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Progression of brain white matter hyperintensities in asymptomatic patients with carotid atherosclerotic plaques and no indication for revascularization

Enrico Ammirati[a,b,*,1], Francesco Moroni[a,1], Marco Magnoni[a], Maria A. Rocca[c], Nicoletta Anzalone[d], Laura Cacciaguerra[c], Simona Di Terlizzi[e], Chiara Villa[e], Federico Sizzano[f], Alessio Palini[f], Isabella Scotti[g], Francesca Besana[h], Pietro Spagnolo[h], Ornella E. Rimoldi[i], Roberto Chiesa[a], Andrea Falini[d], Massimo Filippi[c], Paolo G. Camici[a,**]

[a] Vita-Salute University and IRCCS San Raffaele Hospital, Milan, Italy
[b] De Gasperis Cardio Center, Niguarda Hospital, Milan, Italy
[c] Vita-Salute University and Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy
[d] Vita-Salute University and Department of Neuroradiology, San Raffaele Scientific Institute, Milan, Italy
[e] FRACTAL - Flow Cytometry Resource Advanced Cytometry Technical Applications Laboratory, San Raffaele Scientific Institute, Milan, Italy
[f] Nestlé Institute of Health Sciences, Biobanking & Flow Cytometry Core EPFL, Innovation Park Batiment H, Lausanne, Switzerland
[g] Department of Rheumatology, Istituto Ortopedico Gaetano Pini, Milan, Italy
[h] Cardiovascular Prevention Center, San Raffaele Institute, Milan, Italy
[i] CNR IBFM, Segrate, Italy

HIGHLIGHTS

- Asymptomatic patients with intermediate carotid plaque have brain white matter hyperintensities (WMH) progression.
- Female gender, hypercholesterolemia, and impaired renal function are associated with brain WMH progression at 2 years.
- No plaque characteristics or circulating cellular biomarkers emerge as independent predictors of brain WMH progression.

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ABSTRACT

Background and aims: Brain white matter hyperintensities (WMHs) have been associated with an increased risk of ischemic stroke and considered as markers of brain ischemia. Progression of WMHs in asymptomatic patients with non-hemodynamically significant carotid plaque could represent a putative marker of plaque vulnerability. We prospectively evaluate progression and determinants of WMHs in this population.

Methods: This prospective study included 51 asymptomatic patients with carotid stenosis < 70% that underwent brain magnetic resonance imaging scans at baseline and after a median follow up of 595 days (interquartile range 553–641 days). Patients (mean age of 69 years and 45% females) underwent baseline carotid computed tomography angiography, contrast-enhanced ultrasound for carotid plaque characterization and analysis of subsets of circulating lymphocytes and monocytes by flow cytometry.

Results: Seventeen subjects (33.3%) had carotid stenoses of 50–70% (Doppler flow velocity) while the rest had stenoses of < 50%. In 25 (49.0%) patients, new WMHs, with 5 new lesions on average and a median volume of 134 mm^3, were detected at follow-up. None of the plaque characteristics or of the circulating cellular biomarkers investigated were associated with the global and ipsilateral occurrence of new WMHs whereas, at multivariate analysis, female sex, hypercholesterolemia, and lower glomerular filtration rate (GFR) emerged as independent variables associated with new WMHs.

Conclusions: Half of the patients with carotid plaques of intermediate severity had evidence of WMH progression

* Corresponding author. Cardiothoracic Department, San Raffaele Scientific Institute and University, Via Olgettina 60, 20132, De Gasperis Cardio Center, Niguarda Hospital, Piazza Ospedale Maggiore 3, 20162, Milan, Italy.
** Corresponding author. Vita Salute University and IRCCS San Raffaele Hospital, Via Olgettina 60, 20132, Milan, Italy.
E-mail addresses: enrico.ammirati@ospedaleniguarda.it (E. Ammirati), camici.paolo@hsr.it (P.G. Camici).
[1] These authors contributed equally to this work.

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at follow up. Female gender and systemic factors such as hypercholesterolemia, and lower GFR, but not plaque characteristics or circulating cellular biomarkers, are associated with WMH progression.

1. Introduction

Non-hemodynamically significant plaques (i.e. a plaque with a luminal reduction below 70%) are highly prevalent in asymptomatic patients with carotid atherosclerosis, with an incidence of up to 87% in a recent population-based Norwegian study in a cohort with a mean age of 64 years [1]. Atherosclerosis in the carotid arteries has an etiopathogenic role in the development of ischemic stroke, mainly through athero-embolic mechanisms [2]. In asymptomatic subjects with carotid plaque, the risk of ischemic stroke is low, with an annual incidence ranging from 0.3% to 1.3% and thus the identification of subjects at risk is particularly challenging [2]. In asymptomatic patients with flow-limiting carotid stenoses, evidence of micro-emboli detected by transcranial Doppler was associated with increased risk of stroke and transient ischemic attack (TIA) [3]. On the other hand, individuals with carotid plaques are more susceptible to cerebral small vessels disease [4–8], and micro-embolic events are reported more frequently in subjects with cerebral small vessels disease [9,10], although this issue remains controversial [11,12].

Brain magnetic resonance imaging (MRI) allows identification and quantification of microvascular lesions and silent ischemic strokes. Among microvascular events, white matter hyperintensities (WMHs) have drawn increasing attention due to their prognostic significance in terms of risk of future ischemic stroke and dementia [13,14]. WMHs are lesions of the periventricular and deep white matter that display a high signal intensity on T2 and/or fluid-attenuated inversion recovery (FLAIR) sequences [15,16]. A vascular and, possibly, neuroinflammatory pathogenesis has been recently proposed and WMHs could reflect demyelination and axonal loss as a consequence of ischemia [14]. A recent meta-analysis of observational studies found a significant association between carotid atherosclerosis and the presence of WMHs [8]. Up to date prospective longitudinal data on progression and determinants of WMHs or silent brain infarct in asymptomatic patients with mild to moderate carotid plaques without indication for revascularization are lacking.

Aims of this prospective study in a consecutive cohort of asymptomatic patients with carotid stenosis < 70% were: 1- to assess the rate of appearance of new WMHs or silent brain infarct at an average follow up of 20 months; 2- to identify potential carotid-plaque characteristics (using a multi-imaging approach) and systemic factors and cell markers (by immune-phenotyping) that could predict WMH progression at baseline.

2. Materials and methods

2.1. Study design and population

This prospective, single-center longitudinal study was approved by the Ethics Committee of the San Raffaele Hospital (date of approval January 30th, 2012, protocol name the IMaging Della PLAcca Carotidea [IMPLAC], ClinicalTrials.gov registration number was NCT03333330 and EudraCT number was 2012–000648-83). All subjects provided informed, written consent to participate in the study, in compliance with the Declaration of Helsinki and Good Clinical Practice. Consecutive, asymptomatic patients with carotid stenosis < 70% (Doppler flow velocity) referred to our tertiary medical center (San Raffaele, Milan, Italy) for the assessment of cardiovascular risk profile were screened between April 2012 and November 2015. Exclusion criteria set in order to avoid potential confounding causes of cerebral damage are detailed in Supplemental Table 1. The eligible subjects underwent carotid ultrasound imaging, contrast enhanced ultrasound (CEUS), carotid computed tomography angiography (CTA) and brain MRI at baseline. All these examinations were performed within 3 weeks, to minimize the bias introduced by possible disease progression. At the end of the observation period (approximately 20 months), the patients underwent a second brain MRI to assess the occurrence of new WMHs and WMH volumetric progression. Of the 67 subjects that constituted the baseline population, 66 patients (98.5%) had a complete clinical follow up and 53 patients (79.1%) underwent a second brain MRI. Two of them repeated a brain MRI after carotid endarterectomy and for this reason, were excluded from the final analysis (Supplemental Fig. 1). The cross-sectional study that explored the association between the number and volume of WMHs at baseline and plaque- and patient-related characteristics in these 67 subjects was published previously [17].

2.2. Clinical characteristics

See Supplemental Materials.

2.3. Carotid ultrasound

All patients underwent bilateral Duplex ultrasound evaluation of the carotid arteries using a dedicated ultrasonography equipment (Logiq S8, GE Healthcare, UK) with a 7-MHz linear probe (7L, GE). For all subjects, the degree of carotid stenosis, plaque echogenicity, the total burden of carotid atherosclerosis in terms of total plaque area (TPA) and the number of segments involved by the atherosclerotic process were evaluated as previously described [17]. Each of the 4 segments (common carotid artery [CCA], carotid bulb, internal carotid artery [ICA] and external carotid artery [ECA]) was identified bilaterally according to the Rotterdam Study criteria [18,19]: the 15 mm caudal to the bifurcation was defined as carotid bulb, while cranially we identified ICA and ECA. The common carotid intima-media thickness (CC-IMT) measurement was also performed bilaterally in lateral projection images of the CCA in a semi-automated manner. Doppler velocity measurements were made on longitudinal views at the site of any identifiable lesions within CCA, carotid bulb or ICA. The degree of stenosis was evaluated by velocimetric criteria according to the Society of Radiologists in Ultrasound Consensus Conference [20]. The plaque determining the highest stenosis was considered the main lesion. For the main lesion, we calculated the degree of stenosis according to the European Carotid Surgery Trial (ECST) criteria, as previously described [21]. Briefly, the narrowest diameter and the estimated normal diameter of the artery at the site of the main plaque were measured, and the degree of stenosis was calculated as [1-(narrowest diameter/estimated normal diameter)]%. For each patient, TPA was measured off-line as previously described [22]. Briefly, two independent operators (M.M. and F.M) measured the two-dimensional (2D) area of each identifiable lesion by tracking around the lesion perimeter. The sum of all lesions areas was taken as TPA. The number of segments, CCA, bulb, ICA and ECA bilaterally, involved by atherosclerosis was also registered as an indicator of the extension of the disease. Finally, each plaque was classified according to its echogenicity (Supplemental Materials) [23].

2.4. CEUS of the carotid arteries

CEUS examination was performed using the same dedicated equipment in 47 patients. In 4 patients CEUS examination was deemed unsafe due to a recent history of allergic reactions. Preset contrast-specific modality (pulse inversion) was employed, adjusting image

settings in order to maximize contrast signal visualization and using low mechanical index (0.08–0.12). Five mL of sodium hexafluoride (SonoVue, Bracco Imaging, Italy) were diluted 1:3 in saline. A 3 mL bolus was injected through a peripheral vein and then flushed with 5 mL saline. Not more than a single vial of sodium hexafluoride was used for each examination. Carotid plaque neovascularization was identified as contrast bubbles moving through the plaque as previously described [24]. For each patient, the number of neovascularized plaques, defined as CEUS + lesions was recorded [17]. The intra- and interobserver agreement on plaque grading for neovascularization was 0.94 and 0.88 as previously described [25].

2.5. CTA

Patients (n = 48) were examined with a 64-slice CT scanner (VCT Lightspeed, GE Healthcare, USA), using the previously described protocol [17]. Fifty mL of non-ionic, iso-osmolar contrast material (Iodixanol, 320 mg of iodine per mL, Visipaque 320; GE Healthcare, USA) preheated at 37 °C was injected into an antecubital vein through a 20 or 18-gauge catheter at a rate of 5 ml/s, followed by a saline flush. Data were acquired from the aortic arch to the vertex. Images were reconstructed with a slice thickness of 0.625 mm using a soft-tissue convolution kernel, transferred to an external workstation (AW 4.5, GE Healthcare) for post-processing. Image analysis was carried out by two experienced radiologists (F.B. and P.S.), blinded to other imaging data and clinical information. The average absorbed radiation dose per patient was approximately 0.68 mSv.

2.6. Brain MRI

In all subjects, scans of the brain were collected at baseline and follow up using a 1.5 T scanner (ACHIEVA Philips Medical Systems). Two experienced operators (M.A.R. and L.C.), blinded to each subject clinical and imaging characteristic, identified FLAIR-hyperintense lesions. FLAIR lesion number and volume were subsequently quantified using a local thresholding segmentation technique (Jim 6, Xinapse Systems, West Bergholt, UK; http://www.xinapse.com/), that has been previously validated in a randomized trial where the core lab analysis was performed in our Institute [26]. FLAIR lesion volume and number was reported for the whole brain and for each hemisphere separately, as previously described [17]. Confidence of new FLAIR-hyperintense lesion detection was improved by co-registering baseline to follow-up scans, using a rigid transformation and the FLIRT program from the FMRIB Software Library [27]. No incidental findings were found on brain MRI at baseline and at follow up and no scans needed to be discarded because of poor image quality.

2.7. Lymphocytes and monocytes subpopulations

Whole blood was collected on the same day of the ultrasonographic evaluation using EDTA-anticoagulated vacutainer tubes. Details of staining and analysis of cell subsets are reported in the online supplemental materials. CD14 and CD16 were used to identify classical, intermediate and non-classical monocyte subsets following the classification of the International Union of Immunologic Societies: classical $CD14^{high}CD16$-monocytes; intermediate $CD14^{high}CD16+$; non-classical $CD14^{low}CD16+$ [28]. HLD-DR was used as a marker of cellular activation and the intensity of expression on the subsets of monocytes was expressed as median fluorescence intensity. Further subsets of T-lymphocytes were identified through the combinations of the following markers: $CD3^+$T cells, $CD3^+CD4^+$T cells, naive T cells ($CD3^+CD4^+CD45RO^-$), memory T cells ($CD3^+CD4^+CD45RO^+$),

Table 1
Characteristics of the study population.

	Total (n = 51)	Non progressors (n = 26)	Progressors (n = 25)	*p*
Demographic characteristics				
Age, years	69 ± 8	67 ± 9	69 ± 6	0.35
Female, n (%)	21 (45)	7 (27)	16 (64)	0.007[a]
Follow up duration, days	595 (553–641)	595 (553–659)	588 (552–629)	0.11
Baseline WMH number	29 (5–73)	14 (3–34)	34 (21–115)	0.01[a]
Baseline WMH volume, mm^3	973 (120–4053)	499 (38–1801)	1305 (530–6615)	0.02[a]
Blood tests				
White blood cells (10^3 cells/mm^3)	8 (7–9)	8 (7–9)	7 (6–9)	0.09
Creatinine (mg/dL)	0.85 (0.71–1.01)	0.81 (0.71–1)	0.93 (0.74–1.06)	0.30
eGFR (ml/min)	75 (62–97)	94 (70–113)	65 (52–78)	0.001[a]
Total cholesterol (mg/dL)	177 (158–197)	182 (171–197)	165 (148–200)	0.18
LDL cholesterol (mg/dL)	104 (88–121)	110 (88–120)	99 (88–125)	0.52
HDL cholesterol (mg/dL)	43 (37–49)	43 (36–48)	44 (38–51)	0.59
Cardiovascular risk factors				
Family history of CAD, n (%)	17 (33)	9 (35)	8 (32)	1.0
Family history of stroke, n (%)	5 (9)	2 (8)	3 (12)	1.0
Systemic arterial hypertension, n (%)	40 (78)	20 (77)	20 (80)	1.0
Resistant hypertension, n (%)	9 (17)	3 (12)	6 (24)	0.29
Systolic blood pressure at baseline (mmHg)	130 (125–145)	130 (125–145)	130 (120–140)	0.45
Hypercholesterolemia, n (%)	37 (73)	14 (54)	23 (92)	0.004[a]
Type 2 diabetes mellitus, n (%)	11 (22)	8 (30)	3 (12)	0.17
Current smoker, n (%)	24 (47)	14 (54)	10 (40)	0.40
Obesity (BMI > 30 kg/m^2)	3 (6)	3 (12)	0 (0)	0.24
Cardiovascular history				
Previous ACS, n (%)	6 (12)	3 (12)	3 (12)	1.0
Medical therapy				
ACE inhibitors/ARBs, n (%)	31 (61)	16 (62)	15 (60)	1.0
β-blockers, n (%)	22 (43)	13 (50)	9 (35)	0.40
Diuretics, n (%)	11 (22)	3 (12)	8 (31)	0.09
Calcium channel blockers	13 (25)	3 (12)	10 (40)	0.02[a]
Number of anti-hypertensive agents	2 (0–2)	1 (0–2)	2 (1–3)	0.19
Use of 2 or more anti-hypertensive agents	26 (51)	12 (46)	14 (56)	0.58
Statins, n (%)	29 (57)	9 (35)	20 (80)	0.002[a]
Antiplatelet agent, n (%)	30 (59)	16 (62)	14 (56)	0.77

[a] Statistically significant comparison. Continuous variables are reported as median and interquartile range.

effector memory T cells (HLA-DR$^+$CD3$^+$CD4$^+$CD45RO$^+$) and activated CD4$^+$T cells (HLA-DR + CD3$^+$CD4$^+$) [29].

2.8. Statistical analysis

See Supplemental Materials.

3. Results

3.1. Characteristics of the study population

The mean age of the study population was 69 ± 8 years and 45% were females. Seventy-eight percent (40/51) of the patients were hypertensive, 73% (37/51) had hypercholesterolemia and 22% (11/51) had type 2 diabetes mellitus. Median follow up was 595 (interquartile range [Q1-Q3]: 553–641) days. Clinical characteristics of the study population and the number and volume of WMHs at baseline are reported in Table 1. Approximately one-third of the subjects had a (maximum) carotid stenosis of 50–70%, while the rest had stenosis of < 50% at Doppler flow velocity. Twenty-eight patients (55%) had evidence of a plaque contralateral to the maximum stenosis. The median number of neo-vascularized plaques at CEUS was 1 per subject. Median TPA was 0.76 cm^2, while median total plaque burden on CTA images was 300 mm^3. Table 2 summarizes baseline standard echographic, CEUS and CTA findings of the main plaque and of the atherosclerotic burden of the carotid arteries for each patient.

3.2. Progression of brain WMH

Fifty out of 51 subjects (98.0%) with longitudinal brain MRI evaluation had at least 1 WMH at baseline and 25 patients (49.0%) developed at least 1 significant new WMH at follow up, therefore fulfilling the criteria for progression (defined as progressors). Among those with at least 1 new WMH, the median number of new lesions was 5 (Q1-Q3: 3–10), and median new lesion volume was 134 (Q1-Q3: 51–219) mm^3. Compared with non progressors, progressors, had significantly more baseline WMH both in terms of number and volume (34 *vs.* 14, $p = 0.008$) and volume (1305 mm^3 *vs.* 499 mm^3, $p = 0.02$, Fig. 1A and B and Table 1). Importantly, none had a silent brain infarct either at baseline or follow up.

3.3. Clinical profile and WMH progression

Among the tested clinical and laboratory variables reported in Table 1, only female sex, lower estimated glomerular filtration rate (GFR) and hypercholesterolemia were associated with WMH progression at follow up (all $p < 0.01$; Table 1 and Fig. 1C–E). Female sex conferred a relative risk (RR) of 2.16 (1.18–3.95, $p = 0.007$) of WMH progression at follow up. Hypercholesterolemic subjects were at higher risk for WMH progression, with a RR of 4.35 (1.18–16.1, $p = 0.004$). Use of statins was also associated with WMH progression ($p = 0.002$). Similarly, the use of calcium channel blockers was found to be associated with WMH progression ($p = 0.02$), possibly due to their use in the elderly or in patients with resistant hypertension. Fig. 2 shows 2 representative patients, 1 with new WMH and 1 without new WMHs at follow up.

3.4. Imaging of the carotid plaque, circulating immune cells and WMH progression

Although several characteristics of the main plaque and the global atherosclerotic burden of the carotid arteries were investigated, none of these factors was found to be significantly associated with the occurrence of a new WMH lesion (Table 2). Similarly, none of the analyzed

Table 2
Baseline ultrasonographic and computed tomography angiography characteristics of the population.

Carotid ultrasound characteristics	Total (n = 51)	Non progressors (n = 26)	Progressors (n = 25)	*p*
Main plaque side				
Left, n (%)	15 (30)	9 (35)	8 (32)	0.76
Main plaque characteristics				
Degree of stenosis (Doppler), n (%)				
< 50%	34 (67)	18 (69)	16 (64)	0.77
50–70%	17 (33)	8 (31)	9 (36)	.
ECST (%)	51 ± 10	52 ± 10	48 ± 12	0.25
Grey scale, n (%)				
Lipid-rich plaques (I-II), n (%)	16 (31)	10 (38)	6 (24)	0.36
Fibro-calcific plaques (III–V), n (%)	35 (69)	19 (73)	16 (64)	.
CEUS$^+$, n (%)*	22 (46)	8 (35)	14 (56)	0.15
Atherosclerotic burden evaluation				
CC-IMT (mm)	0.81 (0.75–0.90)	0.81 (0.73–0.88)	0.80 (0.75–1.01)	0.42
TPA (cm^2)	0.76 (0.59–1.09)	0.82 (0.63–1.37)	0.73 (0.56–1.02)	0.21
Number of segments with plaque	4 (3–5)	4 (3–5)	4 (2–5)	0.28
Number of CEUS$^+$ plaques*	1 (0–2)	1 (0–2)	1 (0–2)	0.39
At least one CEUS$^+$ plaque*	30 (64)	18 (75)	12 (52)	0.23
Controlateral plaque > 40%, n (%)	28 (55)	15 (52)	13 (52)	0.78

Carotid CT angiography	N = 48	Non-progressors (n = 25)	Progressors (n = 23)	*p*
Main plaque characteristics				
NASCET area, %	30 (10–40)	30 (10–40)	30 (10–40)	0.91
ECST area, %	55 (38–75)	60 (43–80)	50 (28–66)	0.06
Density, HU	296 (112–560)	303 (122–889)	274 (83–443)	0.33
Volume, mm^3	111 (72–253)	150 (88–336)	95 (39–249)	0.13
Positive remodeling	0.49 (0.36–0.69)	0.48 (0.36–0.62)	0.50 (0.36–0.76)	0.82
Presence of ulcer, n (%)	7 (14)	4 (16)	3 (13)	1.00
Presence of luminal micro-calcification, n (%)	21 (43)	10 (40)	11 (48)	0.77
Plaque burden				
Total plaque volume, mm^3	300 (173–597)	322 (152–764)	256 (178–576)	0.41

ECST, European Carotid Surgery Trial; NASCET, North America Symptomatic Carotid Endarterectomy Trial; CEUS, contrast enhanced ultrasound; CC-IMT, common carotid intima media thickness; TPA, total plaque area.

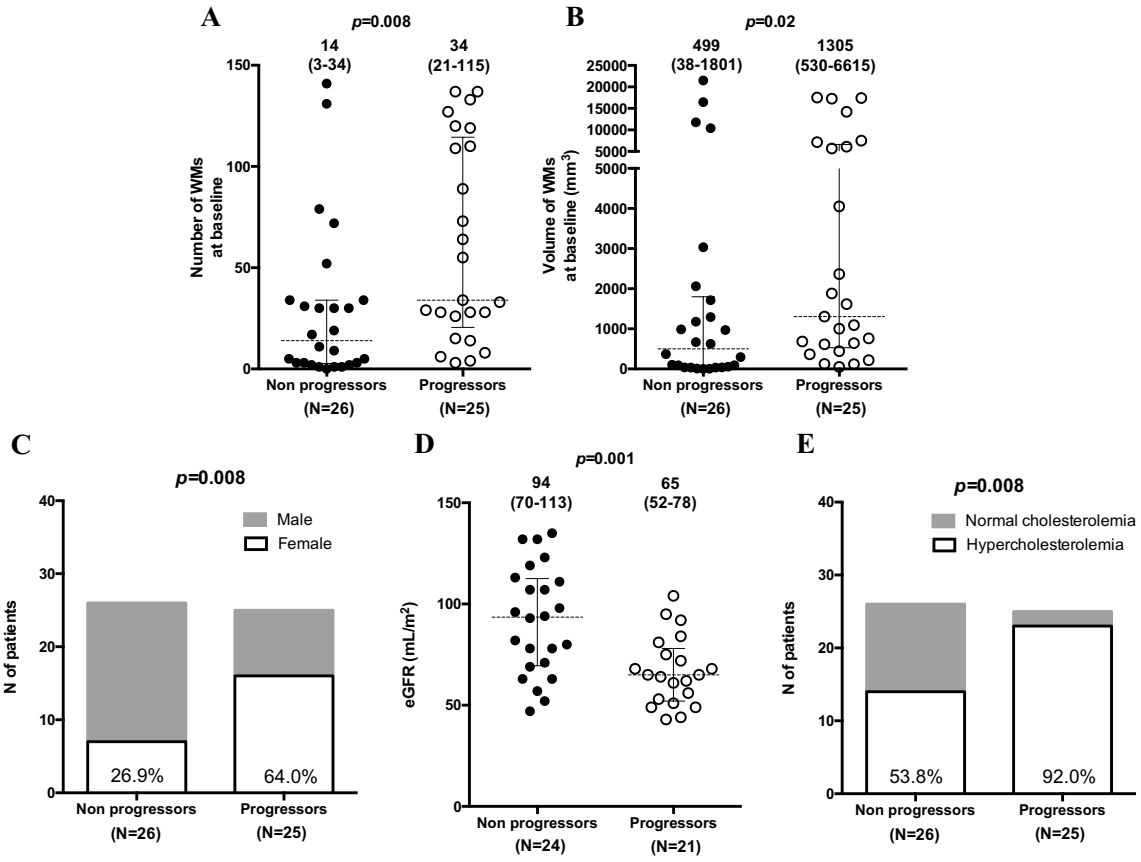

Fig. 1. Variables associated with white matter hyperintensity (WMH) progression.
(A and B) Subjects in which a new WMH was identified were defined as *progressors*, comparing with those without new WMH at follow up that were called *non progressors*. Those subjects with more baseline WMH, both in terms of number and volume, had more WMH progression based on univariate analysis. (C–E) Female sex, lower eGFR, and the presence of hypercholesterolemia emerged as independent variables associated with WMH progression at follow up (all $p < 0.01$) at multivariate analysis.

cellular markers of inflammation, including monocyte and lymphocyte subpopulations, was associated with WMH progression (Supplemental Table 2).

3.5. Multivariate regression analysis

We included in the multivariate model all the variables that were significantly associated with the occurrence of a new WMH lesion at univariate analysis. The resulting model included sex, hypercholesterolemia, statin therapy, treatment with a calcium channel blocker, GFR, and baseline burden of WMH. Female sex, hypercholesterolemia, and GFR remained significantly associated with WMH progression ($p = 0.01$, $p = 0.04$ and $p = 0.007$, respectively) following multivariate analysis. Table 3 shows the coefficients for the multivariable regression analysis.

3.6. Plaque characteristics, degree and side of WMH progression

Amongst the 25 patients defined as progressors, 6 (24%) had WMH progression only in the hemisphere ipsilateral to the main plaque, 18 (72%) had bilateral progression and 1 (4%) had only contralateral progression. Interestingly, among the 6 ipsilateral progressors none (0/6, 0%) had a plaque causing significant stenosis on the contralateral side, while 13 (13/19, 68.4%) of the remaining subjects had bilateral plaques. Thus, the absence of significant contralateral plaque was the only variable significantly associated with ipsilateral WMH progression ($p = 0.005$).

Only maximum stenosis severity was found to be associated with the extent of global WMH progression. Indeed, subjects with a stenosis of 50–70%, according to Doppler ultrasound criteria, had a larger number and volume of new WMH compared with those with stenosis below 50% (number of lesions: 10 [6–11] vs. 3 [1–8] respectively, $p = 0.009$; and total volume of WMH, 208.5 [138.0–309.3] mm^3 *vs.* 58.4 [49.6–134.5] mm^3 respectively, $p = 0.004$). The degree of stenosis evaluated on CTA significantly correlated with the burden of new WMH both in terms of number ($r = 0.531$, $p = 0.009$) and volume ($r = 0.578$, $p = 0.003$). No association was found between the number or volume of new lesions and follow up duration ($p = 0.22$ and $p = 0.21$ respectively). Subjects with a larger baseline volume of WMH had a significantly higher progression at follow up ($r = 0.42$, $p = 0.03$ for the number of new WMH and $r = 0.44$, $p = 0.03$ for the volume of new WMH). Supplemental Fig. 2 shows the described associations. No significant correlation was found between WMH number and volume and circulating lymphocytes and monocytes subpopulations.

4. Discussion

To the best of our knowledge, this is the first prospective longitudinal study that quantitatively investigated the progression of brain WMHs in patients with carotid disease. The main finding is that the progression of WMHs is a common event in asymptomatic patients with a carotid plaque of intermediate severity that are not yet considered candidate for revascularization. In fact, a new WMH occurred in almost half of the patients, with a median increase of 5 new lesions in those who progressed, during a relatively short follow up (20 months). Consequently, even these patients with asymptomatic carotid plaques

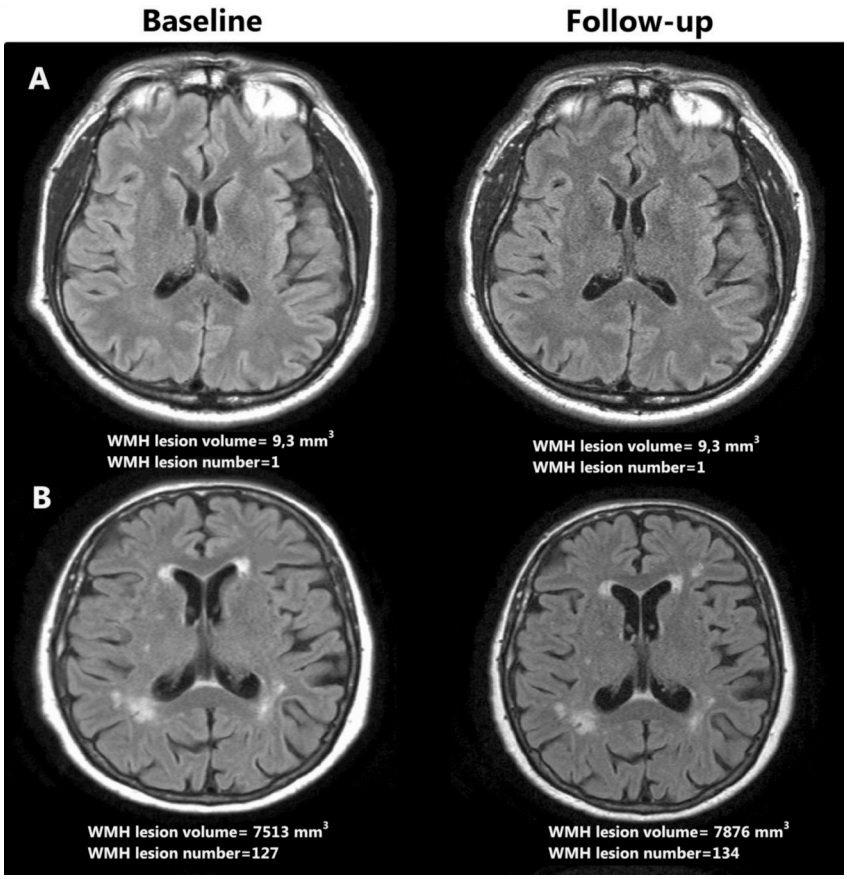

Fig. 2. Two representative cases of brain magnetic resonance imaging analysis at baseline and follow up.
(A) One representative subject with no white matter hyperintensity (WMH) progression (*non progressor*), who had 1 lesion at baseline and 1 lesion at the second scan. (B) One representative patient with progression of WMHs (*progressor*) at second brain scan from 127 to 134 WMHs. A new WMH is underlined in the figure.

Table 3
Multivariate analysis of variables associated with white matter hyperintensity (WMH) progression at univariate analysis.

Variable	β	95% CI	*p*
(Intercept)	0.13	−0.42; 0.68	0.64
Sex	0.27	0.06; 0.49	**0.01**
Hypercholesterolemia	0.29	0.02; 0.56	**0.04**
Statin	0.17	−0.07; 0.42	0.16
Calcium channel blockers	0.21	−0.04; 0.47	0.10
Baseline WMH	0.001	−0.001; 0.003	0.29
eGFR	−0.006	−0.01; 0.002	**0.007**

CI, confidence interval; GFR, glomerular filtration rate.

that are believed to be at low risk for future ischemic stroke, should be considered at risk of developing new WMHs in the short term, with potential impact on cognitive decline in the long-term. This pilot study was not powered to investigate the impact of WMHs at the cognitive level, even if it is recognized that brain WMHs can be seen as an early neuroimaging marker of cognitive impairment [14,30]. Furthermore, patients with a higher number of WMHs are at increased risk of future strokes [31]. The longitudinal study (EVA-MRI cohort study) after a 4-year follow up demonstrated that the presence of a carotid plaque was independently associated with progression of WMHs when compared with subjects without plaques [6]. It has to be pointed out that in the EVA-MRI study, WMH assessment was semi-quantitative, less than 25% of the subjects had a carotid plaque and only 3% had stenosis above 20% [6].

The second main finding of the present study is that patient-related variables and not plaque-related variables were significantly associated with brain WMHs progression. We found that females with an intermediate carotid plaque are at increased risk of WMHs progression compared with males. Of note, all women included in the present study were in menopausal status, therefore, no between-group imbalance in terms of hormonal status can be hypothesized as an underlying confounder. Another hospital-based cross-sectional study observed that female gender was associated with leukoaraiosis even if no quantitative analysis of WMHs was performed [32]. While the biological processes underlying this phenomenon are incompletely understood, it is currently accepted that females have a different susceptibility compared with males to neurodegeneration and neuroinflammatory conditions [33]. Hypercholesterolemia emerged as the main risk factor with the highest impact on WMHs in our population. That could imply that the prompt correction of this cardiovascular risk factor could be valuable not only in the reduction of risk of ischemic stroke but potentially also in the prevention of cognitive impairment on a vascular basis. However, in the present study, the short term follow up and the fact that most patients were on statins at baseline (up to 73%) did not allow to demonstrate a favorable association between the use of statin and WMH progression. On the contrary, there was an association at the univariate level (not at multivariate level) between the use of statin and the progression of WMHs. That could be explained by the fact that the use of statin identifies patients with more severe hypercholesterolemia. A similar finding was also reported in the population-based Cardiovascular Health Study [34].

The third independent variable associated with WMH progression was GFR, with lower GFR values in WHM progressors. The finding that patients with impaired renal function have an increased WMH burden has been reported in several cross-sectional studies both in subjects with cardiovascular risk factors [35,36], and patients with previous stroke [37,38]. Nevertheless, this is the first longitudinal study in patients with a carotid plaque showing a significant association between renal impairment and risk of WMH progression. Nevertheless, we cannot exclude that the association of GFR with WMH progression may be mediated by shared etiologic factors, more likely age, diabetes, and

hypertension.

Our study also suggests that prevention of progression of WMHs can be particularly relevant in those subjects with a high WMH burden at baseline, a factor that has been associated with further WHM progression at the univariate level. Baseline severity of the burden of WMHs emerged as a risk factor for WMH progression also in previous studies, such as in the longitudinal population-based Rotterdam Scan Study [39], and in the community-dwelling Austrian Stroke Prevention Study [40]. In the Rotterdam Scan Study, they showed a WMH progression in 39% of subjects after 3 years of follow up based on a semi-quantitative visual scale analysis [39], and also a significant progression of subcortical WMHs in women [39]. In the Austrian Stroke Prevention Study, they observed a visual progression of leukoaraiosis based on a qualitative visual scale in 18% subjects after 3 years of follow up [40]. Of note, neither hypertension nor age were found to be associated with WMH progression in our cohort, compared with the population-based Rotterdam Scan Study [39]. Indeed, the population of our study was homogeneous in terms of age with a Q1-Q3 between 62 and 75 ages, and this may have masked the effect of this parameter, together with the fact that the pattern of WMH progression is not linear along the lifespan [41]. In fact, previous studies that showed an association between age and WMHs were performed in healthy subjects from the general population with a larger age span and with a low incidence of presence of carotid plaque [39]. It must be noted that also in the Austrian Stroke Prevention Study no association was observed between the progression of leukoaraiosis and age [40]. For what concerns hypertension, in the population examined in our study, the vast majority of subjects, i.e. around 80%, had high blood pressure. This high proportion, together with the small number of individuals included, could have hampered the detection of the effect of hypertension on WMH progression. Nevertheless, it must be noted that in our previous study on a slightly larger population, in which we explored the relationship between cross-sectional WMHs and systemic and plaque-related factors, resistant hypertension emerged as the only factor independently associated both with the number and the volume of WMH [17].

While no association was found between plaque parameters and WMH progression, WMH progression appears to be modulated by the presence of atherosclerosis in the carotid arteries. Indeed, we described for the first time that among patients with evidence of WMH progression, a more severe carotid stenosis (those with a plaque with a degree of stenosis between 50 and 70% vs. those with plaque < 50%) was an independent factor associated with WMH progression. This association was confirmed by 2 independent imaging tools (Doppler flow velocity and CTA). The underlying process is not currently known but lends itself to speculation. On the one hand, more severe stenosis may be associated with more widespread impairment of blood vessels, there including the intracranial circulation, which we could not explore in the present study. Indeed, a recent report by Xu et al. demonstrated an association between the presence and severity of carotid stenoses and intracranial vessels atherosclerosis [42]. Therefore, a more severe carotid stenosis may mark the presence of an impairment of cerebral circulation. On the other hand, limited evidence supports nowadays the idea that more severe atherosclerotic involvement of the carotid arteries is linked to a higher burden of cardiovascular risk factors [43,44]. Therefore, a larger carotid plaque may indicate the presence of a more atherosclerosis-prone environment, and a milieu favoring microvascular damage.

Finally, we tried to explore the issue of laterality of the WMH. Previous studies have yielded contrasting data, on the one hand reporting a significantly higher number of WMH ipsilaterally to the atherosclerotic plaque, on the other hand, no differences in terms of laterality were found [8,30]. In this study population, amongst subjects who progressed only in the hemisphere subtended by the largest plaque, the vast majority had no evidence of plaque contra-laterally. This fact hints at a direct modulatory effect of carotid atherosclerosis on the development of new WMH.

4.1. Study limitations

The main study limitation is related to the small number of subjects that lowers its statistical power. However, compared with previous larger studies [40], the rate of WMH progression was higher in our cohort (49% vs. 18% in the Austrian Stroke Prevention Study). Furthermore, the stringent inclusion and exclusion criteria employed (e.g. the exclusion of subjects with a history of atrial fibrillation or previous cardiac surgery) has probably contributed to limit confounding factors. A few patients did not undergo the second MRI evaluation, thus further limiting the number of cases eligible for the final analysis. Nevertheless, this is a common bias that affected all previous prospective longitudinal studies assessing WMH progression [34,39,40].

In conclusion, we showed that half of the patients with carotid plaques of intermediate severity had evidence of WMH progression in the short term. Female gender and systemic risk factors such as hypercholesterolemia, and lower GFR, but not plaque characteristics or circulating cellular biomarkers, were associated with WMH progression.

Registration number

ClinicalTrials.gov registration number NCT03333330 and EudraCT number 2012-000648-83.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.04.230.

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