Increased Incidence of Ischemic Cerebrovascular Events in Cardiovascular Patients With Elevated Apolipoprotein CIII

Oliviero Olivieri, MD; Manuel Cappellari, MD; Gianni Turcato, MD; Bruno Bonetti, MD; Domenico Girelli, MD, PhD; Francesca Pizzolo, MD, PhD; Simonetta Friso, MD, PhD; Antonella Bassi, MD; Annalisa Castagna, MSc, PhD; Nicola Martinelli, MD, PhD

Background and Purpose—Apo CIII (apolipoprotein CIII), a crucial regulator of lipoprotein metabolism, has been associated with increased activity of coagulation factors and thrombin generation and, in turn, with an increased risk of thromboembolic events in both arterial and venous districts. Thus, we hypothesized that it may affect the risk of acute ischemic cerebrovascular events in cardiovascular patients.

Methods—We systematically checked medical records and quantified cerebral ischemic events in a cohort of 950 subjects (median age 65 with interquartile range, 55–79 years; 30.7% females) with or without angiographically defined coronary artery disease (CAD: 774 CAD and 176 CAD-free, respectively). All the subjects, enrolled between May 1999 and December 2006, were prospectively followed until death or July 31, 2018. Assessments of complete plasma lipid and apolipoprotein profiles, including Apo A-I, B, CIII, and E, were available for all subjects at enrollment.

Results—After a median follow-up of 130 months (interquartile range, 69–189), 95 subjects (10%) suffered ischemic stroke/transient ischemic attack (TIA) events. Stroke/TIA subjects had higher Apo CIII plasma concentration (11.4; interquartile range: 9.3–14.4 mg/dL) at enrollment than those without stroke/TIA (10.4, interquartile range: 8.7–13.0 mg/dL). Subjects with Apo CIII levels above the median value (10.6 mg/dL) exhibited an ≈2-fold increased risk of stroke/TIA, even after adjustment for potential confounders, including sex, age, CAD diagnosis, hypertension, atrial fibrillation, oral anticoagulant treatment, and all plasma lipid parameters (hazard ratio: 2.23 [95% CI, 1.21–4.13]). This result was confirmed in CAD and CAD-free populations, separately, and even by a propensity score matching method, in which 98 CAD and 98 CAD-free subjects were one-to-one matched for all clinical and laboratory characteristics.

Conclusions—These findings suggest that a high Apo CIII plasma concentration may predict an increased risk of ischemic stroke/TIA in cardiovascular patients. **(*Stroke*. 2020;51:61-68. DOI: 10.1161/STROKEAHA.119.026811.)**

Key Words: apolipoproteins ■ cardiovascular diseases ■ coronary artery disease ■ ischemic cerebrovascular events ■ lipids

Ischemic stroke is a leading cause of adult disability and death worldwide[1] with an enormous social-economic impact on Western Health Systems. For this reason, strategies for prevention and early detection of populations at risk are key-measures to tackle this global challenge.

Apo CIII (apolipoprotein CIII) is a crucial regulator of plasma lipids, especially TRLs (triglyceride-rich lipoproteins), and is recognized as a causal risk factor for ischemic heart disease.[2] Increased Apo CIII plasma concentration is indeed a well-known predisposing condition for the development of atherosclerotic disease.[3] Although such atherogenetic role is mainly attributed to altered lipid mechanisms, in the last years other mechanisms have been also demonstrated, involving direct effects on endothelial cells or inflammatory pathways of the arteriosclerotic processes.[4,5]

In earlier works, we demonstrated that elevated circulating levels of Apo CIII—but not other lipids or apolipoproteins—were associated with a progressive increase of FII (factor II) coagulant activity in the plasma of patients with or without coronary artery disease (CAD).[6] Importantly, this relationship was equally strong in CAD as well as in CAD-free patients, thus supporting the view that FII activation is an atherosclerosis-independent effect induced by Apo CIII. Plasma levels of Apo CIII and *APOC3* gene variants have been associated with other coagulation biomarkers, like activated factor VII-antithrombin complex, which is an indicator of tissue factor expression, thereby supporting the hypothesis of an Apo CIII-related prothrombotic diathesis.[7] Consistent with such hypothesis, recently, in a prospective cohort study with long-term follow-up (12 years) aimed to investigate the association between plasma lipids and venous

Received June 26, 2019; final revision received September 20, 2019; accepted October 11, 2019.

From the Department of Medicine, Unit of Internal Medicine, University of Verona, Italy (O.O., D.G., F.P., S.F., A.C., N.M.); Borgo Trento Hospital, Verona, Italy (M.C., B.B.); San Bonifacio Hospital, Verona, Italy (G.T.); and Laboratory of Clinical Chemistry and Hematology, University Hospital of Verona, Italy (A.B.).

The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.119.026811.

Correspondence to Oliviero Olivieri, MD, Unit of Internal Medicine, Department of Medicine, University of Verona, Policlinico G.B. Rossi, Piazzale L.A. Scuro 10, 37134 Verona, Italy. Email oliviero.olivieri@univr.it

thromboembolic events incidence in cardiovascular patients, we demonstrated that subjects with high Apo CIII concentrations (ie, ≥10.6 mg/dL) have an about 3-fold increased risk of venous thromboembolic events.[8]

All these findings suggest that plasma Apo CIII concentration may predict an increased risk of thromboembolic events in both arterial and venous districts. In this context, a possible association between high Apo CIII plasma levels and risk of ischemic cerebrovascular events appears as a plausible working hypothesis. In spite of the abundance of studies investigating the link with ischemic heart disease, the potential relation of Apo CIII with cerebrovascular disease has been substantially neglected and only few studies have provided some support to this possibility so far.[9,10]

Therefore, we prospectively investigated acute nonfatal ischemic cerebrovascular events (ie, ischemic strokes or transient ischemic attack [TIA]) that occurred during a long-term follow-up in a large cardiovascular cohort of CAD and CAD-free subjects for whom complete data of plasma lipids and apolipoproteins at enrollment, including baseline Apo CIII data, were available.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Population

The population for this study was selected from the cohort of VHS (Verona Heart Study). VHS is an ongoing survey that uses cross-sectional and prospective designs aimed to search for new risk factors for CAD in subjects with angiographic documentation of their coronary vessels. The Ethics Committee of our Institution (Azienda Ospedaliera Universitaria Integrata, Verona, Italy) approved the study. Informed written consent was obtained from all the participants after a full explanation of the study. A total of 950 subjects, who lived in the District of Verona and for whom both prospective data on cerebrovascular events and laboratory data on baseline plasma lipids were available, were included in this study. Blood samples and a complete clinical history were obtained in the days preceding the execution of the coronary arteriography at enrollment. According to angiographic evaluation, subjects were classified as CAD-free (individuals who underwent coronary angiography for reasons other than suspected CAD, mainly valvular heart disease) and CAD patients (individuals with at least one of the major epicardial coronary arteries [left anterior descending, circumflex, or right] affected with ≥1 significant stenosis [≥50% lumen reduction]). CAD-free subjects were required to be free of carotid artery lesions (by ultrasonic evaluation) and to have no history or evidence of arteriosclerosis in other vascular districts. Study design and patients' selection criteria have been summarized in Figure 1.

Assessment of Outcome

The subjects were followed until death or July 31, 2018. Study subjects' mortality status was determined by searching in the National Population Register. Survival times were calculated starting from the date of enrollment. The electronic medical records of all the Hospitals in the District of Verona, Northeast Italy, including data of Emergency Units admissions were obtained for all subjects; ambulatory or telephone survey was performed in case of clinical doubt. To be included in the statistical computation, nonfatal ischemic stroke/TIA events had to be confirmed and validated by 2 independent neurologists after careful evaluation of all the available information. As a rule, cerebrovascular events were adjudicated when both of the following criteria were satisfied: (1) clinical signs and symptoms of acute ischemic cerebrovascular events in combination with (2) confirmation on medical imaging (eg, brain computed tomography scan and magnetic resonance imaging).

Laboratory Testing

Samples of venous blood were drawn from each subject at enrollment after an overnight fast. Serum lipids, apolipoproteins, and other routine biochemical parameters were assayed as previously described.[11] Plasma Apo CIII concentration was measured using a fully automated turbidimetric immunoassay.[11] The reagent was obtained from Wako Pure Chemical Industries (Osaka, Japan), and the procedure recommended by the manufacturer was implemented on an RXL Dimension Analyzer (Dade International Inc, Newark, DE). All testing was performed in duplicate. Intraassay coefficients of variation were 1.84%, 2.02%, and 1.98% on 3 pools of control sera with low, medium, and high concentrations of Apo CIII, respectively; interassay coefficients of variation were 4.4%, 3.4%, and 2.29% for low, medium, and high concentration, respectively.

Statistical Analysis

Statistical analyses were performed using the software STATA 13.0 (STATA Corps) and SPSS 23.0 (SPSS Inc, Chicago, IL) statistical packages. Distributions of continuous variables were expressed as median value with interquartile range (IQR). Categorical variables were expressed as proportions. Quantitative data distributions were assessed using the Mann-Whitney test. Qualitative data were analyzed by χ^2 test or χ^2 for linear trend analysis when indicated. Ischemic stroke/TIA event rates during the follow-up period were assessed by using the Kaplan-Meier method with Log-rank statistic and Cox regression. Kaplan-Meier curves were used for survival plots, which stratified the study population according to Apo CIII plasma concentration. Multivariate Cox proportional hazards for ischemic stroke/TIA events were performed considering the Apo CIII median value as threshold and including in the different models potential confounding factors, like sex, age, hypertension, CAD diagnosis, atrial fibrillation, oral anticoagulant therapy, all plasma lipid parameters, and all the variables showing a different distribution between groups with or without ischemic stroke/TIA during the follow-up.

Finally, SPSS 23.0 statistical package was used to conduct propensity score matching of 2 similar groups (CAD versus CAD-free) with 1:1 ratio and match tolerance of 0.001. The propensity score matching was applied on the whole study population for all unbalanced variables with a probability value <0.10. Kaplan-Meier curves and Cox regression models were performed also in this population weighted by propensity score matching.

A value of $P<0.05$ was considered statistically significant.

Results

The main clinical and laboratory characteristics at time of enrollment of the 950 subjects included in this study are shown in Table 1. Data are reported considering the study population either as a whole or subdivided in the CAD (n=774) and CAD-free (n=176) subgroups. As expected, cardiovascular risk factors were more represented among CAD subjects, as in the case of Apo CIII whose plasma concentration was higher in CAD than in CAD-free subjects. The prevalence of both atrial fibrillation and anticoagulant therapy at time of enrollment was higher in CAD-free group, consistent with the fact that the large majority of CAD-free subjects in this study cohort had heart valve disease. However, antiplatelet and lipid-lowering therapies were more represented in CAD group (Table 1). After a median follow-up of 130 months (interquartile range, 69–189), 95 (10% of the total population) nonfatal ischemic stroke, or TIA events occurred, 74 (9.6%) in patients with CAD and 21 (11.9%) in CAD-free subjects (Table 2).

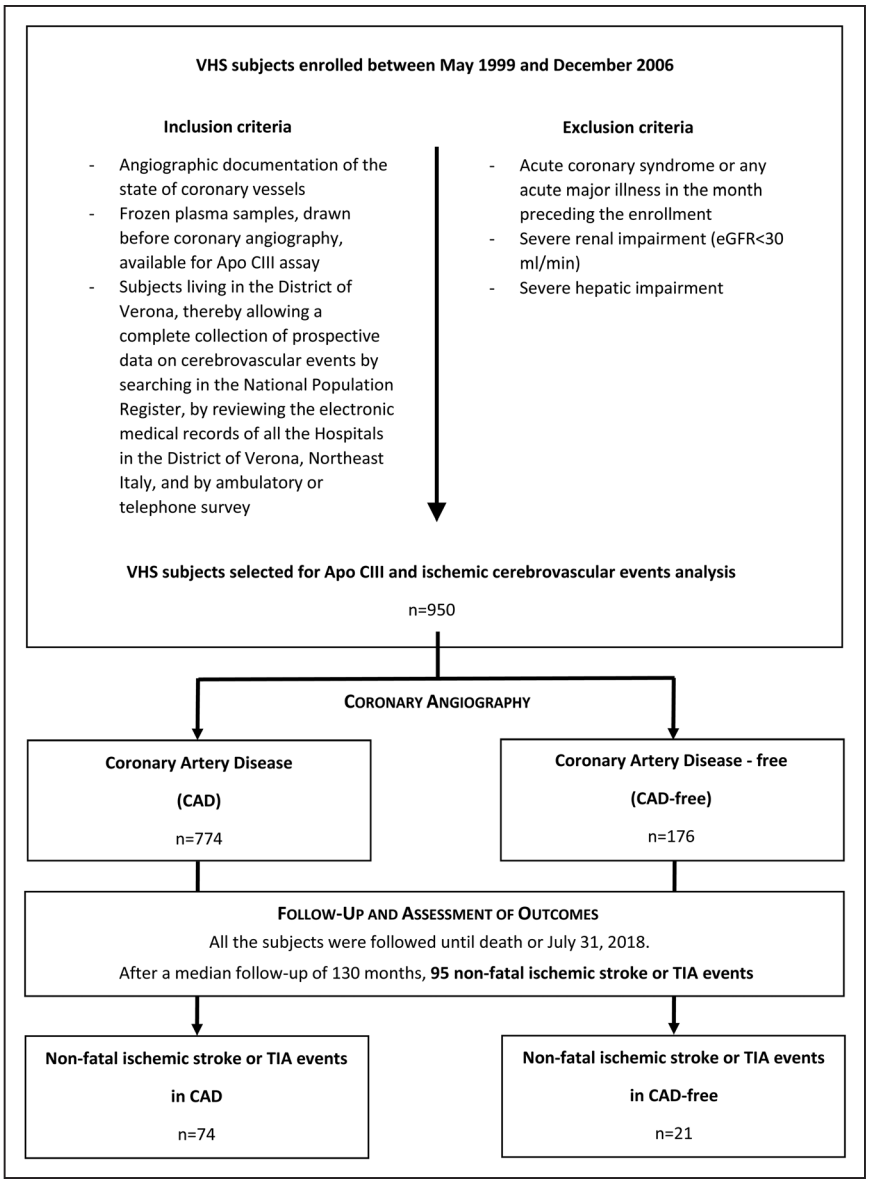

Figure 1. VHS (Verona Heart Study) design for the analysis of Apo CIII (apolipoprotein CIII) and ischemic stroke and transient ischemic attack (TIA) in subjects with or without angiographically demonstrated coronary artery disease (CAD) eFGR indicates estimated glomerular filtration rate.

Subjects with stroke/TIA during the follow-up were older and more represented by females than those without stroke. Atrial fibrillation, smoke, and history of previous stroke/TIA were associated with stroke during follow-up. Oral anticoagulant therapy was more frequent among subjects with stroke/TIA, consistent with the higher prevalence of atrial fibrillation in the same subgroup, while lipid-lowering therapy was more frequent among those without stroke/TIA. As regards plasma lipid parameters, total and HDL (high-density lipoprotein) cholesterol, Apo B, and Apo CIII were higher in subjects with stroke/TIA (Table 2). However, including all the plasma lipid parameters in Cox regression analysis, only Apo CIII maintained a significant association with stroke/TIA (data not shown). Stratifying the study population according to Apo CIII plasma concentration, the incidence rate of stroke/TIA increased from the lowest to the highest quartile (Figure 2). This finding was even more evident if the median value (10.6 mg/dL) was considered as the threshold level. The choice of the median value as threshold level was further supported by the receiver operating characteristic curve analysis (data not shown). As shown by Kaplan-Meier survival curves in Figure 3, subjects with Apo CIII plasma concentration above the median level had an increased rate of ischemic stroke/TIA events during the follow-up period in the whole study population (hazard ratio [HR] 1.89 with 95% CI, 1.24–2.65; Figure 3A), as well as by analyzing separately CAD-free (HR, 2.61 with 95% CI, 1.08–6.32; Figure 3B) and CAD subjects (HR, 1.67 with 95% CI, 1.04–2.68; Figure 3C).

High levels of Apo CIII remained significantly associated with an increased risk of ischemic stroke/TIA by different Cox regression models, even after adjustment for plasma lipid parameters (HR, 2.23 with 95% CI, 1.21–4.13), as well as by including in the regression analysis all the variables showing an association at the univariate analysis (HR, 1.80 with 95% CI, 1.12–2.89; Table 3).

To avoid the potential bias due to confounding variables in CAD or CAD-free subjects, which could be involved by chance in the estimate of the effect, we tried to randomize all these variables by using the propensity score method. For each covariate, randomization implies that the 2 resulting groups

Table 1. Clinical and Laboratory Characteristics of the Study Population at Time of Enrollment Considered as a Whole and Subdivided in the CAD and CAD-Free Subgroups

	All Subjects (n=950)	CAD-Free Subjects (n=176)	CAD Subjects (n=774)	*P* Value*
Demographics				
Age, y; median (IQR)	65 (56–72)	64 (54–71)	65 (57–73)	0.032
Female sex, n (%)	273 (28.7)	80 (45.5)	193 (24.9)	<0.001
Medical history				
Hypertension, n (%)	654 (68.8)	85 (48.3)	569 (73.5)	<0.001
Diabetes mellitus, n (%)	203 (21.4)	19 (10.8)	184 (23.8)	<0.001
Smoke habit, n (%)	573 (60.3)	67 (38.1)	506 (64.4)	<0.001
Previous stroke/TIA, n (%)	34 (3.6)	6 (3.4)	28 (3.6)	1.000
Atrial fibrillation, n (%)	69 (7.3)	26 (14.8)	43 (5.6)	<0.001
Congestive heart failure, n (%)	46 (4.8)	7 (4.0)	39 (5.0)	0.698
Obesity, n (%)	222 (23.4)	31 (17.6)	191 (24.7)	0.048
Antiplatelet therapy, n (%)	720 (75.8)	64 (36.4)	656 (84.8)	<0.001
Oral anticoagulant therapy, n (%)	118 (12.4)	56 (31.8)	62 (8.0)	<0.001
Statin treatment, n (%)	526 (55.4)	20 (11.4)	506 (65.4)	<0.001
Baseline laboratory data				
Creatinine, mmol/L; median (IQR)	87.4 (75.9–99)	86 (75–98.2)	87.4 (75.9–99)	0.654
Total cholesterol, mmol/L; median (IQR)	4.99 (4.34–5.68)	5.06 (4.45–5.88)	4.98 (4.29–5.66)	0.063
LDL-cholesterol, mmol/L; median (IQR)	3.20 (2.74–3.85)	3.24 (2.76–3.93)	3.18 (2.71–3.82)	0.312
HDL-cholesterol, mmol/L; median (IQR)	1.16 (0.97–1.37)	1.28 (1.06–1.63)	1.11 (0.95–1.34)	<0.001
Triglycerides, mmol/L; median (IQR)	1.53 (1.14–2.05)	1.32 (1.00–1.92)	1.56 (.1.17–2.08)	<0.001
Apo AI, g/L; median (IQR)	1.24 (1.08–1.44)	1.33 (1.13–1.56)	1.23 (1.08–1.42)	<0.001
Apo B, g/L; median (IQR)	0.98 (0.79–1.17)	0.95 (0.79–1.13)	0.99 (0.79–1.18)	0.436
Apo CIII, mg/dL; median (IQR)	10.6 (8.8–13.0)	10.0 (8.4–12.3)	10.6 (8.8–13.2)	0.021
Apo E, g/L; median (IQR)	0.037 (0.031–0.046)	0.038 (0.031–0.047)	0.037 (0.031–0.045)	0.404
Outcome				
Stroke/TIA events, n (%)	95 (10.0)	21 (11.9)	74 (9.6)	0.332

Apo indicates apolipoprotein; CAD, coronary artery disease; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; and TIA, transient ischemic attack.

*Mann-Whitney test or χ^2 test, when indicated.

will be balanced on average. Thus, we created a sample of subjects that was comparable, with the exception of age, for all observed covariates to the other one. Ninety-six patients with CAD were one-to-one matched with the same number of patients with CAD-free, and their clinical and laboratory characteristics are showed in Table I in the online-only Data Supplement. The analysis in this selected population confirmed the association between high levels of Apo CIII and stroke/TIA, while no other plasma lipid parameter reached a significant difference by comparing subjects with or without stroke/TIA (Table II in the online-only Data Supplement).

Similarly, as shown in Figure 3D, Kaplan-Meier survival curves in the subpopulation selected by using the propensity score method displayed an increased incidence risk of ischemic stroke/TIA events in subjects with Apo CIII plasma concentrations above the median level (HR, 2.63 with 95% CI, 1.16–5.96). Finally, also in this subpopulation, the association between high levels of Apo CIII and an increased risk of ischemic stroke/TIA was confirmed by different and multiadjusted Cox regression models (Table III in the online-only Data Supplement).

Discussion

To the best of our knowledge, this longitudinal study is the first to investigate the association between Apo CIII plasma concentration and incident ischemic cerebrovascular events in a cohort of cardiovascular patients. Our results show that subjects with Apo CIII plasma levels above the median value (10.6 mg/dL) are 2-times as likely to experience future ischemic stroke/TIA events within a period of about 10 years. Noteworthy, this value was identical to that conferring a substantially increased risk of venous thromboembolic event.[8] In our study population, the incidence rate of ischemic stroke/TIA was about 9 events/1000 persons-year, which was substantially higher

Table 2. Clinical and Laboratory Characteristics of the Study Population Subdivided on the Basis of the Occurrence of Nonfatal Ischemic Stroke or TIA Events During the Follow-Up

	No Stroke/TIA (n=855)	Stroke/TIA (n=95)	*P* Value
Demographics			
Age, y; median (IQR)	65 (56–72)	68 (59–74)	0.050
Female sex, n (%)	232 (27.1)	41 (43.2)	0.002
Medical history			
Hypertension, n (%)	586 (68.5)	68 (71.6)	0.641
Diabetes mellitus, n (%)	184 (21.5)	19 (20.0)	0.793
Smoke habit, n (%)	528 (61.8)	45 (47.4)	0.008
Previous stroke/TIA, n (%)	24 (2.8)	10 (10.5)	0.001
Atrial fibrillation, n (%)	48 (5.6)	21 (22.1)	<0.001
Congestive heart failure, n (%)	41 (4.8)	5 (5.3)	1.000
Obesity, n (%)	200 (23.4)	22 (23.2)	1.000
Antiplatelet treatment, n (%)	648 (75.8)	72 (75.8)	1.000
Oral anticoagulant treatment, n (%)	94 (11.0)	24 (25.3)	<0.001
Statin treatment, n (%)	487 (57.0)	39 (41.1)	0.003
Baseline data			
CAD, n (%)	700 (81.9)	74 (77.9)	0.332
Creatinine, mmol/L; median (IQR)	87.42 (75.9–99.0)	85.0 (76.1–99.8)	0.814
Total cholesterol, mmol/L; median (IQR)	4.99 (4.31–5.66)	5.12 (4.49–6.05)	0.021
LDL-cholesterol, mmol/L; median (IQR)	3.2 (2.74–3.82)	3.15 (2.71–4.06)	0.279
HDL-cholesterol, mmol/L; median (IQR)	1.15 (0.96–1.37)	1.19 (1.04–1.45)	0.039
Triglycerides, mmol/L; median (IQR)	1.52 (1.14–2.05)	1.61 (1.13–2.05)	0.460
Apo AI, g/L; median (IQR)	1.24 (1.08–1.43)	1.27 (1.11–1.49)	0.121
Apo B, g/L; median (IQR)	0.97 (0.79–1.16)	1.06 (0.86–1.22)	0.017
Apo CIII, mg/dL; median (IQR)	10.4 (8.7–13.0)	11.4 (9.3–14.4)	0.028
Apo E, g/L; median (IQR)	0.037 (0.031–0.045)	0.041 (0.031–0.049)	0.165

Apo indicates apolipoprotein; CAD, coronary artery disease; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; and TIA, transient ischemic attack.

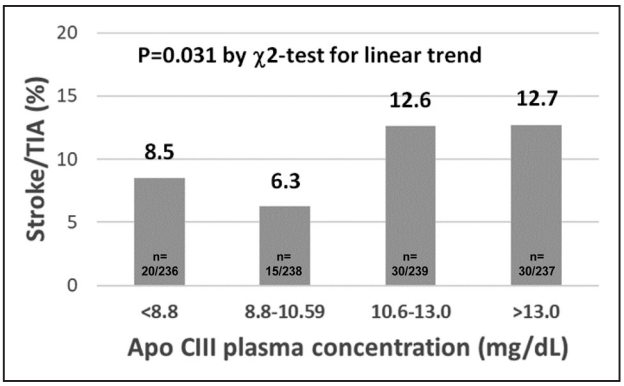

Figure 2. Ischemic stroke and transient ischemic attack (TIA) rate during the follow-up period among subjects stratified into quartiles according to Apo CIII (apolipoprotein CIII) plasma concentration.

than that recognized in the general population living in the same region.[12] Bearing in mind that ischemic stroke can have various etiologies (in primis large-artery atherosclerosis), and CAD and large-artery atherosclerotic stroke share the same predisposing factors,[12] this result was overall expected. However, our population was composed by 2 different types of subjects, that is, with or without arterial disease (defined in an objective way by coronary angiography). In the first case, traditional cardiovascular risk factors (like hypertension, diabetes mellitus, hyperlipidemia, smoke habit, higher levels of triglycerides, and lower levels of HDL-cholesterol) were common, while female sex and atrial fibrillation were prevalent in patients with CAD-free (who were mainly affected by valvular heart disease). Thus, as an interesting feature allowed by such type of selection, CAD and CAD-free subgroups might represent different models of risk for ischemic stroke due to large-arteries arteriosclerosis and cardioembolism, respectively.[13,14] In patients with ischemic stroke due to atherosclerosis, arterial occlusion or the source of artery-to-artery embolism may be found in extracranial or intracranial arteries despite that pathogenesis of extracranial and intracranial atherosclerosis may differ.[15] History of CAD has been reported to be more frequent in patients with extracranial carotid artery atherosclerosis than in those with intracranial middle cerebral artery stenosis.[16,17]

However, when our population was divided based on incident ischemic stroke/TIA events during follow-up, instead of the traditional atherogenic risk factors, conditions known to favor cardioembolic events (female sex, atrial fibrillation) and elevated levels of total cholesterol, Apo B, and Apo CIII were associated with ischemic stroke/TIA (Table 2). High baseline Apo CIII plasma concentration predicted ischemic stroke/TIA events during follow-up, even after adjustment for all plasma lipid parameters and other potential confounding factors, like the traditional atherosclerosis risk factors (Table 3). It is tempting to speculate that Apo CIII may have a detrimental role due to cardioembolic diathesis rather than only atherogenic mechanisms. Such hypothesis is further supported by the observation that the association between high Apo CIII levels and the risk of ischemic stroke/TIA was confirmed in the separate analysis on CAD-free subjects (Figure 3B), in whom the increase of risk appeared even higher than in patients with CAD (Figure 3C). Although preliminary, this novel working hypothesis appeared consistent with our earlier works showing an Apo CIII-related prothrombotic diathesis.[6,7,11] It is also intriguing that the threshold value of Apo CIII, conferring substantially increased risk for venous thromboembolic event,[8] was identical to that associated with the risk of stroke.

As an alternative approach to confirm our results, to avoid the bias due to confounding variables eventually involved by chance in the estimate of the effect, we adopt a propensity

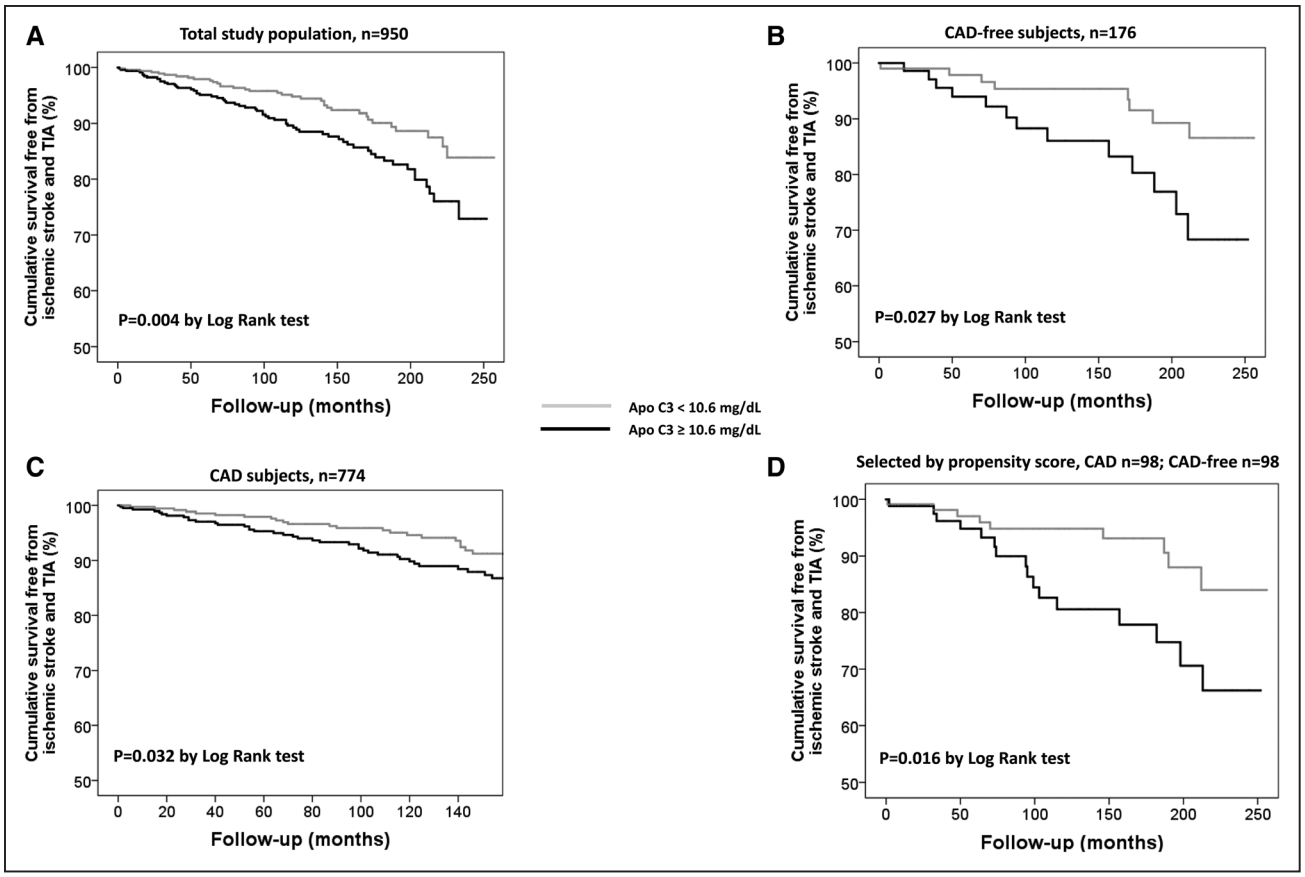

Figure 3. Kaplan-Meier survival curves for ischemic stroke/transient ischemic attack (TIA) in the whole population (**A**), in subjects without coronary artery disease (CAD-free, **B**), in subjects with CAD (**C**), and in the subpopulation selected by using a propensity score matching method, in which 98 CAD and 98 CAD-free subjects were one-to-one matched for all clinical and laboratory characteristics (**D**). Apo indicates apolipoprotein.

score matching method in our study population. By this way, in 2 selected cohorts (CAD and CAD-free) of patients balanced for multiple variables, the regression models were retested. Once again the association of Apo CIII with ischemic stroke/TIA was confirmed, while no significant association was found for the other plasma lipid parameters (Table II in the online-only Data Supplement).

Only a few studies have showed a link between Apo CIII plasma levels and ischemic stroke so far.[9,10] Our findings not only support such link but potentially suggest that over time prothrombotic effects connected with elevated concentrations of Apo CIII may play a role as important as that observed for atherogenesis. According with the results of our previous works,[6,7,12] Apo CIII seems to act as a modulator of prothrombinase activity by favoring the triggering and amplification of FII coagulant activity in the final phase of the coagulation cascade. More precisely, we demonstrated that FII activity is similarly increased in patients with elevated Apo CIII and in carriers of the prothrombin 20210A allele, a well-known prothrombotic mutation.[6] Evidence for a role in ischemic stroke of prothrombin G201210A mutation is largely confirmed in literature.[18–20] If the hypothesis of an Apo CIII-related role in thrombosis and cardiac embolism will be confirmed, it may be of value in clinical practice. Pharmacological treatments able to lower Apo CIII concentrations, such as statins, may exhibit beneficial effects beyond lipid metabolism, for instance by limiting prothrombotic diathesis. A meta-analysis of 18 randomized controlled trials revealed that statins reduced the overall incidence of stroke.[21] Similar evidence was provided by the SPARCL (Stroke Prevention by Aggressive Reduction of Cholesterol Levels)[22,23] although several post hoc analyses of different subgroups following the SPARCL study did not fully confirm such results.[24–27]

However, as an indirect evidence, the MUCH-Italy (Multicenter Study on Cerebral Haemorrhage in Italy) showed that increasing levels of total serum cholesterol were associated with a decreased risk of intracerebral bleeding, while statin use was associated with an increased risk.[27] Thus, the relationship between plasma lipids and risk of ischemic or hemorrhagic stroke may be more complex than expected. The strength of associations between plasma lipids and the risk of either ischemic or hemorrhagic stroke remains to be clarified.[28] Our results could offer new data potentially contributing to explain these mechanisms, namely a role for Apo CIII. A threshold effect for the prothrombotic activity of the Apo CIII concentrations is consistent with the data reported in the present study, in which a clear increase in ischemic stroke/TIA risk was observed only for plasma concentrations of Apo CIII higher than 10.6 mg/dL.

Our data suggest 2 further considerations as possible working hypotheses. The first one is related to the potential use of total Apo CIII plasma concentration for patient-tailored therapy in

Table 3. High Plasma Concentration of Apo CIII Above the Median Level, ≥10.6 mg/dL, as a Predictor of Ischemic Stroke/TIA Events by Different Cox Regression Models (Subjects With Low Plasma Concentration of Apo CIII, <10.6 mg/dL, Are Considered as Reference Group)

	Coefficient B	SE	Hazard Ratio	*P* Value
Unadjusted	0.634	0.213	1.89 (1.24–2.65)	0.003
Model 1	0.555	0.214	1.74 (1.15–2.72)	0.009
Model 2	0.557	0.215	1.75 (1.15–2.66)	0.010
Model 3	0.542	0.216	1.72 (1.13–2.63)	0.012
Model 4	0.804	0.314	2.23 (1.21–4.13)	0.010
Model 5	0.586	0.243	1.80 (1.12–2.89)	0.016

Model 1: sex- and age-adjusted. Model 2: adjusted for sex, age, and CAD diagnosis. Model 3: adjusted for sex, age, CAD diagnosis, atrial fibrillation, and oral anticoagulant treatment. Model 4: adjusted for sex, age, CAD diagnosis, atrial fibrillation, hypertension, oral anticoagulant treatment, and all plasma lipid parameters (ie, total, HDL, and LDL-cholesterol, triglycerides, Apo AI, B, and E). Model 5: adjusted for all variables associated with ischemic stroke/TIA with a probability value <0.10 at the univariate analysis (ie, sex, age, CAD diagnosis, atrial fibrillation, smoke habit, previous ischemic stroke/TIA, oral anticoagulant treatment, statin treatment, total cholesterol, HDL-cholesterol, and Apo B). Apo indicates apolipoprotein; CAD, coronary artery disease; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; and TIA, transient ischemic attack.

clinical practice. Apo CIII may be a predictor of ischemic stroke/TIA risk and also provide information about the prothrombotic propensity of a single patient, especially in a setting of secondary prevention of cardiovascular diseases. Several methods for routinely measuring Apo CIII are available. Since these methods exhibit excellent precision and may be automated[29] they could be easily implemented in the clinical practice.

Second, Apo CIII-lowering drugs may exhibit additional and unexpected antithrombotic effects. It is interesting to note that volanesorsen, an anti-Apo CIII antisense oligonucleotide,[30] has not been approved by the Food and Drug Administration for safety problems related to hemorrhagic complications.[31] These complex effects and the potential interactions with the currently available anticoagulant treatment are largely unexplored. Accurate future investigations and specifically designed clinical trials will be necessary to define all these issues.

Study Limitations

The study presents some limitations that have to be acknowledged. The first one relies on the modality of the follow-up of the patients. It was mainly based on an extensive and careful check of the patients' electronic records, available for care in the hospitals of the District (all the enrolled patients were living in the Verona District). Potentially incomplete data might derive from care in hospitals outside the District. However, indirect evidence of outcomes of previous ischemic events was also checked in the electronic records and finally excluded. Yet theoretically possible, this chance should be very limited and statistically irrelevant. Moreover, possible different roles of Apo CIII in ischemic versus hemorrhagic stroke remain an open question that the present work is structurally unable to answer. Finally, our current results should be replicated in other study populations.

Conclusions

The present study suggests a role of elevated Apo CIII plasma levels in the setting of acute ischemic cerebrovascular events. This result appears consistent with our previous observations linking high Apo CIII concentrations with both enhancement of the coagulation cascade and amplified thrombin generation. However, further studies are needed to validate the potential role of Apo CIII in ischemic stroke and cerebrovascular diseases.

Acknowledgments

This study was performed in part in the LURM (Laboratorio Universitario di Ricerca Medica) Research Center, University of Verona.

Sources of Funding

This work was supported by the Cariverona Foundation (project B36J16002570003).

Disclosures

Dr Cappellari reports consulting for Boehringer-Ingelheim and Pfizer-BMS. The other authors report no conflicts.

References

1. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics–2011 update: a report from the American Heart Association. *Circulation*. 2011;123:e18–e209. doi: 10.1161/CIR.0b013e3182009701
2. Ooi EM, Barrett PH, Chan DC, Watts GF. Apolipoprotein C-III: understanding an emerging cardiovascular risk factor. *Clin Sci (Lond)*. 2008;114:611–624. doi: 10.1042/CS20070308
3. Wyler von Ballmoos MC, Haring B, Sacks FM. The risk of cardiovascular events with increased apolipoprotein CIII: a systematic review and meta-analysis. *J Clin Lipidol*. 2015;9:498–510. doi: 10.1016/j.jacl.2015.05.002
4. Kawakami A, Aikawa M, Alcaide P, Luscinskas FW, Libby P, Sacks FM. Apolipoprotein CIII induces expression of vascular cell adhesion molecule-1 in vascular endothelial cells and increases adhesion of monocytic cells. *Circulation*. 2006;114:681–687. doi: 10.1161/CIRCULATIONAHA.106.622514
5. Kawakami A, Osaka M, Tani M, Azuma H, Sacks FM, Shimokado K, et al. Apolipoprotein CIII links hyperlipidemia with vascular endothelial cell dysfunction. *Circulation*. 2008;118:731–742. doi: 10.1161/CIRCULATIONAHA.108.784785
6. Olivieri O, Martinelli N, Baroni M, Branchini A, Girelli D, Friso S, et al. Factor II activity is similarly increased in patients with elevated apolipoprotein CIII and in carriers of the factor II 20210A allele. *J Am Heart Assoc*. 2013;2:e000440. doi: 10.1161/JAHA.113.000440
7. Martinelli N, Baroni M, Castagna A, Lunghi B, Stefanoni F, Tosi F, et al. Apolipoprotein C-III strongly correlates with activated factor VII-antithrombin complex: an additional link between plasma lipids and coagulation. *Thromb Haemost*. 2019;119:192–202. doi: 10.1055/s-0038-1676817
8. Olivieri O, Turcato G, Moruzzi S, Castagna A, Girelli D, Pizzolo F, et al. Not just arterial damage: increased incidence of venous thromboembolic events in cardiovascular patients with elevated plasma levels of apolipoprotein CIII. *J Am Heart Assoc*. 2019;8:e010973. doi: 10.1161/JAHA.118.010973
9. Wang Y, Yin X, Li L, Deng S, He Z. Association of apolipoprotein C3 genetic polymorphisms with the risk of ischemic stroke in the Northern Chinese Han Population. *PLoS One*. 2016;11:e0163910. doi: 10.1371/journal.pone.0163910
10. Pechlaner R, Tsimikas S, Yin X, Willeit P, Baig F, Santer P, et al. Very-low-density lipoprotein-associated apolipoproteins predict cardiovascular events and are lowered by inhibition of APOC-III. *J Am Coll Cardiol*. 2017;69:789–800. doi: 10.1016/j.jacc.2016.11.065
11. Olivieri O, Martinelli N, Girelli D, Pizzolo F, Friso S, Beltrame F, et al. Apolipoprotein C-III predicts cardiovascular mortality in severe

coronary artery disease and is associated with an enhanced plasma thrombin generation. *J Thromb Haemost*. 2010;8:463–471. doi: 10.1111/j.1538-7836.2009.03720.x
12. Nardetto L, Giometto B, Moretto G, Mantoan D, Saia M. Hub-and-spoke stroke network in the Veneto region: a retrospective study investigating the effectiveness of the stroke pathway and trends over time. *Neurol Sci*. 2017;38:2117–2121. doi: 10.1007/s10072-017-3118-z
13. Arboix A, García-Eroles L, Comes E, Oliveres M, Targa C, Balcells M, et al. Importance of cardiovascular risk profile for in-hospital mortality due to cerebral infarction. *Rev Esp Cardiol*. 2008;61:1020–1029.
14. Bogiatzi C, Hackam DG, McLeod AI, Spence JD. Secular trends in ischemic stroke subtypes and stroke risk factors. *Stroke*. 2014;45:3208–3213. doi: 10.1161/STROKEAHA.114.006536
15. Cabral NL, Gonçalves AR, Longo AL, Moro CH, Costa G, Amaral CH, et al. Incidence of stroke subtypes, prognosis and prevalence of risk factors in Joinville, Brazil: a 2 year community based study. *J Neurol Neurosurg Psychiatry*. 2009;80:755–761. doi: 10.1136/jnnp.2009.172098
16. Bang OY, Lee PH, Yoon SR, Lee MA, Joo IS, Huh K. Inflammatory markers, rather than conventional risk factors, are different between carotid and MCA atherosclerosis. *J Neurol Neurosurg Psychiatry*. 2005;76:1128–1134. doi: 10.1136/jnnp.2004.054403
17. Mathur KS, Kashyap SK, Kumar V. Correlation of the extent and severity of atherosclerosis in the coronary and cerebral arteries. *Circulation*. 1963;27:929–934. doi: 10.1161/01.cir.27.5.929
18. Sarecka-Hujar B, Kopyta I, Skrzypek M, Sordyl J. Association between the 20210G>A prothrombin gene polymorphism and arterial ischemic stroke in children and young adults-two meta-analyses of 3586 cases and 6440 control subjects in total. *Pediatr Neurol*. 2017;69:93–101. doi: 10.1016/j.pediatrneurol.2016.12.013
19. De Stefano V, Chiusolo P, Paciaroni K, Casorelli I, Rossi E, Molinari M, et al. Prothrombin G20210A mutant genotype is a risk factor for cerebrovascular ischemic disease in young patients. *Blood*. 1998;91:3562–3565.
20. Lalouschek W, Schillinger M, Hsieh K, Endler G, Tentschert S, Lang W, et al. Matched case-control study on factor V Leiden and the prothrombin G20210A mutation in patients with ischemic stroke/transient ischemic attack up to the age of 60 years. *Stroke*. 2005;36:1405–1409. doi: 10.1161/01.STR.0000170635.45745.b8
21. Wang W, Zhang B. Statins for the prevention of stroke: a meta-analysis of randomized controlled trials. *PLoS One*. 2014;9:e92388. doi: 10.1371/journal.pone.0092388
22. Amarenco P, Bogousslavsky J, Callahan A 3rd, Goldstein LB, Hennerici M, Rudolph AE, et al; Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) Investigators. High-dose atorvastatin after stroke or transient ischemic attack. *N Engl J Med*. 2006;355:549–559. doi: 10.1056/NEJMoa061894
23. Chaturvedi S, Zivin J, Breazna A, Amarenco P, Callahan A, Goldstein LB, et al; SPARCL Investigators. Effect of atorvastatin in elderly patients with a recent stroke or transient ischemic attack. *Neurology*. 2009;72:688–694. doi: 10.1212/01.wnl.0000327339.55844.1a
24. Goldstein LB, Amarenco P, Lamonte M, Gilbert S, Messig M, Callahan A, et al; SPARCL Investigators. Relative effects of statin therapy on stroke and cardiovascular events in men and women: secondary analysis of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) Study. *Stroke*. 2008;39:2444–2448. doi: 10.1161/STROKEAHA.107.513747
25. Sillesen H, Amarenco P, Hennerici MG, Callahan A, Goldstein LB, Zivin J, et al; Stroke Prevention by Aggressive Reduction in Cholesterol Levels Investigators. Atorvastatin reduces the risk of cardiovascular events in patients with carotid atherosclerosis: a secondary analysis of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial. *Stroke*. 2008;39:3297–3302. doi: 10.1161/STROKEAHA.108.516450
26. Amarenco P, Benavente O, Goldstein LB, Callahan A 3rd, Sillesen H, Hennerici MG, et al; Stroke Prevention by Aggressive Reduction in Cholesterol Levels Investigators. Results of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial by stroke subtypes. *Stroke*. 2009;40:1405–1409. doi: 10.1161/STROKEAHA.108.534107
27. Pezzini A, Grassi M, Iacoviello L, Zedde M, Marcheselli S, Silvestrelli G, et al; Multicenter Study on Cerebral Haemorrhage in Italy (MUCH-Italy) Investigators. Serum cholesterol levels, HMG-CoA reductase inhibitors and the risk of intracerebral haemorrhage. The Multicenter Study on Cerebral Haemorrhage in Italy (MUCH-Italy). *J Neurol Neurosurg Psychiatry*. 2016;87:924–929. doi: 10.1136/jnnp-2015-312736
28. Holmes MV, Millwood IY, Kartsonaki C, Hill MR, Bennett DA, Boxall R, et al; China Kadoorie Biobank Collaborative Group. Lipids, lipoproteins, and metabolites and risk of myocardial infarction and stroke. *J Am Coll Cardiol*. 2018;71:620–632. doi: 10.1016/j.jacc.2017.12.006
29. Chan DC, Chen MM, Ooi EM, Watts GF. An ABC of apolipoprotein C-III: a clinically useful new cardiovascular risk factor? *Int J Clin Pract*. 2008;62:799–809. doi: 10.1111/j.1742-1241.2007.01678.x
30. Gaudet D, Alexander VJ, Baker BF, Brisson D, Tremblay K, Singleton W, et al. Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. *N Engl J Med*. 2015;373:438–447. doi: 10.1056/NEJMoa1400283
31. The Endocrinologic and Metabolic Drugs Advisory Committee (EMDAC). EMDAC meeting for volanesorsen (Waylivra). U.S. Food & Drug Administration (FDA) Briefing Document of the Endocrinologic and Metabolic Drugs Advisory Committee Meeting, May 10, 2018.