 50 year. Among the 12 patients enrolled, 11 (92%) were males, nine (75%) smoked and five (41.7%) had high blood pressure. All patients had a long history of HIV and HCV, respectively a median of 25 and 20 years from the first HIV and HCV testing. Median ART exposure was 15.5 years. Median time with HIV suppression was 4.5 years. Only one patients was being treated with rosuvastatin and acetylsalicylic acid. No patient had diabetes. At baseline, 11 (91.6%) patients showed pathological ultrasonographic findings; of these, two had abnormal IMT and nine had plaques. Findings for one patient were normal. Before shifting to MVC plus PI/r, 10 patients (83%) were receiving two NRTIs plus PI/r, one patient two NRTIs plus non-NRTI

Table I. *Baseline characteristics of the study population (n=12).*

Characteristic	Value
Age, years	50 (48-52)
Male gender	11 (92%)
CDC clinical stage	
A	4 (33.4%)
B	7 (58.4%)
C	1 (8.2%)
Source of HIV infection	
Intravenous drug user	11 (92%)
Other	1 (8%)
Source of HCV infection	
Intravenous drug user	11 (92%)
Other	1 (8%)
Nadir CD4, cells/ml	198 (98.2-271)
CD4 cell count, cells/ml	660 (579.7-931.7)
Time since HIV diagnosis (years)	25 (21-27)
Time since HCV diagnosis (years)	20 (15.7-21.7)
Time on ART (years)	15.5 (8.5-18.7)
Time with HIV viral suppression (years)	4.5 (3-8.7)
HCV RNA, IU×10^6/ml	2.1245 (1.426-5.25075)
HCV genotype	
1	7 (58.4%)
3	2 (16.6%)
4	3 (25%)
Previous history of failure with anti-HCV therapy	6 (50%)
Smoker	9 (75%)
High blood pressure	5 (41.7%)
Use of statins	1 (9%)
Use of acetylsalicylic acid	1 (9%)

CDC: Centre for disease control and prevention; HIV: human immunodeficiency virus; HCV: hepatitis C virus; ART: antiretroviral therapy; CD4: CD4 cells. All values are expressed as median and interquartile range or number (percentage), unless otherwise specified.

and another one three NRTIs. Antiretroviral treatment and switching to MVC plus PI/r are described in Table II.

After 48 weeks of MVC-based therapy, the patient with normal features remained normal; the two patients with abnormal IMT (maximum observed values 1.1 and 1 mm, respectively) showed an amelioration (to 0.9 and 0.76 mm, respectively). Of the remaining nine patients with plaques, four (44%) showed a reduction of the previously diagnosed atheromasic plaque; five (56%) did not show any modification of their baseline ultrasonographic findings; no patients worsened.

Among the nine smokers, eight had plaques at baseline. At follow-up, four (50%) showed an improvement at ultrasonographic examination. Four out of the five patients with high blood pressure had plaques at baseline but only one patient had an improvement at follow-up. The plaque pattern patient being treated with rosuvastatin and acetylsalicylic acid did not change.

The relationship between outcome of the plaques and their characteristics is described in Table III. No significant changes in inflammatory and endothelial adhesion biomarkers, VACS index and FRS were detected, as shown in Table IV. Before shifting to MVC plus PI/r among the four patients with plaques who showed an improvement at 48 weeks, two patients were receiving abacavir/lamivudine with PI/r (lopinavir/r and atazanavir/r), while the other two patients were receiving tenofovir/emtricitabine with darunavir/r once daily.

Liver stiffness. At baseline, one patient had no/mild fibrosis (F0-F1), an F2-F3 stage of fibrosis was observed in eight and a F4 stage in three. After 48 weeks of treatment, three patients (25%) showed an improvement in liver stiffness, whereas in nine (75%) the fibrosis grade using Fibroscan did not change. Median liver stiffness decreased from 9.2 kPa (interquartile range, IQR=8.1-13.7) at baseline to 7.6 kPa (IQR=6.8-11.4) after 48 weeks without achieving statistical significance. No change was observed between median FIB4 value at baseline [1.69 (IQR=1.1-2.8)] and after a follow-up of 48 weeks [1.6 (IQR=1.0-2.8)].

Immunovirological outcome. One patient experienced an HIV virological failure (350 copies of HIV-RNA) after 6 months of treatment with MVC plus atazanavir/r and it was necessary to strengthen the therapy adding tenofovir/emtricitabine. In the following period, HIV remained suppressed and no ultrasonographic changes were observed. All the other patients remained aviremic during the whole period of follow-up.

Discussion

In HIV patients, atherosclerosis is accelerated and chronic inflammatory processes are activated. Cardiovascular disease is one of the most common non-acquired immune deficiency syndrome events, with overall increased morbidity and mortality (20). Likewise a large number of studies suggest an increased risk of cardiovascular disease events in HCV-monoinfected and HIV-HCV co-infected patients compared with uninfected controls (21-24). The risk of cardiovascular disase in HIV-HCV-positive patients is due to several mechanisms: aging, insulin resistance, hepatic steatosis, chronic inflammation, microbial translocation and immune activation (25).

HIV CCR5 co-receptor, which plays a crucial role in HIV viral entry, is also involved in many inflammatory pathways (1-5). MVC is the only available CCR5 antagonist currently used in clinical practice for treating HIV infection. The blockade of CCR5 can have an important effect on plaque progression and systemic inflammation as previously described (1).

In a recent study, Cipriani *et al*. demonstrated that in a murine model of genetic dyslipidemia, MVC reduced

Table II. *Antiretroviral therapy (ART) of each patient at the time of switching and ultrasonographic outcome.*

				Ultrasonography	
Patient no.	Pre-switch ART	Study ART	FPR at baseline (%)	At baseline	Improvement
1	3TC/ABC+ATV/r	MVC+ATV/r	45.1	Pathological	No
2	TDF/FTC+EFV	MCV+ATV/r	94.5	Pathological	No
3	3TC+ABC+AZT	MVC+DRV/r	48.7	Pathological	No
4	TDF/FTC+LPV/r	MVC+LPV/r	29.8	Normal	No
5	3TC/ABC+ATV/r	MVC+ATV/r	30.1	Pathological	Yes
6	3TC/ABC+LPV/r	MVC+LPV/r	39.6	Pathological	Yes
7	3TC/ABC+ATV/r	MVC+ATV/r	95.5	Pathological	No
8	TDF/FTC+DRV/r	MCV+DRV/r	33.2	Abnormal IMT	No
9	3TC/ABC+LPV/r	MVC+LPV/r	46.5	Abnormal IMT	No
10	TDF/FTC+ DRV/r	MVC+DRV/r	13.5	Pathological	Yes
11	TDF/FTC+DRV/r	MVC+DRV/r	59.2	Pathological	Yes
12	TDF/FTC +LPV/r	MVC+LPV/r	35.3	Pathological	No

3TC: Lamivudine; ABC: abacavir; ATV: atazanavir; DRV: darunavir; FPR: false-positive rate; FTC: emtricitabine; IMT: *intima media* thickness; LPV: lopinavir; MVC: maraviroc; r: ritonavir; TDF: tenofovir.

Table III. *Baseline characteristics of carotid intima media thickness (IMT) and plaques, and outcome at follow-up in nine patients with pathological ultrasonography.*

Patient no.	Iso-hypoechogenicity	Homogeneity	Smooth surface	Cleavage plain	Shadow cones	After 48-week follow-up
1	−	−	−	+	+	No improvement
2	−	−	−	+	−	No improvement
3	−	−	−	+	−	No improvement
5	+	+	+	−	−	Improvement
6	+	+	+	−	−	Improvement
7	−	−	−	+	−	No improvement
10	+	+	+	−	−	Improvement
11	+	+	+	−	−	Improvement
12	−	−	−	+	+	No improvement

+: Present; −: absent.

Table IV. *Modification of serum levels of metabolic, immunological and inflammatory biomarkers at baseline and after a follow-up of 48 weeks.*

Variable	Baseline	Follow-up (48 week)	*p*-Value
Total cholesterol, mg/dl	163 (152.7-198.2)	180 (156.7-217.2)	0.17
LDL cholesterol,mg/dl	89.5 (71.7-105)	103.5 (85.2-123.5)	0.3
Triglycerides, mg/dl	156.5 (93-285)	146.5 (107.2-434)	0.33
hs-CRP, ng/ml	0 (0-31)	0 (0-20)	0.12
IL6, pg/ml	5.2 (4.5-5.57)	5.5 (4.9-5.67)	0.4
D-Dimers, ng/ml	289 (191-368)	200 (122-278)	0.24
ICAM1, pg/ml	12068 (11392-13650)	11831 (11236-13347)	0.67
VCAM1, pg/ml	9971 (9358-10255)	10121 (9798-10911)	0.06
VACS index	20 (11-27.5)	17 (17-28.2)	0.52
Framingham score,%	10 (3.75-15.7)	11.5 (3.2-20.2)	0.36

LDL: Low-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein; IL6: interleukin-6; ICAM1: intercellular adhesion molecule 1; VCAM1: vascular cell adhesion protein 1; VACS index: Veterans Aging Cohort Study index. Data are median (interquartile range) values.

atherosclerosis progression by lowering macrophage infiltration and expression of adhesion molecules and RANTES (regulated on activaction, normal T-cell expressed, and secreted) inside the plaques and reversed the proinflammatory profile in a mouse model characterized by a ritonavir–induced inflammation (1).

In the present study, the potential benefit of MVC on atherosclerosis progression and on the inflammatory profile was evaluated in a group of HIV-HCV co-infected patients who were shifted to a MVC plus PI/r dual therapy from their triple ART. Our data revealed that a number of patients shifted to a MVC plus PI/r regimen showed an improvement of their baseline carotid lesions: both the patients with pathological IMT findings showed an amelioration, as did 4/9 (44%) of the patients with plaques.

No difference in impact of the previous ART was observed between patients who achieved an improvement. Of the four patients with improved plaques, two patients were receiving abacavir/lamivudine with PI/r (lopinavir/r and atazanavir/r) at baseline, while the other two patients were receiving tenofovir/emtricitabine with darunavir/r once a day. However, because of the small number of patients, a possible role of the prior ART in those achieving an improvement of the atherosclerotic plaque cannot be definitely excluded. In fact, after 48 weeks, an increase of low-density lipoprotein cholesterol was observed however, without, a statistical significance. This might be explained by the loss of lipid-lowering effect observed with tenofovir but also by a slight improvement in liver function. Only one patient with plaque was treated with rosuvastatin and acetylsalicylic acid, but no improvement was evident.

Aging in HIV-positive patients is associated with an increased frequency of non-HIV-related comorbidities. This appears to be related to chronic inflammation, immune activation and immunosenescence (26). Furthermore levels of inflammatory and immune activation such as IL6, hs-CPR and D-dimer are associated with increased risk for non-HIV-related events such as cardiovascuar disease and atherosclerosis progression, and all-cause mortality (20). All patients in our study had a long history of HIV and HCV infection, as shown in Table I, but they also had a long period of HIV viral suppression. For this reason, it is likely that the long period of HIV undetectability and the persistence of viral suppression during the period of observation did not show an impact on changes of inflammation, endothelial dysfunction and immune activation biomarkers.

Nine patients were smokers and eight had plaques at baseline. At follow-up, four (50%) showed an improvement at ultrasonographic examination. Undiagnosed high blood pressure was found in five patients, four of them had plaques at baseline but only one patient had an improvement, highlighting the importance of a periodic proper control of blood pressure. Only one patient with arterial hypertension started anti-hypertensive therapy (ramipril), however no modification in ultrasonographic finding was observed at follow-up.

Among the four patients with improved plaques, two showed an improvement in liver stiffness and in two there was no modification in their fibrosis grade using Fibroscan. The possible association between progression of atherosclerosis and liver stiffness modification is worthy of future investigation.

Even if no worsening was registered in our study, only plaques with specific characteristics such as iso-hypoechogenic structure, smooth endoluminal surface and absence of a cleavage plain had an improvement, as showed in Table III. These characteristics of the ultrasound images are due to the inflammatory aspect of the lesions: inflammatory plaques are mainly composed of watery and cellular content. On the other hand, the atheromasic plaque is a phenomenon involving only the endothelium and the luminal portion of the intima, thus accounting for the typical presence of a cleavage plain between the lesion and the underlying tissues. In contrast, a higher degree of echogenicity is proportional to the fiber content and shadow cones are due to the presence of calcifications. Moreover, the endoluminal surface of a typical atheromasic plaque is generally irregular (27). Two examples of plaques are shown: at baseline, the first patient had a plaque with an inflammatory aspect (Figure 1A) which had improved at follow-up (Figure 1B); conversely, the second patient had a plaque with atheromasic characteristics at baseline (Figure 1C) that did not show improvement at follow-up (Figure 1D).

This evidence suggests that CCR5 inhibition might reduce the development of atherosclerosis, especially in the inflammatory and non-calcific stage, and could play a role in the blockade of atheromasic plaque progression. Of note, the amelioration of plaques is a phenomenon that does not involve the classic risk factors, as documented by VACS index and FRS, which remained unchanged. For this reason, we could hypothesize a direct effect of the pharmacological CCR5 abrogation not mediated by other inflammatory or endothelial adhesion biomarkers, which did not show any modification during the study period. However, the role of an NRTI-sparing dual regimen in ameliorating vascular lesions cannot be excluded and further investigations to clarify this point are warranted.

The main limitation of our study is the small number of patients enrolled. This was also a single-arm study and there was no control group. It would be interesting in further investigations to compare our end-points in patients who switch to an MVC-based regimen to those who do not switch in a larger population for a longer time. To the best of our knowledge, even if the potential activity of MVC in reducing the progression of plaque has been already described (1) and a

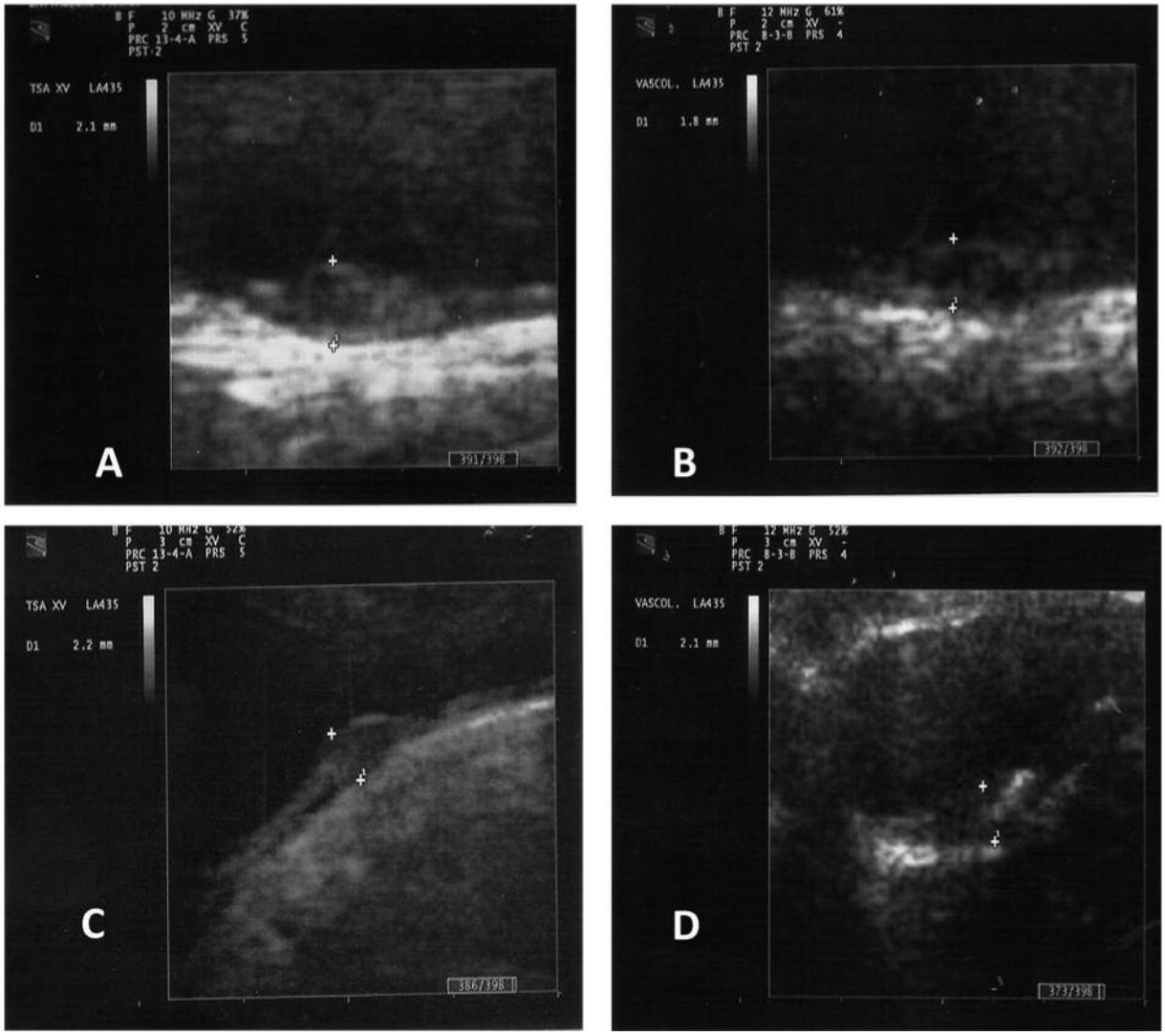

Figure 1. *Two examples of plaque evolution from baseline to follow-up. Patient at baseline (A) and improvement after 48 weeks (B). Patient at baseline (C) and no change after 48 weeks (D).*

recent study of Piconi *et al*. showed that MVC reduces arterial stiffness in a patients with similar characteristics (28), this is the first study evaluating the impact of MVC on the IMT and on the progression of plaque using echo color-Doppler ultrasonography in a clinical practice setting. An evaluation on a larger population is warranted to confirm our observations.

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