The Risk of Carotid Plaque Instability in Patients with Metabolic Syndrome: It is Higher in Women with Hypertriglyceridemia

Francesca Servadei

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Lucia Anemona

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Marina Cardellini

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Manuel Scimeca

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Manuela Montanaro (✉ manuela.montanaro@uniroma2.it)

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata <https://orcid.org/0000-0001-8441-3731>

Valentina Rovella

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Francesca Di Daniele

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Erica Giacobbi

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Jacopo Maria Legramante

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Annalisa Noce

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Rita Bonfiglio

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Patrizia Borboni

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Nicola Di Daniele

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Arnaldo Ippoliti

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Massimo Federici

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata

Alessandro Mauriello

University of Rome Tor Vergata: Università degli Studi di Roma Tor Vergata <https://orcid.org/0000-0002-7351-5676>

Original investigation

Keywords: Metabolic syndrome, carotid, histology, hypertriglyceridemia, post-menopause

Posted Date: February 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-165616/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

**The risk of carotid plaque instability in patients with metabolic syndrome:
it is higher in women with hypertriglyceridemia**

Francesca Servadei^{1, #}, Lucia Anemona^{1, #}, Marina Cardellini^{2, #}, Manuel Scimeca^{1,3,4}, Manuela Montanaro¹, Valentina Rovella⁵, Francesca Di Daniele⁶, Erica Giacobbi¹, Iacopo Maria Legramante², Annalisa Noce², Rita Bonfiglio^{1,7}, Patrizia Borboni², Nicola Di Daniele², Arnaldo Ippoliti⁸, Massimo Federici² and Alessandro Mauriello^{1,9}

[#]Francesca Servadei, Lucia Anemona and Marina Cardellini equally first author.

¹ Anatomic Pathology, Department of Experimental Medicine, University of Rome Tor Vergata, Via Montpellier 1, 00133 Rome, Italy;

² Department of Systems Medicine, University of Rome Tor Vergata, Italy

³ San Raffaele University, Via di Val Cannuta 247, 00166 Rome, Italy;

⁴ Saint Camillus International University of Health Sciences, Via di Sant'Alessandro, 8, 00131 Rome, Italy;

⁵ UOC of Internal Medicine – Center of Hypertension, University of Rome Tor Vergata, Italy

⁶ PhD School of Applied Medical, Surgical Sciences, University of Rome Tor Vergata, Italy

⁷ Fondazione Umberto Veronesi (FUV), Piazza Velasca 5, 20122 Milano (MI), Italy;

⁸ Vascular Surgery, Department of Biomedicine and Prevention, University of Rome Tor Vergata, Italy

⁹ Tor Vergata Oncoscience Research (TOR), University of Rome "Tor Vergata", Italy.

22

23 **Corresponding Author**

24 **Prof. Alessandro Mauriello, MD**

25 Department of Experimental Medicine, University "Tor Vergata", Via Montpellier 1, Rome
26 (RM) 00133 Italy; Email: alessandro.mauriello@uniroma2.it phone: +39 0620903908

27

28 **Abstract**

29 **Background:** Metabolic syndrome certainly favors growth of carotid plaque; however, it is
30 uncertain if it determines plaque destabilization. Furthermore, it is likely that only some
31 components of metabolic syndrome are associated with increased risk of plaque destabilization.
32 Therefore, we evaluated the effect of different elements of metabolic syndrome, individually
33 and in association, on carotid plaques destabilization.

34 **Methods:** A total of 186 carotid endarterectomies from symptomatic and asymptomatic
35 patients were histologically analysed and correlated with major cardiovascular risk factors.

36 **Results:** Metabolic syndrome, regardless of the cluster of its components, is not associated
37 with a significant increase in risk of plaque destabilization, rather with the presence of stable
38 plaques. The incidence of unstable plaques in patients with metabolic syndrome is quite low
39 (43.9%), when compared with that seen in the presence of some risk factors, but significantly
40 increases in the subgroup of female patients with hypertriglyceridemia, showing an odds ratio
41 of 3.01 (CI 95% 0.25 - 36.30).

42 **Conclusions:** Our data may help to identify patients with real increased risk of acute
43 cerebrovascular diseases and may support the hypothesis that the control of

hypertriglyceridemia should be a key point on prevention of carotid atherosclerotic plaque destabilization, especially in post-menopausal female patients.

Keywords: Metabolic syndrome, carotid, histology, hypertriglyceridemia, post-menopause.

Background

Many studies have demonstrated that Metabolic Syndrome (MetS), defined as a cluster of interconnected metabolic risk factors (including abdominal obesity, elevated fasting glucose, hypertriglyceridemia, hypertension, and low High-Density Lipoprotein (HDL) cholesterol levels), increases the risk for atherosclerosis and cardiovascular disease [1-6]. In addition, the recent interest of the scientific community has focused on the possible involvement of insulin resistance as a linking factor [7-10].

Atherosclerosis is a chronic inflammatory disease, to whose clinical manifestations multiple cellular and systemic events contribute [11]. Two types of atherosclerotic disease are described: a stable form (with low embolic risk, constant and slow growth of the plaque over time) and an unstable form (with a high embolic risk, linked to the presence of a significant inflammation which causes the rupture of the plaque, with thrombosis and subsequent fragment's detachment resulting in embolic complications and leading to acute clinical manifestations) [12-14]. Despite it is known that the cardiovascular risk factors certainly favor the development and growth of atherosclerotic plaque, it is not yet fully understood exactly which in particular favor its destabilization by accelerating plaque growth with consequent rupture and thrombosis [15-17]. It is possible to hypothesize that the main cardiovascular risk factors can play a crucial role in the destabilization process of atheromatous lesions through the modification of the histological composition of fibroatheromatous plaques. The identification of specific risk

factors associated with plaque destabilization is particularly important in the prevention and treatment of cerebrovascular syndromes. According to American Heart Association (AHA) and American College of Cardiology (ACC) guidelines, severe carotid stenosis is the main predisposing condition for acute cerebrovascular events and it still represents the most important criterion to identify patients who need surgical treatment [18-21]. Nevertheless, whether it is necessary to perform carotid endarterectomy in all asymptomatic patients with stenosis >70% (even if most of them have stable plaque that is not at risk of thromboembolism), is still an open question. Indeed, it is now clear that the degree of stenosis alone is not sufficient to accurately identify patients at high risk of developing an acute cerebrovascular event. Thus, in this scenario, the identification of risk factors correlated with plaque destabilization becomes necessary to calculate the most real probability of ischemic cerebrovascular complications, stratifying patients with carotid atherosclerosis.

Previous clinical studies have consistently demonstrated a significant association between MetS and incidence of ischemic stroke [2, 6, 22]. However, the relationship between MetS and the morphological characteristics of carotid plaque instability remains uncertain. Indeed, previous studies have mainly used the imaging, in particular the measurement of carotid Intimal-Medial Thickness (IMT) to assess the risk of ischemic stroke [8, 23-24]. This represents a valid approach to evaluate the degree of atherosclerosis, but it does not provide information on the possible destabilization of plaques, that can only be assessed by histological methods. Furthermore, it has not yet been clarified which of plaque's individual components were associated with an increased stroke risk.

Therefore, in this study we investigate each main factor involved in MetS, in order to estimate their impact on the destabilization of the carotid plaque. Specifically, to quantify their possible synergistic effect, the relative risk of different components of metabolic syndrome, individually and in association with each other, were calculated.

Methods

Cases selection and histology

A total of 186 carotid plaques from symptomatic (major stroke or transient ischemic attack - TIA) and asymptomatic patients submitted to surgical carotid endarterectomy (CEA) at the University of Tor Vergata (Rome, Italy) from 2016 to date were analysed. The sampling collection and analysis methods have been previously reported [13]. In short, samples were fixed in 10% buffered formalin, briefly decalcified using the Surgipath Decalcifer II (LEICA, Western Road, Stratford Upon Avon, UK) in order to allow the cutting without artifacts and to preserve the recognition of perfect morphology of the plaque, cut transversely every 5 mm, embedded in paraffin, and stained with haematoxylin-eosin. Only intact carotid plaques, from patients with a complete clinical and laboratory assessment of the major cardiovascular risk factors were histologically analysed and included in the study.

According to the modified AHA classification, atherosclerosis plaques have been histologically distinguished into unstable and stable [25]. Unstable plaques consisted of: (a) thrombotic plaques associated with rupture or erosion of the cap; (b) healed plaque with a thrombus in organization; (c) vulnerable plaque or thin-cap fibro-atheroma (TCFA) characterized by a fibrous cap less than 165 μ m thick, heavily infiltrated by macrophages CD68 positive (>25 per high magnification field), without plaque rupture. The other plaques, classified as stable, included: (a) fibroatheromata, such as plaques with a large lipidic necrotic core and thick non-inflamed cap, (b) fibrocalcific plaques with large calcification without extensive inflammation and (c) fibrous plaques mainly constituted by fibrous tissue (Figure 1). Immunohistochemical analysis was performed in all plaques in order to characterize the inflammatory infiltrate using CD68 antibody anti-monocyte/macrophages cells (rabbit monoclonal, clone KP-1; Ventana, Tucson, AZ, USA) and CD3 antibody anti-T cells (rabbit monoclonal, clone 2GV6; Ventana).

118 Histopathologic examination was performed, utilizing the definitions reported above, by two
119 blinded pathologists (A.M., F.S.). Inter-observer reliability was >98%.

120 Risk factors definition

121 Clinical records were reviewed for all cases in order to determine risk factors profile.

122 According to AHA Scientific Statements [1] the presence of any 3 of 5 following conditions
123 constitutes a diagnosis of metabolic syndrome: (a) elevated waist circumference (abdominal
124 obesity) >94 cm in men and >80 cm in women; (b) hypertriglyceridemia: patients with serum
125 triglycerides levels ≥ 150 mg/dL (> 1.70 mmol/L); (c) reduced HDL-C, < 40 mg/dL in men or
126 < 50 mg/dL in women; (d) elevated blood pressure, systolic ≥ 130 and/or diastolic ≥ 85 mm
127 Hg; (e) elevated fasting glucose ≥ 100 mg/dL.

128 The presence of hypertension was also evaluated according to the American College of
129 Cardiology/American Heart Association (ACC/AHA) [26], the European Society of
130 Cardiology (ESC) and European Society of Hypertension (ESH) Guidelines [27].

131 The other following major risk factors were considered: (a) hypercholesterolemia: patients with
132 total cholesterol level > 200 mg/dL (> 5.18 mmol/L); (b) diabetes mellitus: patients with fasting
133 blood glucose > 126 mg/dL and/or following oral treatment or insulin therapy; (c) patients with
134 tobacco dependence were categorized as: smokers, if the consumption was more than 10
135 cigarettes/day, while who had stopped smoking for > 5 years was considered as non-smokers.

136 Statistical analysis

137 Data were analyzed using SPSS version 16.0 (SPSS Inc, Chicago, Ill) software. Continuous
138 variables were expressed as the mean $\pm$ SD or $\pm$ SE. The Shapiro-Wilk test was used to
139 statistically assess the normal distribution of the data. Comparisons between continuous
140 variables were performed using the independent Student t-test or the Wilcoxon rank sum test.
141 Categorical data were analysed using the chi-square test or the Fisher exact test.

Multivariate analysis using stepwise logistic regression (using the “enter” method for variable selection) was utilized to identify independent risk factors which significantly correlate with the presence of plaque destabilization. The following variables were included: age, gender, hypertension, diabetes, smoking habit, hypercholesterolemia, low HDL and hypertriglyceridemia, abdominal obesity, metabolic syndrome. Multivariate analysis was performed in 3 models: (1) using the definition of hypertension according the ACC/AHA, (2) according the pressure cut-off of ESC/ESH Guidelines; (3) when the metabolic syndrome was considered in the multivariate analysis, hypertension, diabetes, hypertriglyceridemia, low HDL, and abdominal obesity were excluded. The odds ratio of an unstable plaque for the different risk factors was evaluated by logistic regression using the value of $\text{EXP}(B)$, where B represents the logistic coefficient.

Moreover, multivariate logistic regression was also used in order to evaluate the effect of each component of metabolic syndrome in plaque destabilization. In this analysis only patients with the metabolic syndrome were considered.

A 2-tailed p value <0.05 was considered statistically significant.

Results

Baseline data

Baseline data of patients are reported in *Table 1*. **(Please, insert Table 1 here).**

The mean age of 186 patients at time of surgical CEA was 72.6 ± 8.6 years, 131 (70.4%) were male and 55 (29.6%) were female. Seventy-two (38.7%) patients were symptomatic (affected by ipsilateral major stroke or TIA), while 114 (61.3%) were asymptomatic who underwent CEA for high grade carotid stenosis ($>60\%$), assessed by echography or, in rare cases, by bilateral CT angiography.

A metabolic syndrome was observed in 85 out of 186 patients (45.7%). All patients included in this study presented at least one risk factor. Among single risk factors, the hypertension was

the most frequently observed when it was evaluated according to both the ACC/AHA (162 patients, 87.1%) and to the ESC/ESH criteria (112 patients, 60.2%). The low incidence of hypercholesterolemia (only 15.6% of cases) may probably be explained by the 128 patients (68.8%) who underwent statins treatments. Continuous treatment with aspirin (100 md/die) was administered to all patients in the post-operative and follow-up periods.

In 82 out of 186 patients (44.1%) unstable plaques were found (*Figure 1*). They consisted in 48 thrombotic plaques (all from symptomatic patients) associated with rupture of a thin fibrous cap rich in inflammatory cells, 16 TCFA (10 from symptomatic and 6 from asymptomatic patients) and 16 plaques with an organized acute thrombus (all symptomatic) characterized by a network of large, thin-walled vascular channels and a variable number of macrophagic cells loaded with hemosiderin within the area of an acute thrombus. In the remaining 2 unstable plaques, a calcified nodule was found protruding into the lumen covered by extremely thin fibrous cap. The remaining 104 carotids (55.9% of cases) showed a stable plaque, characterized by a variable lipid-necrotic core containing extracellular lipid, cholesterol crystals and necrotic debris covered by a thick fibrous cap with few inflammatory cells (*Figure 1*). In 33 plaques (both stable and unstable) a large calcification was observed.

Plaque instability and risk factors

The presence of unstable carotid plaques was not correlated with the finding of specific risk factors, as demonstrated by the uni- and multivariate analysis (see *Table 2*).

Table 2. Plaque instability and risk factors

Stable plaques (104 cases)	Unstable plaques (82 cases)	Odds ratio (95% CI)	P uni- variate analysis	P multi- variate analysis
----------------------------------	-----------------------------------	------------------------	----------------------------------	------------------------------------

Age (yrs $\pm$ SD)	72.5 $\pm$ 8.1	72.8 $\pm$ 9.3	0.99 (0.96-1.03)	0.80	0.79
Gender					
Male	65 (62.5%)	66 (80.5%)	0.41 (0.20-0.82)	0.008	0.01
Female	39 (37.5%)	16 (19.5%)			
Hypertension acc. AHA	90 (86.5%)	72 (87.8%)	1.38 (0.54-3.56)	0.80	0.50
Diabetes	48 (46.2%)	32 (39.0%)	0.69 (0.35-1.36)	0.33	0.28
Smoking habit	23 (22.1%)	17 (20.7%)	0.81 (0.37-1.75)	0.82	0.58
Hypercholesterolemia	17 (16.3%)	12 (14.6%)	0.72 (0.29-1.76)	0.75	0.47
Hypertriglyceridemia	35 (33.7%)	35 (42.7%)	1.75 (0.87-3.51)	0.21	0.12
Low-HDL	48 (46.2%)	38 (46.3%)	1.22 (0.62-2.40)	0.98	0.57
Abdominal obesity	20 (19.2%)	9 (11.0%)	0.46 (0.19-1.12)	0.12	0.09
Hypertension acc. ESC	62 (59.6%)	50 (61.0%)	1.11 (0.58-2.14)	0.85	0.74
Metabolic syndrome	49 (47.1%)	36 (43.9%)	0.86 (0.46-1.58)	0.66	0.62

187

188 Only a significant correlation with the gender was observed, as males showed a higher
189 incidence of unstable plaques than females (p=0.01). In fact, unstable plaques were observed
190 in 66 of the 131 (50.45%) male patients undergoing CEA and only in 16 of the 55 (29.1%)
191 female patients.

192 In particular, the incidence of unstable plaques in patients with metabolic syndrome was 42.4%
193 (36 out of 85 cases), with no significant differences compared to that of stable plaques (p =
194 0.62).

195 When only the individual risk factors were considered in the statistical analysis, the greatest
196 odds ratio for an unstable plaque was observed in patients with hypertriglyceridemia (1.75,
197 95% CI 0.87-3.51) or hypertension (according to ACC/AHA) (1.38, 95% CI 0.54-3.56).

Considering the metabolic syndrome instead of hypertension, diabetes, hypertriglyceridemia, low HDL-C and abdominal obesity, a low odds ratio for plaque instability (0.86, 95% CI 0.46-1.58) were found by multivariate analysis.

Components of metabolic syndrome and plaque instability

Taking into account only the patients with metabolic syndrome, we evaluated the effect of single risk factors on plaque destabilization.

Patients with hypertriglyceridemia were those who presented a greater risk of destabilization of plaques (an odds-ratio of 1.43, CI95% 0.44 - 3.52). Since a significant inverse correlation between age and triglyceridemia values was observed ($r = -0.44$, $p = 0.001$), the risk was calculated in two subgroups of patients with metabolic syndrome, with an age of ± 70 years old. Patients < 70 years old had an odds ratio of 1.55 (CI95% 0.25-9.54), while in the elderly it was reduced to 1.00 (CI95% 0.17 - 5.76). The risk was significantly increased in women with hypertriglyceridemia compared to males. In fact, the odds ratio was 1.28 (CI95% 0.37 - 4.48) in the male subgroup and 3.01 (CI95% 0.25 - 36.30) in the female one.

The other possible combinations of the metabolic syndrome components did not achieve a significant increase in risk.

Discussion

The results obtained in our study show that MetS is not associated with a significant increase in the risk of carotid plaque destabilization, regardless of the analyzed risk factors. On the contrary, MetS showed a correlation with the presence of stable plaques according to the histological features released by the ACC/AHA [26]. Indeed, the incidence of unstable plaques in patients with MetS is rather low (43.9%), when compared with that observed in the presence of other individual risk factors such as hypertension. However, the risk of carotid plaque

destabilization significantly increases in the subgroup of female patients with hypertriglyceridemia, showing an odds ratio of 3.01.

Carotid stenosis still remains the criterion of choice to undergo a preventive endarterectomy, especially in asymptomatic patients [18-21]. According to our previous studies, results of the present study showed that more than 50% of asymptomatic patients undergoing surgery had a stable carotid plaque that evolves very slowly over time [13-14, 28]. This problem is particularly important in those asymptomatic patients with unilateral carotid stenosis who have no other comorbidities that may increase the risk of an ischemic stroke. The prevention of atherosclerosis is based on the control of the major cardiovascular risk factors and each patient has many of them, whose interaction must be taken into consideration. The various risk factors probably promote the slow growth of the carotid plaque. However, there are conflicting data which lead to the hypothesis that they can significantly determine not only the plaque growth but also its rupture and thrombosis.

The results of the multivariate analysis reported in our study showed that none of the considered risk factors were independently associated with an increased risk of destabilization of the carotid plaque (*Table 2*). In particular, unstable carotid plaques were more frequent in hypertensive patients but, according to both the AHA [26] and ESC Guidelines [27], the assessed incidence of hypertension was equally high even in patients with stable plaques ($p=0.50$ and 0.74 , respectively). About 40% of patients with unstable plaque were also affected by the MetS itself; nevertheless, a similar incidence was observed in those with stable plaque ($p=0.62$). However, our results do not contradict some clinical meta-analyses, that propose the MetS as a relevant risk of developing cerebrovascular disease [5-6, 29]. Among them, *Mottillo et al.* performed a large clinical meta-analysis by investigating data of 87 studies, comprising 951,083 subjects [29]. The increased risk, as our results have demonstrated, is not due to MetS itself but it varies on the basis of the combination of MetS components present in each patient.

Only few and contradictory studies have assessed all possible combinations of MetS components in order to determine which elements are most strongly associated with atherosclerosis [8, 30-32]. In particular, *Golden et al.* found a strong independent association between selected groupings of MetS components and excess carotid intimal-medial thickness, suggesting a synergy for atherosclerosis risk beyond what would have been expected from a merely additive effects [8]. The evaluation of carotid intimal-medial thickness represents only a subclinical assessment of atherosclerosis [8, 23-24] and does not distinguish between a stable plaque and ones with risk of rupture and thrombosis. A precise risk evaluation of plaque rupture can only be performed with histological methods (as in the present study) or with high-definition imaging approaches.

The major component of MetS that significantly correlates with an increased risk of destabilization of the carotid plaque was the hypertriglyceridemia. In our series, hypertriglyceridemia represents the major component of dyslipidemia associated with the progression of atherosclerotic carotid plaque (*Table 2*). In fact, it should be emphasized that in our study, patients underwent endarterectomy (symptomatic or asymptomatic but potentially with risk of acute cerebrovascular syndrome), showed a very low incidence of hypercholesterolemia (15.6% of cases). This can be explained by the fact that 128 out of 186 patients (68.8%) were taking statins which, as it is well known, have a lowering effect on both LDL-C levels and anti-inflammatory effects.

Recent studies have long considered that elevated triglyceride levels increase the risk of cardiovascular disease that persist despite statin treatment [2, 33-34]. Normal or low triglyceride levels are associated with reduced cardiovascular event, as demonstrated by PROVE-IT TIMI (Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction) trial [35]. Patients who received statin treatment after hospitalization for acute coronary syndrome had a lower risk of further coronary heart disease events if

triglyceride levels were <150 mg/dL [35]. These results were confirmed by the recent REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention) clinical trial carried out on 8170 patients under statin therapy, with elevated fasting triglyceride levels and normal LDL-C levels [36]. This trial demonstrated a significant reduction in ischemic events in subjects taking Icosapent Ethyl (a molecule which decreases triglyceride levels without modifying LDL-C) as compared to subjects taking placebo, thus demonstrating how the reduction of triglyceride levels is associated with a lower risk of cardiovascular events among subjects with normal LDL-C levels [36]. Despite the normalization of LDL-C levels for the intake of statins, our results confirm that also in carotid district the increase in triglycerides is associated with an increase in the destabilization of atherosclerotic plaque, providing an evident morphological basis.

Hypertriglyceridemia could favor the destabilization of the carotid plaques by increasing local inflammation, damaging the endothelium, and stimulating the expression of cellular adhesion molecules [37-38]. High levels of triglycerides are also associated with increased remnant lipoprotein particles that induce the expression of pro-inflammatory cytokines (TNF- α , IL-6, VCAM1, etc.) which have a cytotoxic effect on the endothelium [39].

The observation that the risk of plaque instability is higher in female patients with metabolic syndrome and hypertriglyceridemia, than in those without hypertriglyceridemia, confirms the conclusions of a recent meta-analysis by Li et al. [6]. The study reported that patients with MetS had a significantly higher risk of incident stroke, as compared to those without MetS, and that this effect was most remarkable among females (RR, 1.83 [95% CI, 1.31–2.56]) compared with males (RR, 1.47 [95% CI, 1.22–1.78]).

Sex-difference in cardiovascular disease is now widely accepted [40]. Most likely, sex hormones play an atherosclerotic protective role in women, due to their action on endothelial function and lipid homeostasis. Estrogens might have plaque stabilization properties and

effects on plaque inflammatory status [41] [42] . The effects of estrogens are probably age-dependent, reducing inflammation in younger age, while an opposite pro-inflammatory effect could be observed in older women, as those analyzed in the present study [43] [44].

Conclusions

Data here reported suggest that a better understanding of how each individual component of MetS influences the stroke risk correlated to carotid plaque destabilization, rupture, and thrombosis, may help to stratified patients according to the real risk of acute cerebrovascular diseases. Moreover, our results support the hypothesis that the control of hypertriglyceridemia should be a key point on prevention of the destabilization of atherosclerotic carotid plaque, especially in post-menopausal female patients. However, data on sex-difference are still limited, and further studies are recommended to better define which differences may have implications for clinical prevention and management of acute complications of carotid artery disease.

List of abbreviations

MetS: Metabolic Syndrome.

HDL-C: High-Density Lipoprotein Cholesterol.

IMT: Intimal-Medial Thickness.

TIA: Transient Ischemic Attack.

CEA: Carotid Endarterectomy.

TCFA: Thin-Cap Fibro-Atheroma.

LDL-C: Low-Density Lipoprotein Cholesterol.

318 **Declarations**

319 **Ethics approval and consent to participate:** Informed consent was obtained from each
320 patient. The study protocol was approved by the Institutional Review Board of the University
321 of Rome “Tor Vergata”.

322

323 **Consent for publication:** All authors have agreed with the submission in its present (and
324 subsequent) forms.

325

326 **Availability of data and materials:** The data that support the findings of this study are
327 available from the corresponding author upon reasonable request.

328 **Competing interests:** The authors declare that they have no competing interests. The study is
329 original, and the manuscript has not been published yet and is not being considered for
330 publication.

331 **Funding:** This study was not supported by specific funding.

332

333 **Author Contributions:** Conceptualization: F.S., L.A., M.C., M.F, and A.M.; Methodology:
334 F.S., L.A., M.C., V.R., M.S., and M.M; Formal Analysis: F.S., M.M., E.G., R.B., and P.B.;
335 Data Curation: M.C., M.S., M.M., I.M.L.and F.D.D.; Writing: Original Draft Preparation:
336 F.S., L.A., and A.M.; Writing: Review and Editing: M.C., V.R., M.S., M.M., F.D.D., E.G.,
337 R.B., P.B., M.F., I.M.L., N.D.D. and A.M.; Supervision, M.F., N.D.D. and A.M. All authors
338 have read and agreed to the published version of the manuscript.

339

340 **Acknowledgements:**

341 Not applicable.

References

1. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr.: Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009, 120(16):1640-1645.
2. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR *et al*: Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation* 2019, 139(10):e56-e528.
3. Li X, Fang F, Fu X, Lin H, Gao Q: Is Metabolic Syndrome Associated with the Risk of Recurrent Stroke: A Meta-Analysis of Cohort Studies. *J Stroke Cerebrovasc Dis* 2017, 26(12):2700-2705.
4. Roever L, Resende ES, Diniz ALD, Penha-Silva N, O'Connell JL, Gomes PFS, Zanetti HR, Roerver-Borges AS, Veloso FC, Fidale TM *et al*: Metabolic syndrome and risk of stroke: Protocol for an update systematic review and meta-analysis. *Medicine (Baltimore)* 2018, 97(15):e9862.
5. Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, Montori VM: Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *J Am Coll Cardiol* 2007, 49(4):403-414.
6. Li X, Lin H, Fu X, Lin W, Li M, Zeng X, Gao Q: Metabolic syndrome and stroke: A meta-analysis of prospective cohort studies. *J Clin Neurosci* 2017, 40:34-38.

- 367 7. Kernan WN, Inzucchi SE, Viscoli CM, Brass LM, Bravata DM, Shulman GI, McVeety
368 JC, Horwitz RI: Impaired insulin sensitivity among nondiabetic patients with a recent
369 TIA or ischemic stroke. *Neurology* 2003, 60(9):1447-1451.
- 370 8. Golden SH, Folsom AR, Coresh J, Sharrett AR, Szklo M, Brancati F: Risk factor
371 groupings related to insulin resistance and their synergistic effects on subclinical
372 atherosclerosis: the atherosclerosis risk in communities study. *Diabetes* 2002,
373 51(10):3069-3076.
- 374 9. Kernan WN, Viscoli CM, Furie KL, Young LH, Inzucchi SE, Gorman M, Guarino PD,
375 Lovejoy AM, Peduzzi PN, Conwit R *et al*: Pioglitazone after Ischemic Stroke or
376 Transient Ischemic Attack. *N Engl J Med* 2016, 374(14):1321-1331.
- 377 10. Cardellini M, Rizza S, Casagrande V, Cardolini I, Ballanti M, Davato F, Porzio O,
378 Canale MP, Legramante JM, Mavilio M *et al*: Soluble ST2 is a biomarker for
379 cardiovascular mortality related to abnormal glucose metabolism in high-risk subjects.
380 *Acta Diabetol* 2019, 56(3):273-280.
- 381 11. Hansson GK, Libby P: The immune response in atherosclerosis: a double-edged sword.
382 *Nat Rev Immunol* 2006, 6(7):508-519.
- 383 12. Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R: Concept of
384 vulnerable/unstable plaque. *Arterioscler Thromb Vasc Biol* 2010, 30(7):1282-1292.
- 385 13. Spagnoli LG, Mauriello A, Sangiorgi G, Fratoni S, Bonanno E, Schwartz RS, Piepgras
386 DG, Pistolese R, Ippoliti A, Holmes DR, Jr.: Extracranial thrombotically active carotid
387 plaque as a risk factor for ischemic stroke. *JAMA* 2004, 292(15):1845-1852.
- 388 14. Mauriello A, Servadei F, Sangiorgi G, Anemona L, Giacobbi E, Liotti D, Spagnoli LG:
389 Asymptomatic carotid plaque rupture with unexpected thrombosis over a non-canonical
390 vulnerable lesion. *Atherosclerosis* 2011, 218(2):356-362.

- 391 15. Mauriello A, Sangiorgi GM, Virmani R, Trimarchi S, Holmes DR, Jr., Kolodgie FD,
392 Piepgras DG, Piperno G, Liotti D, Narula J *et al*: A pathobiologic link between risk
393 factors profile and morphological markers of carotid instability. *Atherosclerosis* 2010,
394 208(2):572-580.
- 395 16. Scimeca M, Anemona L, Granaglia A, Bonfiglio R, Urbano N, Toschi N, Santeusano
396 G, Schiaroli S, Mauriello S, Tancredi V *et al*: Plaque calcification is driven by different
397 mechanisms of mineralization associated with specific cardiovascular risk factors. *Nutr*
398 *Metab Cardiovasc Dis* 2019, 29(12):1330-1336.
- 399 17. Cardellini M, Rovella V, Scimeca M, Anemona L, Bischetti S, Casella S, Saggini A,
400 Bonanno E, Ballanti M, Davato F *et al*: Chronic Kidney Disease Is Linked to Carotid
401 Nodular Calcification, An Unstable Plaque Not Correlated to Inflammation. *Aging Dis*
402 2019, 10(1):71-81.
- 403 18. Kernan WN, Ovbiagele B, Black HR, Bravata DM, Chimowitz MI, Ezekowitz MD,
404 Fang MC, Fisher M, Furie KL, Heck DV *et al*: Guidelines for the prevention of stroke
405 in patients with stroke and transient ischemic attack: a guideline for healthcare
406 professionals from the American Heart Association/American Stroke Association.
407 *Stroke* 2014, 45(7):2160-2236.
- 408 19. Eckstein HH: European Society for Vascular Surgery Guidelines on the Management
409 of Atherosclerotic Carotid and Vertebral Artery Disease. *Eur J Vasc Endovasc Surg*
410 2018, 55(1):1-2.
- 411 20. Halliday A, Harrison M, Hayter E, Kong X, Mansfield A, Marro J, Pan H, Peto R,
412 Potter J, Rahimi K *et al*: 10-year stroke prevention after successful carotid
413 endarterectomy for asymptomatic stenosis (ACST-1): a multicentre randomised trial.
414 *Lancet* 2010, 376(9746):1074-1084.

- 415 21. Tsivgoulis G, Safouris A, Kim DE, Alexandrov AV: Recent Advances in Primary and
416 Secondary Prevention of Atherosclerotic Stroke. *J Stroke* 2018, 20(2):145-166.
- 417 22. Guembe MJ, Fernandez-Lazaro CI, Sayon-Orea C, Toledo E, Moreno-Iribas C: Risk
418 for cardiovascular disease associated with metabolic syndrome and its components: a
419 13-year prospective study in the RIVANA cohort. *Cardiovasc Diabetol* 2020,
420 19(1):195.
- 421 23. Kumar P, Sharma R, Misra S, Kumar A, Nath M, Nair P, Vibha D, Srivastava AK,
422 Prasad K: CIMT as a risk factor for stroke subtype: A systematic review. *Eur J Clin*
423 *Invest* 2020, 50(11):e13348.
- 424 24. Willeit P, Tschiderer L, Allara E, Reuber K, Seekircher L, Gao L, Liao X, Lonn E,
425 Gerstein HC, Yusuf S *et al*: Carotid Intima-Media Thickness Progression as Surrogate
426 Marker for Cardiovascular Risk: Meta-Analysis of 119 Clinical Trials Involving 100
427 667 Patients. *Circulation* 2020, 142(7):621-642.
- 428 25. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM: Lessons from sudden
429 coronary death: a comprehensive morphological classification scheme for
430 atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 2000, 20(5):1262-1275.
- 431 26. Whelton PK, Carey RM, Aronow WS, Casey DE, Jr., Collins KJ, Dennison
432 Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW *et al*: 2017
433 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for
434 the Prevention, Detection, Evaluation, and Management of High Blood Pressure in
435 Adults: A Report of the American College of Cardiology/American Heart Association
436 Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol* 2018, 71(19):e127-e248.
- 437 27. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, Clement DL,
438 Coca A, de Simone G, Dominiczak A *et al*: 2018 ESC/ESH Guidelines for the
439 management of arterial hypertension: The Task Force for the management of arterial

hypertension of the European Society of Cardiology and the European Society of Hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension. *J Hypertens* 2018, 36(10):1953-2041.

28. Mauriello A, Sangiorgi G, Virmani R, Servadei F, Trimarchi S, Holmes DR, Jr., Kolodgie F, Biondi Zoccai G, Leuzzi C, Spagnoli LG: Evidence of a topographical link between unstable carotid plaques and luminal stenosis: can we better stratify asymptomatic patients with significant plaque burden? *Int J Cardiol* 2012, 155(2):309-311.

29. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, Rinfret S, Schiffrin EL, Eisenberg MJ: The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol* 2010, 56(14):1113-1132.

30. Haffner SM, D'Agostino R, Jr., Mykkanen L, Tracy R, Howard B, Rewers M, Selby J, Savage PJ, Saad MF: Insulin sensitivity in subjects with type 2 diabetes. Relationship to cardiovascular risk factors: the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 1999, 22(4):562-568.

31. Sheu WH, Jeng CY, Young MS, Le WJ, Chen YT: Coronary artery disease risk predicted by insulin resistance, plasma lipids, and hypertension in people without diabetes. *Am J Med Sci* 2000, 319(2):84-88.

32. Rovella V, Anemona L, Cardellini M, Scimeca M, Saggini A, Santeusano G, Bonanno E, Montanaro M, Legramante IM, Ippoliti A *et al*: The role of obesity in carotid plaque instability: interaction with age, gender, and cardiovascular risk factors. *Cardiovasc Diabetol* 2018, 17(1):46.

33. Toth PP, Shah PK, Lepor NE: Targeting hypertriglyceridemia to mitigate cardiovascular risk: A review. *Am J Prev Cardiol* 2020, 3:100086.

- 465 34. Schwartz GG, Abt M, Bao W, DeMicco D, Kallend D, Miller M, Mundl H, Olsson AG:
466 Fasting triglycerides predict recurrent ischemic events in patients with acute coronary
467 syndrome treated with statins. *J Am Coll Cardiol* 2015, 65(21):2267-2275.
- 468 35. Miller M, Cannon CP, Murphy SA, Qin J, Ray KK, Braunwald E: Impact of triglyceride
469 levels beyond low-density lipoprotein cholesterol after acute coronary syndrome in the
470 PROVE IT-TIMI 22 trial. *J Am Coll Cardiol* 2008, 51(7):724-730.
- 471 36. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, Doyle RT, Jr.,
472 Juliano RA, Jiao L, Granowitz C *et al*: Cardiovascular Risk Reduction with Icosapent
473 Ethyl for Hypertriglyceridemia. *N Engl J Med* 2019, 380(1):11-22.
- 474 37. Marz W, Scharnagl H, Winkler K, Tiran A, Nauck M, Boehm BO, Winkelmann BR:
475 Low-density lipoprotein triglycerides associated with low-grade systemic
476 inflammation, adhesion molecules, and angiographic coronary artery disease: the
477 Ludwigshafen Risk and Cardiovascular Health study. *Circulation* 2004, 110(19):3068-
478 3074.
- 479 38. Nordestgaard BG: Triglyceride-Rich Lipoproteins and Atherosclerotic Cardiovascular
480 Disease: New Insights From Epidemiology, Genetics, and Biology. *Circ Res* 2016,
481 118(4):547-563.
- 482 39. Doi H, Kugiyama K, Oka H, Sugiyama S, Ogata N, Koide SI, Nakamura SI, Yasue H:
483 Remnant lipoproteins induce proatherothrombogenic molecules in endothelial cells
484 through a redox-sensitive mechanism. *Circulation* 2000, 102(6):670-676.
- 485 40. Sangiorgi G, Roversi S, Biondi Zoccai G, Modena MG, Servadei F, Ippoliti A,
486 Mauriello A: Sex-related differences in carotid plaque features and inflammation. *J*
487 *Vasc Surg* 2013, 57(2):338-344.
- 488 41. Burke AP, Farb A, Malcom G, Virmani R: Effect of menopause on plaque morphologic
489 characteristics in coronary atherosclerosis. *Am Heart J* 2001, 141(2 Suppl):S58-62.

- 490 42. Iemolo F, Martiniuk A, Steinman DA, Spence JD: Sex differences in carotid plaque
491 and stenosis. *Stroke* 2004, 35(2):477-481.
- 492 43. Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, Trevisan M, Black
493 HR, Heckbert SR, Detrano R *et al*: Estrogen plus progestin and the risk of coronary
494 heart disease. *N Engl J Med* 2003, 349(6):523-534.
- 495 44. Xing D, Nozell S, Chen YF, Hage F, Oparil S: Estrogen and mechanisms of vascular
496 protection. *Arterioscler Thromb Vasc Biol* 2009, 29(3):289-295.

501 **Table 1. Baseline characteristics of patients**

	<i>N(%) or mean (SD)</i>
Total	N = 186
Age	72.6 (8.6)
Gender	
Male	131 (70.4%)
Female	55 (29.6%)
Cerebrovascular disease	
Symptomatic patients	74 (39.8%)
Ipsilateral major stroke	44 (23.7%)
TIA	30 (16.1%)
Asymptomatic patients	112 (60.2%)
Risk factors	
Hypertension AHA [26]	162 (87.1%)
Hypertension ESC [27]	112 (60.2%)
Diabetes	80 (43.0%)
Smoking habit	40 (21.5%)
Hypercholesterolemia	29 (15.6%)
Hypertriglyceridemia	70 (37.6%)

Low-HDL	86 (46.2%)
IRC	56 (30.1%)
Metabolic syndrome	85 (45.7%)
Drugs	
Statins	128 (68.8%)
Diuretics	153 (82.3%)
Associated vascular disease	
Acute cardiovascular disease	45 (24.2%)
Previous myocardial infarction	37 (19.9%)
Unstable angina	8 (4.3%)
Peripheral arterial disease	61 (32.8%)
Aortic aneurysm	11 (5.9%)
Histological type of carotid plaque	
Stable plaques	104 (55.9%)
Fibroatheromata	71 (38.2%)
Fibrocalcific	33 (17.7%)
Unstable plaques	82 (44.1%)
Thrombotic plaque	48 (25.8%)
With a thrombus in organization	16 (8.6%)
TCFA	16 (8.6%)
Calcified nodule	2 (1.1%)

502 **Legend of Figures**

503 **Figure 1. Histology of carotid plaques**

504 Panels A and B) Stable plaque characterized by a thick fibrous cap and a large lipidic necrotic
505 core with few inflammatory cells (Movat, 2x); Panels C and D) Unstable plaque constituted by
506 a thrombotic plaque associated to the cap rupture (Movat, 2x).

507 ***Cap**: fibrous cap; **Ath**: lipidic necrotic core; **Thr**: acute thrombus; **Rup**: site of cap rupture.

Figures

Figure 1

Histology of carotid plaques Panels A and B) Stable plaque characterized by a thick fibrous cap and a large lipidic necrotic core with few inflammatory cells (Movat, 2x); Panels C and D) Unstable plaque constituted by a thrombotic plaque associated to the cap rupture (Movat, 2x). *Cap: fibrous cap; Ath: lipidic necrotic core; Thr: acute thrombus; Rup: site of cap rupture.