

Not Just Arterial Damage: Increased Incidence of Venous Thromboembolic Events in Cardiovascular Patients With Elevated Plasma Levels of Apolipoprotein CIII

Oliviero Olivieri, MD; Gianni Turcato, MD; Sara Moruzzi, MD, PhD; Annalisa Castagna, MSc, PhD; Domenico Girelli, MD, PhD; Francesca Pizzolo, MD, PhD; Simonetta Friso, MD, PhD; Marco Sandri, MSc, PhD; Antonella Bassi, MD;* Nicola Martinelli, MD, PhD

Background—Apolipoprotein CIII (apo CIII) is a crucial player in triglyceride-rich lipoprotein metabolism, but may also act pleiotropically, provoking inflammatory responses and stimulating coagulation. Elevated apoCIII plasma levels have been associated with increased activity of coagulation factors. Since these features of prothrombotic diathesis are linked with venous thromboembolism (VTE), we hypothesized that apo CIII plays a role in VTE.

Methods and Results—We recorded nonfatal VTE events in 1020 patients (age 63.3 ± 11.4 years; 29.1% women) with or without coronary artery disease (79.1% with coronary artery disease and 20.9% without coronary artery disease) during a long follow-up. Complete plasma lipid and apolipoproteins were available for all patients. Forty-five patients (4.4%) experienced nonfatal VTE events during a median follow-up period of 144 months. Apo CIII plasma concentration at enrollment was higher in patients with VTE compared with patients without VTE (12.2 [95% CI, 11.10–13.5] mg/dL vs 10.6 [95% CI, 10.4–10.9] mg/dL, respectively; $P=0.011$). Patients with apo CIII levels above the median value (10.6 mg/dL) exhibited an increased risk of VTE (incidence rate, 6.0 [95% CI, 4.0–8.0] vs 1.8 [95% CI, 0.7–2.9] VTE events/1000 person-years; unadjusted hazard ratio [HR], 3.42 [95% CI, 1.73–6.75]; $P<0.001$). This association was confirmed after adjustment for sex, age, coronary artery disease diagnosis, body mass index, hypertension, and anticoagulant treatment at enrollment (HR, 2.66; 95% CI, 1.31–5.37 [$P=0.007$]), with inclusion of lipid parameters in the Cox model (HR, 3.74; 95% CI, 1.24–11.33 [$P=0.019$]), and even with exclusion of patients who died at follow-up (HR, 3.92; 95% CI, 1.68–9.14 [$P=0.002$]) or patients taking anticoagulants (HR, 3.39; 95% CI, 1.72–6.69 [$P<0.001$]).

Conclusions—Our results suggest that high plasma apo CIII concentrations may predict an increased risk of VTE in patients with cardiovascular disease. (*J Am Heart Assoc.* 2019;8:e010973. DOI: 10.1161/JAHA.118.010973.)

Key Words: apolipoprotein CIII • plasma lipids • triglyceride • venous thromboembolism

Apolipoprotein CIII (apo CIII) is the most important marker of triglyceride-rich lipoproteins,¹ and it is a causal risk factor for cardiovascular diseases. Increased apo CIII serum concentrations are a primary predisposing condition for the development of atherosclerotic disease (see reference 2 for a review and meta-analysis). Recent data reported that rare

loss-of-function genetic variants of *APOC3* gene substantially reduce cardiovascular risk, further and strongly supporting the direct atherogenic effects of apo CIII.³ High levels of apo CIII have been demonstrated to be an important predictor of total and cardiovascular mortality in the setting of secondary prevention for coronary artery disease (CAD).⁴ Apo CIII primarily influences lipid and lipoprotein metabolism, and it also provokes inflammatory and atherogenic responses in monocytes and endothelial cells.^{5,6} Accordingly, a great deal of the scientific evidence is focused on the harmful role of apo CIII in arterial vessels and in atherosclerosis-related diseases.

Several years ago, we suggested a novel potential detrimental role of apo CIII by demonstrating that elevated circulating levels of apo CIII (but not other lipids or apolipoproteins) were involved in a progressive increase in factor II (FII) coagulant activity in the plasma of patients with or without CAD.⁷ The extent of this increase in patients in the top quartile of apo CIII concentration was comparable to that in carriers of the FII G20210A allele. Therefore, from this functional point of view, elevated apo CIII concentrations were equivalent to carrying the

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

*Dr Antonella Bassi is currently located at the Azienda Socio Sanitaria di Mantova, Strada Lago Paiolo, n. 10 Mantova, Italy.

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

Received September 17, 2018; accepted November 21, 2018.

© 2019 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Clinical Perspective

What Is New?

- High plasma levels of apolipoprotein CIII (apo CIII), which is a crucial regulator of lipoproteins and a recognized risk factor for atherosclerosis and ischemic heart disease, may predict an increased risk of venous thromboembolic events in a cohort of patients with cardiovascular disease.
- This result appears consistent with earlier works suggesting that apo CIII is associated with a prothrombotic diathesis.

What Are the Clinical Implications?

- Apo CIII may provide information about the individual procoagulant propensity and the related risk of venous thromboembolism, potentially being a useful index for personalized therapeutic choices in clinical practice.
- Moreover, speculatively, apo CIII-lowering drugs, including the new anti-apo CIII antisense oligonucleotides, may exhibit additional and unexpected antithrombotic effects.

G20210A allele, a genetic condition well known as a predisposing factor for both arterial and venous thrombotic disease. This evidence implies an increased propensity to form venous thrombosis in patients exhibiting high apo CIII plasma levels.

However, after our first report, the relationship of apo CIII with the coagulation pathway and its association with the incidence of venous thromboembolism (VTE) has not been investigated further. Moreover, an objective limitation of our previous results is that they were obtained from a retrospective analysis and were intrinsically flawed by the cross-sectional study design.⁵ Therefore, as a natural corollary of the first study, we planned a follow-up study of the population of previously enrolled patients for whom basal values of apo CIII were available to investigate the clinical consequences of the potential procoagulant effects of high plasma apo CIII concentrations. Specifically, we systematically collected, recorded, and quantified nonfatal VTE events that occurred in patients who survived for a sufficiently long period of follow-up to verify whether patients with higher apo CIII concentrations exhibited an increased risk of VTE, which would provide clinical support for our hypothesis of apo CIII-related prothrombotic diathesis.

Methods

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

Study Population

The population for this study was selected from the cohort of the VHS (Verona Heart Study). The VHS is an ongoing survey

that uses cross-sectional and prospective designs aimed to search for new risk factors for CAD in patients using objective, angiographic documentation of their coronary vessels. Details of the enrollment criteria have been carefully described elsewhere (reference 3, appendix material 4,7). The ethics committee of our institution (Azienda Ospedaliera Universitaria Integrata, Verona, Italy) approved the study. Informed written consent was obtained from all participants after a full explanation of the study.

A total of 1020 patients (723 men and 297 women) who lived in the District of Verona and for whom prospective data 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. On the basis of an angiographic evaluation, the patients were classified as CAD-free (n=213, individuals who underwent coronary angiography for reasons other than suspected CAD, primarily valvular heart disease) and patients with CAD (n=807 individuals with at least 1 of the major epicardial coronary arteries [left anterior descending, circumflex, and right] affected by ≥ 1 significant stenosis [$\geq 50\%$ lumen reduction]).

Assessment of Outcome

The patients were followed until death or June 2017. Survival times were calculated starting from the date of enrollment (ie, some days before the execution of the coronary angiography, which was requested for the enrollment in our cardiovascular cohort). The status of the study patients was determined via search of the national population register to ascertain mortality status. The electronic medical records of all hospitals in the District of Verona, Northeast Italy, including data of emergency unit admissions, were obtained for all patients. Ambulatory or telephone survey was performed in case of clinical doubt. To be included in the statistical computation, nonfatal VTE events had to be confirmed and validated by 2 independent researchers after careful evaluation of all the available information. As a rule, events were adjudicated when both of the following criteria were met: (1) clinical signs and symptoms of deep venous thrombosis or pulmonary embolism in combination with (2) confirmation on medical imaging (eg, compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan, or pulmonary angiography).

Laboratory Testing

Samples of venous blood were drawn from each patient at enrollment after an overnight fast. Serum lipids, apolipoproteins, and other routine biochemical parameters were assayed as previously described.⁴ Plasma apo CIII

concentrations were measured using a fully automated turbidimetric immunoassay.⁴ The reagent was obtained from Wako Pure Chemical Industries, and the procedure recommended by the manufacturer was implemented on an RXL Dimension Analyzer (Dade International Inc.). All testing was performed in duplicate. The intra-assay 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. The interassay coefficients of variation were 4.4%, 3.4%, and 2.29% for low, medium, and high apo CIII concentrations, respectively.

Statistical Analysis

All calculations were performed using SPSS 23.0 (SPSS Inc) and R version 3.5.1 (R Foundation for Statistical Computing, 2018. <https://www.R-project.org/>, accessed November 2018) statistical packages. Distributions of continuous variables in groups are expressed as the means±SDs. Skewed variables, including apo CIII, apolipoprotein E, triglyceride, high-sensitivity C-reactive protein, and serum creatinine, were logarithmically transformed, and geometric means with 95% CIs are reported. Quantitative data were assessed using Student *t* test. The correlation between quantitative variables was assessed using Pearson correlation coefficient (*R*). Categorical data were analyzed using the chi-square test and chi-square for linear trend analysis when indicated. VTE event rates during the follow-up period were assessed using the Kaplan–Meier method (log-rank statistic) and Cox regression. Kaplan–Meier curves were used for survival plots, which stratified the study population on the basis of median apo CIII value. Multivariate Cox proportional hazards for VTE events were performed considering the median apo CIII value as the threshold and including sex, age, CAD diagnosis, body mass index, hypertension, anticoagulant treatment (the main demographic and clinical characteristics or the factors associated with VTE events based on univariate analysis), and all plasma lipids and other apolipoproteins that correlated with the plasma apo CIII concentration in the different models. Incidence rates and incidence rate ratios with 95% CIs were estimated organizing data into an event-time table and estimating on this table a Poisson regression model with person-years as an offset.⁸ *P*<0.05 was considered statistically significant.

Results

After a median follow-up duration of 144 months (interquartile range, 84–197 months), 45 of the 1020 patients (4.4%) experienced nonfatal VTE events (deep venous thrombosis=29 [64.4%], deep venous thrombosis+pulmonary embolism=6 [13.3%], pulmonary embolism=10 [22.2%]). No VTE

events occurred during the first month of follow-up, and all VTE events were reported as first (no recurrent) events.

Table 1 summarizes the main clinical and laboratory characteristics of the study population, which are reported as a whole and subdivided on the basis of VTE events during the follow-up period. The composition of the population according to the initial angiographic diagnosis (CAD or CAD-free) was similar in patients with and without VTE events. Several differences were observed between these 2 groups. Female sex was more represented in the VTE group, and anticoagulant treatment at enrollment was uniquely recorded in patients who did not experience VTE events during the follow-up period. Patients with VTE tended to exhibit an increased body mass index and higher prevalence of hypertension, but without formal statistical significance ($0.10 > P > 0.05$). The main traditional cardiovascular risk factors in clinical history, traditional plasma lipids, high-sensitivity C-reactive protein, and serum creatinine were not differently distributed between patients with or without VTE events. A total of 128 patients were reported to be treated with anticoagulant therapy at discharge from the hospitalization during which they were enrolled in the VHS. All of these patients were reported to take warfarin for atrial fibrillation.

Although plasma lipids were interrelated (Table 2), only the plasma apo CIII concentration at enrollment was significantly elevated in VTE-positive patients compared with VTE-negative patients (12.2 [95% CI, 11.0–13.5 mg/dL] vs 10.6 [95% CI, 10.4–10.9 mg/dL], *P*=0.011). Stratifying the study population into quartiles according to the apo CIII concentration revealed that VTE prevalence increased from the lowest quartile to the highest quartile (*P*=0.001 by chi-square for linear trend, Figure 1). Most patients who experienced a VTE were characterized by high plasma apo CIII concentrations and were included in the third and fourth quartiles, indicating a threshold value at the median level (10.6 mg/dL).

The Kaplan–Meier survival curves (Figure 2) demonstrated that patients with a plasma apo CIII concentration above the median level exhibited an increased risk of VTE (*P*<0.001, log-rank test). High levels of apo CIII were associated with an ≈3-fold increased risk of VTE in different Cox regression models. This association was confirmed after adjustments for sex, age, CAD diagnosis, body mass index, hypertension, and anticoagulant treatment at enrollment, even with inclusion of all plasma lipid parameters in the regression model (Table 3). These results were confirmed in subgroup analyses of surviving patients (*n*=643; hazard ratio, 3.92 [95% CI, 1.68–9.14], *P*=0.002) and patients who were not taking anticoagulant drugs at enrollment (*n*=892; hazard ratio, 3.39 [95% CI, 1.72–6.69], *P*<0.001). All of these results were confirmed by incidence rates and incidence rate ratio analyses, with

Table 1. Clinical and Laboratory Characteristics of the Study Population, Considered as a Whole and Stratified According to VTE Events During Follow-Up

	Total Study Population (N=1020)	No VTE (n=975)	VTE (n=45)	P Value*
Age, y	63.3±11.4	63.2±11.5	63.8±10.9	NS [†]
Women, %	29.1	28.4	44.4	0.021 [‡]
CAD/CAD-free, %	79.1/20.9	79.0/21.0	82.2/17.8	NS [‡]
BMI, kg/m ²	26.6±3.8	26.5±3.8	27.7±3.5	0.072 [†]
Hypertension, %	75.5	75.0	86.7	0.075 [‡]
Diabetes mellitus, %	21.3	21.1	24.4	NS [‡]
Smoking, %	57.4	57.7	48.9	NS [‡]
Serum creatinine, μmol/L	87.9 (86.3–89.6)	87.8 (86.2–89.4)	92.0 (77.6–109.1)	NS [†]
hs-CRP, mg/L	3.34 (3.04–3.66)	3.33 (3.03–3.66)	3.46 (2.09–5.71)	NS [†]
Total cholesterol, mmol/L	5.05±1.06	5.05±1.07	5.01±0.82	NS [†]
LDL cholesterol, mmol/L	3.29±0.87	3.30±0.87	3.14±0.64	NS [†]
HDL cholesterol, mmol/L	1.22±0.35	1.22±0.35	1.22±0.31	NS [†]
Triglyceride, mmol/L	1.53 (1.49–1.57)	1.53 (1.49–1.57)	1.56 (1.38–1.76)	NS [†]
Apo A, g/L	1.29±0.28	1.28±0.27	1.32±0.28	NS [†]
Apo B, g/L	0.99±0.28	0.99±0.27	0.99±0.28	NS [†]
Apo CIII, mg/dL	10.7 (10.4–10.9)	10.6 (10.4–10.9)	12.2 (11.10–13.5)	0.011 [†]
Apo E, g/L	0.037 (0.036–0.038)	0.037 (0.036–0.038)	0.036 (0.032–0.042)	NS [†]
Anticoagulant therapy at enrollment, % [§]	12.5	13.1	0.0	0.009 [‡]

Apo A indicates apolipoprotein A; Apo B, apolipoprotein B; Apo CIII, apolipoprotein CIII; Apo E, apolipoprotein E; BMI, body mass index, CAD, coronary artery disease; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NS, not significant; VTE, venous thromboembolism.

* $P < 0.10$ are reported.

[†]By *t* test.

[‡]By chi-square test.

[§]A total of 128 patients were reported to be treated with anticoagulant therapy at discharge from the hospitalization during which they were enrolled in the Verona Heart Study. All of these patients were reported to take warfarin for atrial fibrillation.

patients with high apo CIII levels having a >3-fold increased incidence rate of VTE than those with low apo CIII levels (6.0 [95% CI, 4.0–8.0] vs 1.8 [95% CI, 0.7–2.9] VTE events/1000 person-years, $P < 0.001$), even in adjusted models and after the exclusion of patients taking anticoagulant drugs at enrollment (Tables 4 and 5).

Discussion

To the best of our knowledge, this prospective cohort study is the first to investigate the association between plasma apo CIII concentration and VTE incidence in humans. Our results demonstrate that patients with cardiovascular disease with

Table 2. Correlations of Lipid Parameters in the Entire Study Population Using Pearson Test

	Apo E	Apo CIII	Apo B	Apo AI	Triglyceride	HDL Cholesterol	LDL Cholesterol
Total cholesterol	0.252*	0.359*	0.724*	0.223*	0.342*	0.271*	0.901*
LDL cholesterol	0.194*	0.135*	0.692*	0.005	0.121*	0.012	
HDL cholesterol	0.085 [†]	0.083 [†]	−0.065	0.735*	0.375*		
Triglyceride	0.197*	0.587*	0.393*	0.177*			
Apo AI	0.103*	0.289*	0.046				
Apo B	0.255*	0.354*					
Apo CIII	0.287*						

Apo AI indicates apolipoprotein AI; Apo B, apolipoprotein B; Apo CIII, apolipoprotein CIII; Apo E, apolipoprotein E; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

* $P < 0.01$.

[†] $P < 0.05$.

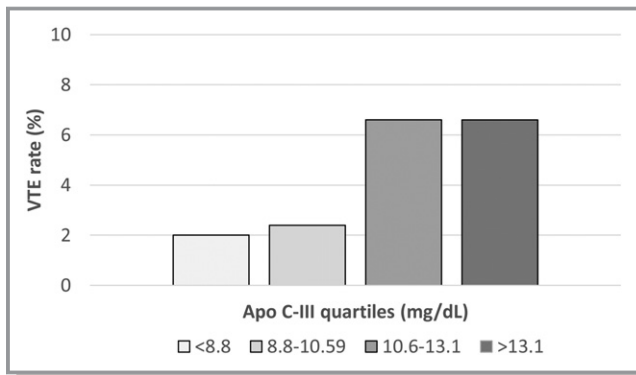


Figure 1. Venous thromboembolism (VTE) rate during the follow-up period among patients stratified into quartiles according to apolipoprotein CIII (apo CIII) levels.

high apo CII concentrations, which is an important marker of circulating triglyceride-rich lipoproteins, are 3 times as likely to experience future VTE events within a long-term period (eg, the median follow-up duration in our study was ≈ 12 years).

Forty-five VTE events were recorded in ≈ 1000 patients affected by coronary or valvular heart disease, which is a VTE incidence rate of 3.7 events/1000 person-years. This rate was higher than that in the unselected general population (1.0–1.8 events/1000 person-years).^{9–11} Thus, as an initial observation, the present data suggest that the VTE incidence rate in patients with cardiovascular disease is clearly higher (by ≈ 2 -fold) than that in healthy patients. Similar results (incidence rate of ≈ 3.0 events/1000 person-years) and conclusions were reported recently in a large population of patients (47 611 patients followed for 3 years) with atherosclerotic vascular disease,¹² which supports the hypothesis that arterial disease (rather than CAD specifically) confers an increased risk of VTE.

Prandoni et al¹³ first demonstrated the association between VTE and subsequent arterial cardiovascular diseases, which raised great interest^{14–16} but remained controversial.^{17,18}

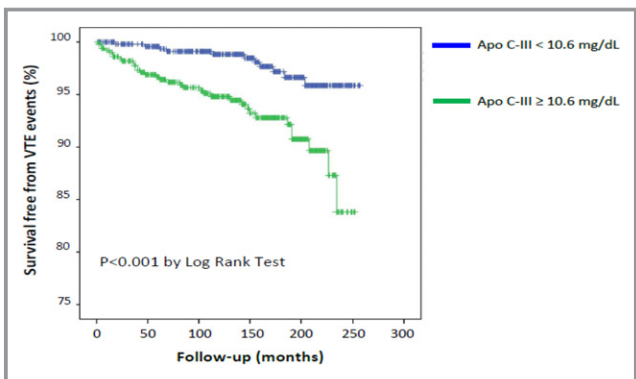


Figure 2. Kaplan–Meier survival curves for venous thromboembolism (VTE) among patients stratified by the median level of apolipoprotein CIII (apo CIII).

Table 3. Plasma Concentrations of Apo CIII Above the Median Level (≥ 10.6 mg/dL) as a Predictor of VTE in Different Cox Regression Models

	Coefficient B	Standard Error	Hazard Ratio	P Value
Unadjusted	1.229	0.347	3.42 (1.73–6.75)	<0.001
Model 1	1.154	0.349	3.17 (1.60–6.29)	0.001
Model 2	1.119	0.350	3.06 (1.54–6.08)	0.001
Model 3	0.977	0.359	2.66 (1.31–5.37)	0.007
Model 4	1.320	0.565	3.74 (1.24–11.33)	0.019

Model 1: adjusted for sex and age; model 2: adjusted for sex, age, and coronary artery disease (CAD) diagnosis; model 3: adjusted for sex, age, CAD diagnosis, body mass index (BMI), hypertension, and anticoagulant therapy at enrollment; model 4: adjusted for sex, age, CAD diagnosis, BMI, hypertension, anticoagulant therapy at enrollment, and all plasma lipid parameters (ie, total and high- and low-density lipoprotein cholesterol, triglyceride, and apolipoproteins AI, B, and E). Apo CIII indicates apolipoprotein CIII; VTE, venous thromboembolism.

Consequently, the reverse association was also investigated, and the hypothesis that traditional cardiovascular risk factors of atherosclerosis (eg, smoking, high blood pressure, diabetes mellitus, obesity, and dyslipidemia) are involved in the occurrence of VTE was substantially confirmed.^{19,20} A recent report on a prospective cohort of patients with VTE suggested that the cumulative burden of conventional risk factors, rather than a single element, is relevant to the risk of VTE occurrence.²¹

From this evidence, it is warranted to note the peculiar features of our study: both groups of patients with cardiovascular disease with or without VTE were fully balanced for all major traditional risk factors for arterial disease at the time of enrollment (Table 1). Therefore, the finding that the initially elevated apo CIII concentrations were asymmetrically distributed between the groups of patients who chronically experienced (or not) VTE events was unexpected and suggestive of a possible causal link. The incidence rates of VTE for the 2 groups of patients stratified according to the median apo CIII level (10.6 mg/dL) were 1.8 and 6.0 events/1000 person-years, respectively. Given that the estimated incidence of VTE in the general population is 1.0 to 1.8 events/1000 person-years,^{9,10} the group with low apo CIII levels exhibited a substantially similar incidence rate as compared with the general population (Table 4), while the group with high apo CIII levels exhibited an ≈ 3 -fold increase in incidence rate (Table 4), which suggests a role for apo CIII in VTE risk. No other laboratory characteristics, including any other plasma lipid parameters, or traditional cardiovascular risk factors were distributed so differently between the groups with or without VTE. Women were more represented in the VTE group (Table 1). Treatment with oral anticoagulants at enrollment (128 patients were taking warfarin for atrial fibrillation) into our study is an important potential bias regarding the results of the current study. Notably, such

Table 4. IRs and IRRs With 95% CIs for VTE According to Plasma Concentrations of Apo CIII, Estimated by Different Poisson Regression Models in the Entire Study Population (N=1020)

	IR		IRR	P Value
	VTE Events/1000 Person-Y, No.			
	Low Apo CIII <10.6 mg/dL	High Apo CIII ≥10.6 mg/dL	High Apo CIII vs Low Apo CIII	
Unadjusted	1.8 (0.7–2.9)	6.0 (4.0–8.0)	3.36 (1.76–6.94)	<0.001
Model 1	1.9 (0.8–3.0)	5.6 (3.6–7.6)	3.03 (1.58–6.3)	0.002
Model 2	1.9 (0.8–3.0)	5.5 (3.5–7.5)	2.97 (1.55–6.18)	0.002
Model 3	1.8 (0.6–2.9)	4.3 (2.3–6.2)	2.42 (1.23–5.09)	0.014
Model 4	1.3 (0.1–2.4)	4.9 (2.1–7.6)	3.88 (1.41–11.99)	0.012

Model 1: adjusted for sex and age; model 2: adjusted for sex, age, and coronary artery disease (CAD) diagnosis; model 3: adjusted for sex, age, CAD diagnosis, body mass index (BMI), and hypertension; model 4: adjusted for sex, age, CAD diagnosis, BMI, hypertension, and all plasma lipid parameters (ie, total and high- and low-density lipoprotein cholesterol, triglyceride, and apolipoproteins A1, B, and E). Apo CIII indicates apolipoprotein CIII; IR, incidence rate; IRR, incidence rate ratio; VTE, venous thromboembolism.

treatment was recorded only in a proportion of patients without VTE but not in patients with VTE events during the follow-up period (Table 1). The results did not change when the risk estimate was reassessed in the warfarin-free group, which excludes any interference of this therapy in our results. Different regression models confirmed the association of VTE incidence with apo CIII after adjustment for all possible confounding factors (Table 3).

These findings reflect a perspective survey over time, but do not provide a direct demonstration of a causal or mechanistic role for apo CIII in VTE. However, the results are an ideal continuation and clinical accomplishment of previous work from our group, in which several coagulation activities were evaluated in the relationship with apo CIII concentrations.^{4,7} Notably, the earlier results of studies on coagulation biomarkers^{4,7} closely match the current study.

We first demonstrated that thrombin generation, which was assessed using endogenous thrombin potential, was amplified in patients with elevated apo CIII concentrations.⁴ Thrombin generation occurs in the terminal portion of the coagulation cascade, which depends on numerous

coagulation factors. For this reason, we subsequently examined the relationship of apo CIII with the plasma coagulant activities of FII, factor V, factor VIII, activated factor VII, and activated factor X.⁷ The strongest association was observed for factor II coagulant activity, which exhibited an increase comparable to that in carriers of the G20210A allele, a well-known genetic predisposing factor for both arterial and venous thrombotic disease. In the analysis, although less strong, statistical associations of apo CIII with factor V-mediated coagulant activity, activated factor VII levels, and activated factor X generation were also observed.⁷ Experiments with the specific thrombin inhibitor hirudin were performed to separate the effects on thrombin generation from the upstream formation of activated factor X as a result of factor VII/tissue factor activation. This relationship was experimentally excluded, and our interpretation was that apo CIII acts as a modulator of prothrombinase activity by favoring the triggering and amplification of factor II coagulant activity in the terminal portion of the coagulation cascade.

Taken together, these data suggest that the apo CIII-related effect on prothrombinase activity occurs in both CAD

Table 5. IRs and IRRs With 95% CIs for VTE According to Plasma Concentrations of Apo CIII, Estimated by Different Poisson Regression Models in Patients Not Taking Anticoagulant Therapy at Enrollment (n=892)

	IR		IRR	P Value
	VTE Events/1000 Person-Y, No.			
	Low Apo CIII <10.6 mg/dL	High Apo CIII ≥10.6 mg/dL	High Apo CIII vs Low Apo CIII	
Unadjusted	2.1 (0.9–3.3)	6.9 (4.6–9.2)	3.33 (1.74–6.88)	0.001
Model 1	2.1 (0.8–3.3)	6.6 (4.3–8.9)	3.16 (1.65–6.56)	0.001
Model 2	2.1 (0.9–3.4)	6.5 (4.2–8.8)	3.09 (1.61–6.42)	0.001
Model 3	2.2 (0.9–3.6)	5.6 (3.3–8.0)	2.52 (1.29–5.31)	0.010
Model 4	1.2 (0.2–2.4)	4.7 (1.8–7.7)	3.99 (1.39–12.92)	0.014

Model 1: adjusted for sex and age; model 2: adjusted for sex, age, and coronary artery disease (CAD) diagnosis; model 3: adjusted for sex, age, CAD diagnosis, body mass index (BMI), and hypertension; model 4: adjusted for sex, age, CAD diagnosis, BMI, hypertension, and all plasma lipid parameters (ie, total and high- and low-density lipoprotein cholesterol, triglyceride, and apolipoproteins A1, B, and E). Apo CIII indicates apolipoprotein CIII; IR, incidence rate; IRR, incidence rate ratio; VTE, venous thromboembolism.

and CAD-free patients independently of “atherosclerosis.” Apo CIII may exert procoagulant activity on the arterial side and the venous compartment. Moreover, taking into account the pivotal role of thrombin in several biological pathways beyond blood coagulation, including its major role as a pathophysiological mediator bridging inflammation and clotting,²² the detrimental and pleiotropic consequences of elevated apo CIII concentrations to both arterial and venous vessels appear evident. A recent prospective nested case-control study of apparently healthy patients revealed an association of apo CIII with CAD and high-sensitivity C-reactive protein, a well-recognized inflammatory marker.²³

If the hypothesis of an apo CIII-related prothrombotic diathesis is true, then as a practical consequence, pharmacological treatments that lower apo CIII concentrations may exhibit beneficial effects beyond lipid metabolism, ie, suppressing thrombin generation and decreasing factor II coagulant activity. Several cardiovascular protective compounds (peroxisome proliferator-activated receptor agonists, omega-3 polyunsaturated fatty acid, niacin, ezetimibe, and statins) are effective apo CIII-lowering drugs.²⁴ Volanesorsen, a new anti-apo CIII antisense oligonucleotide, was demonstrated in phase II clinical trials to be a powerful new therapy for triglyceride-rich lipoprotein-associated dyslipidemia.²⁵ While there is no similar evidence for the other above-mentioned drugs, statins (especially rosuvastatin, which is particularly efficient in reducing apo CIII levels) have been consistently demonstrated to reduce VTE events.²⁶ A systematic review and meta-analysis of observational cohort studies and randomized controlled trials (13 cohort studies comprising 3 148 259 participants and 23 randomized controlled trials of statins vs placebo or no treatment comprising 118 464 participants) recently confirmed this beneficial effect.²⁷

The coagulation cascade requires lipids, and it is greatly accelerated by lipid binding. However, the mechanism by which statin treatment reduces VTE risk is not clear. Our previous and current results offer a new contribution explaining this mechanism, namely, a role of apo CIII. A “threshold effect” for the procoagulant activity of apo CIII concentrations is consistent with the data in the present work, in which a net increase in VTE risk was observed only for individuals with apo CIII concentrations >10.6 mg/dL. Statins appear to lower apo CIII concentrations to different degrees, depending on the dosage and type of statin.²⁸

Study Limitations

A possible limitation of the study is that symptomatic events were retrospectively ascertained using a formal adjudication process of 2 blinded specialists. The retrospective nature of event collection may lead to an underascertainment of events.

Therefore, the observed event rate may underestimate the “true” event rate. However, it is reassuring that the frequency of patients with VHS is comparable to the literature for analogous populations.¹² Notably, the relatively few events collected may intrinsically limit the statistical power of the study, but the number of VTE events is consistent with the expected frequency in real life for a population of ≈1000 patients with cardiovascular disease followed for 12 years.

Our data suggest 2 further considerations as working hypotheses. The first hypothesis is related to the potential use of total apo CIII as a useful index for personalized therapeutic choices in clinical practice. Apo CIII may be a more precise predictor of cardiovascular disease risk than triglyceride concentrations and may provide information about the prothrombotic and procoagulant propensity of a single patient. Several methods for routinely measuring total apo CIII are available, and these methods exhibit excellent precision and may be automated.²⁹ Apo CIII in very low-density lipoprotein and low-density lipoprotein subfractions may be preferable in research settings, but this methodology may be cumbersome in practice because it requires ultracentrifugation. The present findings suggest that a total apo CIII assay is sufficient for clinical purposes. Second, all of the drugs that lower apo CIII concentrations, including the new anti-apo CIII antisense oligonucleotides, may exhibit additional and unexpected antithrombotic effects. Formal evidence of efficacy in reducing VTE risk are lacking for many of these agents, with statins as the only notable exceptions. Therefore, future investigations and specifically designed clinical trials are needed. In this context of uncertainty, verification of drug interactions and quantification of the hemorrhagic risk in cases of associated anticoagulant treatment are required.

Conclusions

The data in the present work provide, for the first time, clinical proof of the prothrombotic role of elevated plasma apo CIII concentrations in the setting of VTE. This conclusion is consistent with our previous observations that high apo CIII concentrations enhance the coagulation cascade via increased factor II coagulant activity and amplified thrombin generation, which are known to play crucial roles in VTE.

Acknowledgments

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

Sources of Funding

Oliviero Olivieri and Nicola Martinelli acknowledge the support of Fondazione CariVerona (project number B36J16002570003).

Disclosures

None.

References

- Jin JL, Guo YL, Li JJ. Apoprotein C-III: a review of its clinical implications. *Clin Chim Acta*. 2016;460:50–54.
- Wyer 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.
- TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute, Crosby J, Peloso GM, Auer PL, Crosslin DR, Stitzel NO, Lange LA, Lu Y, Tang ZZ, Zhang H, Hindy G, Masca N, Stirrups K, Kanoni S, Do R, Jun G, Hu Y, Kang HM, Xue C, Goel A, Farrall M, Duga S, Merlini PA, Asselta R, Girelli D, Olivieri O, Martinelli N, Yin W, Reilly D, Speliotes E, Fox CS, Hveem K, Holmen OL, Nikpay M, Farlow DN, Assimes TL, Franceschini N, Robinson J, North KE, Martin LW, DePristo M, Gupta N, Escher SA, Jansson JH, Van Zuydam N, Palmer CN, Wareham N, Koch W, Meitinger T, Peters A, Lieb W, Erbel R, König IR, Kruppa J, Degenhardt F, Gottesman O, Bottinger EP, O'Donnell CJ, Psaty BM, Ballantyne CM, Abecasis G, Ordovas JM, Melander O, Watkins H, Orho-Melander M, Ardissono D, Loos RJ, McPherson R, Willer CJ, Erdmann J, Hall AS, Samani NJ, Deloukas P, Schunkert H, Wilson JG, Kooperberg C, Rich SS, Tracy RP, Lin DY, Altshuler D, Gabriel S, Nickerson DA, Jarvik GP, Cupples LA, Reiner AP, Boerwinkle E, Kathiresan S. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med*. 2014;371:22–31.
- Olivieri O, Martinelli N, Girelli D, Pizzolo F, Friso S, Beltrame F, Lotto V, Annarumma L, Corrocher R. 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.
- 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.
- Kawakami A, Osaka M, Tani M, Azuma H, Sacks FM, Shimokado K, Yoshida M. Apolipoprotein CIII links hyperlipidemia with vascular endothelial cell dysfunction. *Circulation*. 2008;118:731–742.
- Olivieri O, Martinelli N, Baroni M, Branchini A, Girelli D, Friso S, Pizzolo F, Bernardi F. 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
- Preston DL. Poisson regression in epidemiology. In: Armitage P, Colton T, eds. *Encyclopedia of Biostatistics Volume 6*. 2nd ed. Chichester: John Wiley & Sons; 2005:4124–4127.
- Heit JA. Epidemiology of venous thromboembolism. *Nat Rev Cardiol*. 2015;12:464–474.
- Tagalakis V, Patenaude V, Kahn SR, Suissa S. Incidence of and mortality from venous thromboembolism in a real-world population: the Q-VTE Study Cohort. *Am J Med*. 2013;126:832.e13–832.e21.
- Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR, Jiménez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Mackey RH, Matsushita K, Mozaffarian D, Mussolino ME, Nasir K, Neumar RW, Palaniappan L, Pandey DK, Thiagarajan RR, Reeves MJ, Ritchey M, Rodriguez CJ, Roth GA, Rosamond WD, Sasson C, Towfighi A, Tsao CW, Turner MB, Virani SS, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2017 update: a report from the American Heart Association. *Circulation*. 2017;135:e146–e603.
- Cavallari I, Morrow DA, Creager MA, Olin J, Bhatt DL, Steg PG, Storey RF, Cohen M, Scirica BS, Piazza G, Goodrich EL, Braunwald E, Sabatine MS, Bonaca MP. Frequency, predictors, and impact of combined antiplatelet therapy on venous thromboembolism in patients with symptomatic atherosclerosis. *Circulation*. 2018;137:684–692.
- Prandoni P, Bilora F, Marchiori A, Bernardi E, Petrobelli F, Lensing AWA, Prins MH, Girolami A. An association between atherosclerosis and venous thrombosis. *N Engl J Med*. 2003;348:1435–1441.
- Prandoni P, Ghirarduzzi A, Prins MH, Pengo V, Davidson BL, Sørensen H, Pesavento R, Iotti M, Casiglia E, Illiceto S, Pagnan A, Lensing AW. Venous thromboembolism and the risk of subsequent symptomatic atherosclerosis. *J Thromb Haemost*. 2006;4:1891–1896.
- Sørensen HT, Horvath-Puho E, Pedersen L, Baron JA, Prandoni P. Venous thromboembolism and subsequent hospitalisation due to acute arterial cardiovascular events: a 20-year cohort study. *Lancet*. 2007;370:1773–1779.
- Sørensen HT, Horvath-Puho E, Lash TL, Christiansen CF, Pesavento R, Pedersen L, Baron JA, Prandoni P. Heart disease may be a risk factor for pulmonary embolism without peripheral deep venous thrombosis. *Circulation*. 2011;124:1435–1441.
- van der Hagen PB, Folsom AR, Jenny NS, Heckbert SR, O'Meara ES, Reich LM, Rosendaal FR, Cushman M. Subclinical atherosclerosis and the risk of future venous thrombosis in the Cardiovascular Health Study. *J Thromb Haemost*. 2006;4:1903–1908.
- Reich LM, Folsom AR, Key NS, Boland LL, Heckbert SR, Rosamond WD, Cushman M. Prospective study of subclinical atherosclerosis as a risk factor for venous thromboembolism. *J Thromb Haemost*. 2006;4:1909–1913.
- Agno W, Becattini C, Brighton T, Selby R, Kamphuisen PW. Cardiovascular risk factors and venous thromboembolism. A meta-analysis. *Circulation*. 2008;117:93–102.
- Gresele P, Momi S, Migliacci R. Endothelium, venous thromboembolism and ischaemic cardiovascular events. *Thromb Haemost*. 2010;103:56–61.
- Gaertner S, Cordeanu EM, Mirea C, Frantz AS, Auger C, Bilbault P, Ohlmann P, Schini-Kerth V, Stephan D. Increased risk and severity of unprovoked venous thromboembolism with clustering cardiovascular risk factors for atherosclerosis: results of the REMOTEV registry. *Int J Cardiol*. 2018;252:169–174.
- Borisoff JI, Spronk HM, ten Cate H. The hemostatic system as a modulator of atherosclerosis. *N Engl J Med*. 2011;364:1746–1760.
- van Capelleveen JC, Bernelot Moens SJ, Yang X, Kastelein JJP, Wareham NJ, Zwiderman AH, Stroes ESG, Witztum JL, Hovingh GK, Khaw KT, Boekholdt SM, Tsimikas S. Apolipoprotein C-III levels and incident coronary artery disease risk. *Arterioscler Thromb Vasc Biol*. 2017;37:1206–1212.
- Norata GD, Tsimikas S, Pirillo A, Catapano AL. Apolipoprotein C-III: from pathophysiology to pharmacology. *Trends Pharmacol Sci*. 2015;36:675–687.
- Gaudet D, Alexander VJ, Baker BF, Brisson D, Tremblay K, Singleton W, Geary RS, Hughes SG, Viney NJ, Graham MJ, Crooke RM, Witztum JL, Brunzell JD, Kastelein JJ. Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. *N Engl J Med*. 2015;373:438–447.
- Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Ridker PM. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. *N Engl J Med*. 2009;360:1851–1861.
- Kunutsor SK, Seidu S, Khunti K. Statins and primary prevention of venous thromboembolism: a systematic review and meta-analysis. *Lancet Haematol*. 2017;4:e83–e93.
- Khemasuwana D, Chae YK, Gupta S, Carpio A, Yun JH, Neagu S, Lucca AB, Valsecchi ME, Mora JL. Dose-related effect of statins in venous thrombosis risk reduction. *Am J Med*. 2011;124:852–859.
- 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.