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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-