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Accelerated In Vivo Thrombin Formation Independently Predicts the Presence and Severity of CT Angiographic Coronary Atherosclerosis

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OBJECTIVES This study sought to investigate the association between thrombin generation in plasma and the presence and severity of computed tomography angiographically defined coronary atherosclerosis in patients with suspected coronary artery disease (CAD).

BACKGROUND Besides its pivotal role in thrombus formation, experimental data indicate that thrombin can induce an array of pro-atherogenic and plaque-destabilizing effects. Although thrombin plays a role in the pathophysiology of atherosclerosis progression and vascular calcification, the clinical evidence remains limited.

METHODS Using 64-slice coronary computed tomographic angiography, we assessed the presence and characteristics of CAD in patients ($n = 295$; median age 58 years) with stable chest pain. Coronary artery calcification was graded as absent (Agatston score 0), mild (Agatston score 1 to 100), moderate (Agatston score 101 to 400), and severe (Agatston score >400). Calibrated automated thrombography was used to assess endogenous thrombin potential in plasma in vitro. Thrombin-antithrombin complex (TATc) levels were measured as a marker for thrombin formation in vivo.

RESULTS TATc plasma levels were substantially higher in patients with CAD versus patients without CAD ($p = 0.004$). Significant positive correlations were observed between steadily increasing TATc levels and the severity of CAD ($r = 0.225$, $p < 0.001$). In multinomial logistic regression models, after adjusting for established risk factors, TATc levels predicted the degree of coronary artery calcification: mild (odds ratio: 1.56, $p = 0.006$), moderate (odds ratio: 1.56, $p = 0.007$), and severe (odds ratio: 1.67, $p = 0.002$). Trends were comparable between the groups when stratified according to the degree of coronary luminal stenosis.

CONCLUSIONS This study provides novel clinical evidence indicating a positive independent association between enhanced in vivo thrombin generation and the presence and severity of coronary atherosclerosis, which may suggest that thrombin plays a role in the pathophysiology of vascular calcification and atherosclerosis progression. (J Am Coll Cardiol Img 2012;5:1201–10) © 2012 by the American College of Cardiology Foundation

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Atherosclerosis is a multifactorial chronic inflammatory vascular disorder (1,2). Given the abundant experimental evidence showing extensive interactions between the hemostatic, immune, and inflammation systems, we have proposed a role for the clotting proteins in modulating atherosclerosis progression and atherosclerotic plaque phenotype (3). In particular, thrombin, which is the most central coagulation protein, is also recognized as a strong pro-inflammatory mediator. Endowed with a potent cell signaling capacity, thrombin can induce an array of pro-atherogenic and plaque-destabilizing effects such as inflammation, vascular smooth muscle cell migration and proliferation, leukocyte chemotaxis, proteolysis, apoptosis, and angiogenesis (3,4). Recently, we demonstrated that thrombin, as well as other coagulation proteins, are widely expressed and functionally active throughout distinct compartments of the arterial vessel wall (5), supporting an active cell-based coagulation network within human atherosclerotic plaques. G-protein–coupled protease-activated receptors, which are selectively cleaved by thrombin, are also abundantly distributed in the vasculature under normal conditions and overexpressed in atherosclerotic lesions (6). Experimental animal studies have clearly indicated that variations in the clotting activity affect the progression and thrombogenicity of atherosclerotic plaques (3).

Antithrombotic therapy is a cornerstone in the management and prevention of atherothrombosis in patients (2). Experimental data demonstrate that direct thrombin inhibition substantially attenuates atherosclerosis development in ApoE-null mice (7) and protects against severe plaque progression in prothrombotic mice (8). However, the role of blood coagulation proteins in atherogenesis, in particular thrombin, has not been adequately addressed in previously conducted clinical research. Cardiac computed tomographic angiography (CCTA) is a well-established noninvasive imaging modality, which has high diagnostic accuracy for detection and characterization of coronary atherosclerotic plaques (9,10). Using CCTA, we investigated the association between thrombin formation in plasma and the presence and severity of coronary atherosclerosis in patients with suspected coronary artery disease (CAD).

ABBREVIATIONS AND ACRONYMS

AUROC = area under the receiver-operating characteristic curve

CAC = coronary artery calcification

CAD = coronary artery disease

CAT = calibrated automated thrombography

CCTA = cardiac computed tomographic angiography

CI = confidence interval

CT = computed tomography

ETP = endogenous thrombin potential

FRS = Framingham Risk Score

OR = odds ratio

TATc = thrombin-antithrombin complex

METHODS

Study population. We studied 295 adult patients who were referred from the cardiology outpatient department for CCTA because of stable chest pain, suspected for CAD. Scans were performed in our university medical center between January 2008 and June 2010 as part of the diagnostic work-up in these patients. Included were patients with a recent history of (a)typical chest pain, who underwent a coronary calcium score scan as well as CCTA. Excluded were patients with acute chest pain suspected for an acute coronary syndrome; patients with a history of acute myocardial infarction, percutaneous coronary intervention, and/or coronary artery bypass grafting surgery; patients with missing data regarding their cardiac risk profile; patients with an inconclusive computed tomography (CT) scan; and patients currently on anticoagulation therapy (oral vitamin K antagonist/selective anticoagulants or low-molecular-weight heparins). In vitro hemolysis of blood samples was also an exclusion criterion. We calculated the Framingham Risk Score (FRS) in all patients to estimate the 10-year risk of having a myocardial infarction or cardiovascular death (11). The Institutional Review Board and Ethics Committee at the Maastricht University Medical Center approved the study, and all patients gave written informed consent.

CCTA protocol. Scans were performed using a 64-slice multidetector-row CT scanner (Brilliance 64; Philips Healthcare, Best, the Netherlands) with a 64 × 0.625-mm slice collimation, a gantry rotation time of 420 ms, and a tube voltage of 80 to 120 kV. Tube current varied from 150 to 210 mAs for the prospectively gated "step and shoot" protocol and from 600 to 1,000 mAs for the retrospectively gated "helical" protocol, depending on patients' weight and height. Patients received 50 mg metoprolol tartrate orally, 2 h before CCTA. When indicated, an additional dose of 5 to 20 mg metoprolol tartrate (AstraZeneca, Zoetermeer, the Netherlands) was administered intravenously to lower the heart rate to <65 beats/min. A dose of 0.8 mg nitroglycerin spray (Pohl-Boskamp, Hohenlockstedt, Germany) was given sublingually just prior to CCTA. Heart rate and electrocardiogram were monitored during CCTA.

A nonenhanced scan was performed to determine the amount of coronary artery calcification (CAC), using the Agatston method (12). Subsequently, CCTA was performed using 85 to 110 ml of contrast agent (Xenetix 350, Guerbet, Roissy CdG Cedex, France), which was injected in the antecubital vein at a rate of 6.0 ml/s, directly followed by 40 ml intrave-

nous saline (6.0 ml/s) using a dual-head power injector. A prospectively gated "step and shoot" protocol was used in all patients with a stable heart rate <65 beats/min. In patients with an irregular heart rate or a stable heart rate >65 beats/min, we used a retrospectively gated "helical" protocol with dose modulation to obtain the best image quality at minimal radiation dose (13,14).

CCTA analysis. All scans were independently analyzed by 2 cardiologists with level III expertise in coronary CT angiography, blinded for patient details, using source images in the Cardiac Comprehensive Analysis software (Philips Healthcare). In case of disagreement, consensus was reached by reviewing findings jointly.

CAC was expressed as the Agatston score using calcium scoring software (Philips Healthcare) with a threshold of 130 Hounsfield units (HU). The coronary artery tree was analyzed for the presence and severity of CAD, according to the 16-segment classification of the American Heart Association (15). Coronary plaques were defined as visible structures within or adjacent to the coronary artery lumen, which could be clearly distinguished from the vessel lumen and the surrounding pericardial tissue. Plaques were categorized as calcified (exclusively content with density >130 HU), noncalcified (exclusively content with density <130 HU), or mixed (characteristics of both calcified and noncalcified plaques). The degree of CAD was classified as absent (no luminal stenosis), mild (<50% luminal stenosis), moderate (50% to 70% luminal stenosis), or severe (>70% luminal stenosis) (16). The degree of CAC was classified as absent (Agatston score 0), mild (Agatston score 1 to 100), moderate (Agatston score 100 to 400), or severe (Agatston score >400) (17).

Blood samples and laboratory measurements. Blood samples were taken just before the scan and processed within 2 h, and plasma was stored at −80°C until analysis. Continuous thrombin generation in clotting platelet-poor plasma was monitored in vitro by using the Calibrated Automated Thrombography (CAT) method (Thrombinoscope B.V., Maastricht, the Netherlands) (18). The reaction was triggered by adding 5pM tissue factor (PPP Reagent, Thrombinoscope B.V.) in the presence of 4 μM phospholipids and 16 mM added $CalCl_2$ (in duplicate). Endogenous thrombin potential (ETP) was analyzed (corresponds to the area under the curve). ETP values were normalized on the basis of platelet-poor normal pooled plasma obtained from healthy volunteers, the latter used as a reference (19). Data are expressed as percent of normal pooled plasma (20). In addition, using a commercially available microenzyme immunoassay kit (Enzygnost TAT Micro, Siemens Healthcare Diagnostics, Deerfield, Illinois) we established thrombin-antithrombin complex (TATc) levels in all patients (in duplicate) as a highly specific marker for thrombin formation in vivo.

Statistical analysis. Statistical analyses were performed using IBM SPSS Statistics 19.0.0 (SPSS Inc., Chicago, Illinois). Categorical variables are presented as numbers (percentages), whereas continuous data are expressed as mean ± SD, unless otherwise indicated. TATc plasma levels were normalized by natural logarithm transformation. We used the score plus 1 to also include patients with a TATc plasma level below 1 ng/ml. Demographic differences between patients with or without CAD were tested using either Student's *t* test or Mann-Whitney *U* test, depending on the distribution characteristics of the data. Pearson's chi-square test was used to compare proportions (binary or categorical), whereas continuous variables were analyzed via 1-way analysis of variance test, including Bonferroni correction. Correlations are presented as Pearson or Spearman coefficients according to the observed distribution. Multivariate analyses were conducted using binary/multinomial logistic regression, computed in a multiple main effects or forward stepwise manner, including variables with $p < 0.05$. Pearson's chi-square test and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to determine which variables demonstrated significant independent associations with atherosclerotic plaque presence, degree of luminal stenosis, and CAC. Receiver-operating characteristic analysis was carried out to evaluate the potential of using TATc and FRS (separately or in combination) for determining the presence or absence of CAD. Areas under the receiver-operating characteristic curve (AUROC) were compared using the Hanley and McNeil's method. We performed the net reclassification index to evaluate the incremental effect of adding TATc to the FRS for predicting the presence of coronary plaques. A 2-sided p value <0.05 was considered statistically significant.

RESULTS

Study population characteristics. The study population consisted of 295 individuals (182 men [61.7%] and 113 women [38.3%]) with a median age of 57 years (minimum to maximum: 30 to 87). A total of 226 patients underwent a "step and shoot" scan

Table 1. Baseline Characteristics of Subjects Stratified Into Groups per Presence of CT-Angiographic CAD

	All Participants (n = 295)	Patients Without CAD* (n = 90)	Patients With CAD† (n = 205)	p Value
Age, yrs	57 (30–87)	54 (30–76)	59 (36–87)	<0.001
Male	182 (61.7)	48 (53.3)	134 (65.4)	0.053
BMI, kg/m^2	26.4 (24.2–29.4)	26.6 (23.8–29.7)	26.4 (24.3–29.3)	0.620
Systolic blood pressure, mm Hg	143 (130–156)	139 (127–153)	145 (132–157)	0.023
Diastolic blood pressure, mm Hg	80 (73–87)	80 (71–86)	81 (74–87)	0.615
Smoking	71 (24.1)	18 (20.0)	53 (25.9)	0.304
Diabetes mellitus	26 (8.8)	6 (6.7)	20 (9.8)	0.505
Positive family history	126 (42.7)	36 (40.0)	90 (43.9)	0.609
Lipid-lowering therapy	122 (41.4)	22 (24.4)	100 (48.8)	<0.005
Anticoagulation therapy	0 (0)	0 (0)	0 (0)	—
Framingham Risk Score	18.9 (12.3–30.0)	14.1 (9.8–21.4)	21.6 (13.4–33.1)	<0.001
Total cholesterol, mg/dl	213 (174–244)	217 (192–240)	205 (170–247)	0.077
LDL, mg/dl	128 (101–166)	135 (116–163)	124 (97–166)	0.043
HDL, mg/dl	46 (35–58)	46 (35–57)	44 (37–58)	0.980
Triglycerides, mg/dl	134 (90–208)	146 (91–231)	130 (89–202)	0.180
Coronary atherosclerotic lesions	2 (0–5)	0 (0–0)	4 (2–7)	<0.001
CAC (Agatston score)	27 (0–214)	0 (0–0)	117 (23–355)	<0.001

Values are median (interquartile range), or n (%). *Patients with no angiographically detected coronary atherosclerotic plaques. †Patients with ≥1 angiographically detected coronary atherosclerotic plaque(s). Statistical significance at the $p < 0.05$ level.
BMI = body mass index; CAC = coronary artery calcification; CAD = coronary artery disease; CT = computed tomography; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

(mean radiation dose 3.6 mSv), whereas 69 patients underwent a "helical" scan (mean radiation dose 11.6 mSv). CAD was detected in 205 (69.5%) patients. The prevalence of absent, mild, moderate, and severe CAD was 30.5%, 22.7%, 26.4%, and 20.3%, respectively. Compared to the non-CAD group, patients with CAD were predominantly male (65.4%) and older, showed increased systolic blood pressure, and had lower low-density lipoprotein plasma concentrations. However, the prevalence of statin use in the CAD group was significantly higher compared with the non-CAD group (100 [48.8%] vs. 22 [24.4%]). Baseline characteristics are presented in Table 1.

Increased in vivo thrombin formation independently reflects the presence of coronary atherosclerotic plaques. As depicted in Figure 1A, among the population with CAD (n = 205), the average baseline (lg 10 transformed) TATc levels were significantly higher compared with the group without CAD (mean 0.41 [95% CI: 0.38 to 0.45] vs. mean 0.32 [95% CI: 0.29 to 0.36]; p = 0.001). Multivariate logistic regression analysis showed that higher TATc levels (OR: 1.47, 95% CI: 1.10 to 1.97, p = 0.010), in addition to other established risk factors such as male gender (OR: 3.36, 95% CI: 1.75 to 6.45, p < 0.001), age (OR: 1.09, 95% CI: 1.05 to 1.12, p < 0.001), and smoking (OR: 2.17, 95% CI: 1.09 to 4.33, p < 0.001), were all independently associated with the presence of CAD (data not shown). AUROC for coronary plaque presence, calculated by using the FRS, was 0.663 (95% CI: 0.61 to 0.72, p < 0.001). Addition of TATc as a marker to the FRS improved the predictive value, resulting in a significant increase of the AUROC to 0.676 (95% CI: 0.62 to 0.73, p = 0.048 − difference between areas). The net reclassification index to assess the incremental value of TATc over the FRS in predicting the presence of coronary plaques was 3.1%, which was not significant (p = 0.68).

CAT measurement, which was carried out to assess the potential to generate thrombin in vitro, resulted in ETP values, which were almost the same in the non-CAD group and the CAD group (Fig. 1B).

TATc as determinant of CAC and luminal stenosis. In the entire study population, TATc levels showed a significant positive association with the degree of CAC (Agatston score, r = 0.209, p < 0.001), as presented in Figure 2A. In contrast, no significant relationship was noted between TATc concentrations and the number of noncalcified plaques (r = 0.052, p = 0.376). Multivariate logistic regression analyses, using multiple main effects and forward stepwise techniques, identified higher TATc formation as an independent risk factor for developing CAC (Table 2). Compared to a reference group, consisting of all patients without any coronary

calcifications, the ORs associated with CAC burden were as follows: mild CAC (OR: 1.60, 95% CI: 1.18 to 2.16, $p < 0.005$); moderate CAC (OR: 1.58, 95% CI: 1.16 to 2.15, $p < 0.005$); and severe CAC (OR: 1.71, 95% CI: 1.26 to 2.33, $p < 0.005$). As shown in Figure 2B, we also found a significant difference in the distribution of the TATc quartiles (Q1 to Q4) between the different CAC groups ($p = 0.002$). While in the no CAC group, 35.9% of the patients had TATc values in the lowest quartile (Q1) and only 14.6% of them had values in the highest quartile (Q4), the distribution of the TATc quartiles in the severe CAC group was 9.3% and 41.9% in Q1 and Q4, respectively.

There was a strong positive association between Agatston score and severity of CAD ($r = 0.712$, $p < 0.001$). Nevertheless, we also tested the relationship between TATc formation and the degree of luminal stenosis by performing multivariate logistic regression analyses with degree of luminal stenosis as a dependent variable. Similarly, TATc concentrations accurately identified worsening atherosclerosis. The ORs associated with mild, moderate, and severe CAD are depicted in Table 3.

Furthermore, we found a *U*-shaped relationship between the potential to generate thrombin in vitro and the extent of CAD (Fig. 3). Within the group of patients with detected CAD, ETP did not correlate to the extent of CAC ($r = -0.036$, $p = 0.604$), whereas it was significantly associated with the degree of CAD ($r = 0.271$, $p < 0.001$). In a multivariate logistic regression analysis, when compared with mild CAD, the ORs associated with moderate and severe CAD were as follows: moderate (OR: 1.02, 95% CI: 1.00 to 1.04, $p = 0.056$) and severe (OR: 1.04, 95% CI: 1.02 to 1.06, $p < 0.001$).

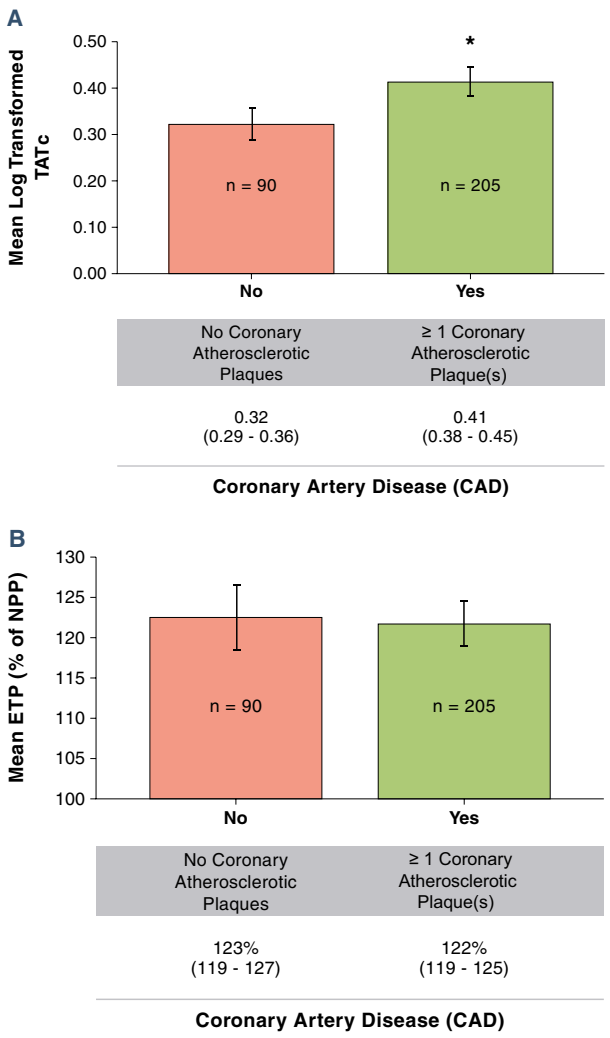

Figure 1. In Vivo/In Vitro Thrombin Formation and the Presence of CAD

Panel A shows that patients with coronary plaques exhibit significantly higher in vivo thrombin generation as compared with patients without coronary plaques ($p = 0.001$). **Bars** represent lg 10 transformed thrombin-antithrombin complex (TATc) levels, presented as mean (95% confidence interval). The corresponding numbers are given **below the panel**. **Panel B** shows that patients with coronary plaques show comparable thrombin generation potential as compared with patients without coronary plaques ($p < 0.004$). **Bars** represent endogenous thrombin potential (ETP) levels, presented as mean (95% confidence interval). The corresponding numbers are given **below the panel**. NPP = normal pool plasma.

DISCUSSION

Major findings. The present study examines the relationship between thrombin formation and CAD. In a cohort of 295 patients with suspected CAD, we established baseline TATc concentrations in plasma and used CCTA imaging to assess the presence and severity of coronary atherosclerotic plaques. We found several novel findings of potential clinical relevance. The primary observation of this study is that higher TATc levels, the latter considered a sensitive marker of thrombin formation in vivo, are independently related to the presence and severity of CAD. Net reclassification index analysis showed that incorporation of TATc as an additional test did not significantly improves the cardiovascular risk stratification capacity of the FRS. On the other hand, the amount of CAC and degree of luminal stenosis were consistently higher with increasing thrombin generation, indicating that TATc measurement is useful to detect even mild-grade CAC or stenosis. In daily practice, TATc concentrations may therefore contribute to predict which patients are more likely to have CAD. However, as a single biomarker it seems not to have enough power to be a substitute for other

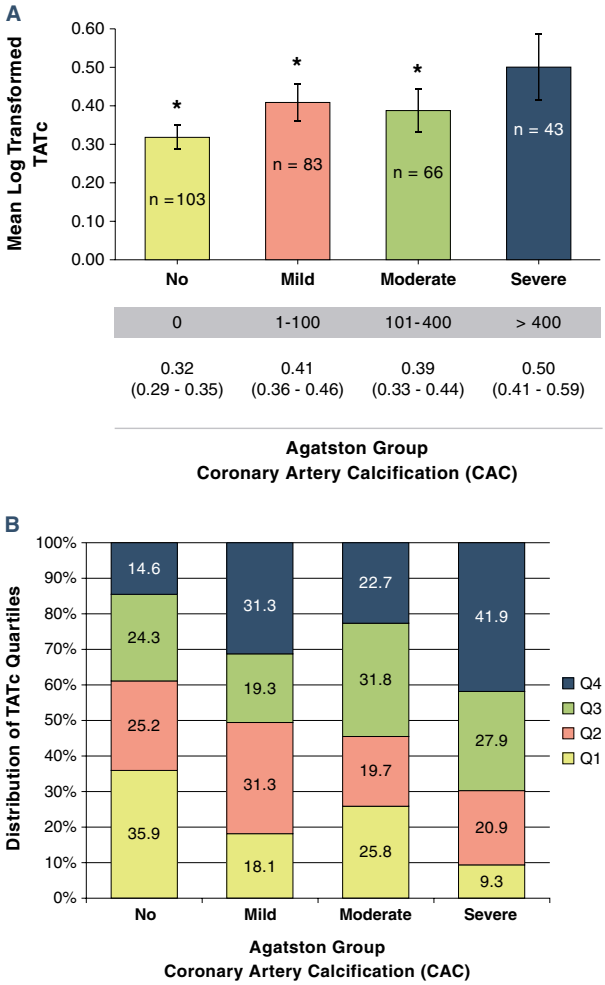

Figure 2. TATc as an Independent Predictor of CAC

(A) Association between gradually increasing thrombin-antithrombin complex (TATc) concentrations and severity of coronary artery calcification (CAC). **Bars** represent lg 10 transformed TATc levels, presented as mean (95% confidence interval), stratified per CAC group. *Statistical significance at the $p < 0.05$ level when compared with the severe CAC group. **(B)** Distribution of TATc quartiles between CAC score groups. Q = quartile.

diagnostic imaging tools like CCTA, magnetic resonance imaging, ultrasound, nuclear imaging tests, or invasive coronary angiography.

The detrimental role of thrombin in atherothrombosis is not a matter of dispute (21). Numerous previously published reports have documented increased rates of thrombin synthesis and blood coagulation activation upon the onset of major adverse cardiovascular events (22–25). However, besides being linked to blood thrombogenicity and determining the magnitude of thrombus formation upon atherosclerotic plaque rupture, thrombin activity per se may be also relevant to the pathophysiology of atherosclerosis progression (3,4). Several research groups have attempted to study the relationship between hypercoagulability and atherosclerosis progression by assessing ankle brachial pressure index in patients with peripheral artery disease (26–28) or evaluating other markers of subclinical atherosclerosis such as carotid intima-media thickness (29,30). Nevertheless, these studies do not provide sufficient insight into this problem due to the very limited potential of ankle brachial pressure index and carotid intima-media thickness techniques to evaluate CAD. To our knowledge, this is the first study to precisely examine the relationship between thrombin generation and the angiographic presence, severity, and calcification of coronary artery plaques by using CCTA in a population with suspected, but not previously established CAD.

Enhanced in vivo thrombin generation during atherogenesis: potential clinical implications. Given the capacity of thrombin to modulate pro-atherogenic actions related to plaque destabilization, it becomes important to define what the clinical implications of these findings may be. Previously, we have demonstrated that early atherosclerotic lesions exert an enhanced pro-coagulant state in comparison to stable advanced ones (5). This phenomenon was partially explained by the increased activity of many key coagulation proteins (including thrombin), but also by the ability of different vascular cell types to synthesize coagulation factors at a local level. There is abundant histopathological and experimental evidence to demonstrate that thrombosis occurs long before an atherosclerotic plaque ruptures, named as subclinical or "buried" thrombosis (8,31,32). The latter is also considered a potential trigger of plaque vulnerability (33–35). Furthermore, blood coagulation is an important component of the host-defense system to fight tissue injury and infection (36). Despite that the exact mechanisms of the enhanced TATc formation in blood during atherosclerosis progression remain unclear to date, one may speculate that inflammation and coagulation operate in a perpetual mode to repair worsening atherosclerosis vascular damage. While oral vitamin K antagonists and antiplatelet therapy remain the cornerstone in primary and secondary prevention therapy against atherothrombosis and reduce cardiovascular mortality by ~30%, numerous clinical trials have failed to account for a clear atheroprotective effect (3). In contrast, a few experimental studies have indicated that administration of direct thrombin inhibitors in atherosclerotic mice substantially inhibits plaque volume and results in plaque stability (7,8). Given the improved safety profile that these novel therapeutic agents show (37,38), it

Table 2. Multivariate Models of Factors Associated With Odds of CAC

Variable	Mild CAC AS 1–100		Moderate CAC AS 100–400		Severe CAC AS >400	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Model 1: "Multinomial Logistic Regression: Main Effects Model"						
Reference (no CAC/AS 0)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Age	1.05 (1.01–1.09)	0.021	1.12 (1.07–1.17)	<0.005	1.15 (1.09–1.22)	<0.005
Gender (male = 0)	2.61 (1.22–5.57)	0.014	3.46 (1.50–8.01)	0.004	6.09 (2.17–17.07)	<0.005
Smoking (yes = 1)	0.71 (0.32–1.53)	0.377	0.51 (0.21–1.22)	0.132	0.34 (0.12–0.95)	0.039
Diabetes mellitus (yes = 1)	1.04 (0.30–3.61)	0.951	0.71 (0.19–2.61)	0.603	0.50 (0.12–2.17)	0.355
Positive family history (yes = 1)	0.60 (0.31–1.16)	0.127	0.42 (0.20–0.88)	0.021	0.41 (0.16–1.01)	0.053
BMI	0.99 (0.91–1.08)	0.788	1.02 (0.93–1.12)	0.687	0.94 (0.83–1.06)	0.329
Total cholesterol	0.88 (0.66–1.15)	0.346	0.96 (0.71–1.29)	0.769	0.84 (0.59–1.21)	0.354
Systolic blood pressure	1.01 (0.99–1.04)	0.219	1.02 (0.99–1.04)	0.130	1.03 (1.00–1.06)	0.051
Diastolic blood pressure	1.00 (0.96–1.03)	0.911	1.00 (0.96–1.04)	0.973	0.99 (0.95–1.04)	0.752
TATc	1.56 (1.14–2.15)	0.006	1.56 (1.13–2.15)	0.007	1.67 (1.21–2.31)	0.002
Model 2: "Multinomial Logistic Regression: Forward Stepwise Model"						
Reference (no CAC/AS 0)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Age	1.05 (1.01–1.09)	0.010	1.12 (1.07–1.17)	<0.005	1.16 (1.10–1.21)	<0.005
Gender (male = 0)	2.45 (1.25–4.81)	0.009	3.14 (1.49–6.61)	<0.005	4.86 (1.98–11.95)	<0.005
Positive family history (yes = 1)	0.59 (0.31–1.11)	0.104	0.40 (0.20–0.82)	0.012	0.40 (0.17–0.93)	0.034
TATc	1.60 (1.18–2.16)	<0.005	1.58 (1.16–2.15)	<0.005	1.71 (1.26–2.33)	<0.005

Statistical significance at the $p < 0.05$ level.
AS = Agatston score; BMI = body mass index; CAC = coronary artery calcification; CI = confidence interval; OR = odds ratio; TATc = thrombin-antithrombin complex.

becomes important to further investigate the effects of selective thrombin inhibition on plaque volume and phenotype determination in patients.

Although the CAT method is meant to determine the potential to generate thrombin in plasma in vitro, the *U*-shaped association between ETP and CAD, which we demonstrate, may also be of physiological relevance. We have previously reported that early atherosclerotic lesions show an increased thrombin generation potential in comparison to stable advanced lesions (5). Since the absence of angiographic CAD does not exclude early-stage morphological changes of the arterial vessel wall (e.g., mild pathological intimal thickening), one may assume that the increased thrombin-forming capacity in the non-CAD group may be due to first signs of atherosclerotic alterations. Thrombin is a central enzyme in the coagulation-inflammation axis and represents a potential therapeutic target via which atherosclerosis might be modulated. Moreover, thrombin is well known for its dual-faceted character in both hemostasis and cell signaling (4). At very low concentrations, thrombin can mediate numerous atheroprotective effects such as endothelial barrier protection, reduction in apoptosis and transendothelial migration of leukocytes, and promote atheroprotective interleukin-10 synthesis (39–41). Some of those actions are dependent on the occupancy of endothelial protein C receptor by its natural ligand protein C/activated protein C. Hence, the net effect of specific long-term thrombin inhibition remains hard to predict.

Other findings. The role of CAC in inducing plaque vulnerability remains controversial. Clinical evidence shows that CAC is associated with coronary vasomotor dysfunction and reduced myocardial perfusion, even in the absence of luminal stenosis (42). Novel concepts of plaque vulnerability propose that atherosclerotic plaque hypoxia may induce angiogenesis, intraplaque hemorrhage, and increased risk for rupture (43,44). Besides serving as a precise indicator of the presence and severity of coronary plaque burden (45), the Agatston score is considered a better predictor of cardiovascular outcomes than the FRS (46). In the current study, we present new evidence indicating higher thrombin generation as an independent determinant of CAC. Despite that the clinical significance of these findings remains to be further investigated, a recent ex vivo human study reports that aortic valve calcification can be induced through increased thrombin generation (47).

Study limitations. First, we performed a single-center study in which all patients were of Western European descent. Second, the patient number was relatively small, which limits the options for analyzing the

Table 3. Multinomial Logistic Regression Models for CAD Severity as the Dependent Variable

Variable	Mild CAD Stenosis <50%		Moderate CAD Stenosis 50–70%		Severe CAD Stenosis >70%	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Model 1: "Multinomial Logistic Regression: Main Effects Model"						
Reference (no CAD [0%])	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Age	1.08 (1.03–1.12)	<0.005	1.09 (1.04–1.13)	<0.005	1.10 (1.05–1.15)	<0.005
Gender (male = 0)	2.50 (1.12–5.55)	0.025	3.02 (1.37–6.69)	0.006	5.70 (2.33–13.94)	<0.005
Smoking (yes = 1)	0.62 (0.26–1.47)	0.276	0.54 (0.23–1.28)	0.161	0.28 (0.12–0.67)	0.004
Diabetes mellitus (yes = 1)	0.69 (0.20–2.36)	0.557	0.82 (0.23–2.89)	0.756	0.98 (0.23–4.28)	0.980
Positive family history (yes = 1)	0.60 (0.30–1.22)	0.160	0.62 (0.31–1.25)	0.177	0.63 (0.30–1.34)	0.226
BMI	0.98 (0.90–1.08)	0.704	1.04 (0.95–1.14)	0.356	1.00 (0.90–1.10)	0.944
Total cholesterol	0.77 (0.57–1.04)	0.093	0.82 (0.61–1.11)	0.198	0.97 (0.72–1.32)	0.861
Systolic blood pressure	1.01 (0.98–1.03)	0.524	1.02 (0.99–1.04)	0.207	1.02 (0.99–1.04)	0.232
Diastolic blood pressure	1.00 (0.96–1.04)	0.939	1.00 (0.97–1.04)	0.870	1.01 (0.97–1.05)	0.745
TATc	1.37 (0.99–1.88)	0.056	1.57 (1.16–2.14)	0.004	1.47 (1.07–2.02)	0.017
Model 2: "Multinomial Logistic Regression: Forward Stepwise Model"						
Reference (no CAD [0%])	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Age	1.08 (1.03–1.12)	<0.005	1.09 (1.05–1.13)	<0.005	1.09 (1.05–1.14)	<0.005
Gender (male = 0)	2.46 (1.20–5.08)	0.014	3.12 (1.52–6.41)	0.002	5.35 (2.38–12.03)	<0.005
Smoking (no = 0)	0.73 (0.32–1.65)	0.443	0.65 (0.29–1.46)	0.296	0.31 (0.14–0.69)	0.004
TATc	1.39 (1.02–1.89)	0.036	1.59 (1.18–2.14)	0.002	1.50 (1.11–2.04)	0.009

Statistical significance at the $p < 0.05$ level.
Abbreviations as in Table 2.

relevance of TATc for predicting follow-up events. Moreover, because the relatively healthy population, we did not found a high rate of cardiovascular events, especially not in the short term, which is in line with other CT studies. Third, while we screened for angiographic CAD, these findings may reflect other existing atherosclerosis settings (carotid or peripheral artery lesions), which we did not assess in this study. Therefore, the association between thrombin formation and atherosclerosis in other vascular beds remain open. Fourth, the purpose of this study was to investigate the association of thrombin formation and CAD and was not meant to unravel this complex causal relationship.

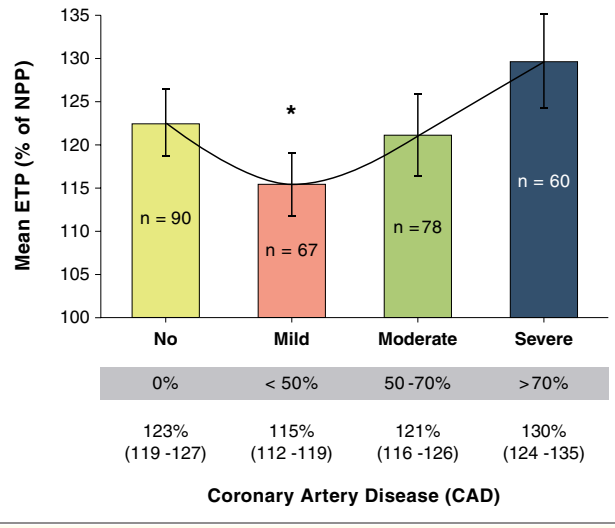

Figure 3. *U*-Shaped Relationship Between ETP and CAD

Bars represent endogenous thrombin potential (ETP) values (percent of normal pool plasma [NPP]), presented as mean (95% confidence interval), stratified according to degree of luminal stenosis. *Statistical significance at the $p < 0.05$ level when compared with the severe coronary artery disease group.

CONCLUSIONS

Thrombin formation is a useful tool in determining the presence and severity of CAD, but more importantly, may be also involved in the pathophysiology of vascular calcification and atherosclerosis progression.

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