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Full Length Article

Increased plasma thrombin potential is associated with stable coronary artery disease: An angiographically-controlled study

Federica Tosi [a,1], Roberta Micaglio [a], Marco Sandri [a], Annalisa Castagna [a], Diego Minguzzi [a], Filippo Stefanoni [a], Carmela Chiariello [a], Ilaria Franzese [b], Giovanni Battista Luciani [b], Giuseppe Faggian [b], Domenico Girelli [a], Oliviero Olivieri [a], Nicola Martinelli [a,*,1]

[a] Department of Medicine, University of Verona, Verona, Italy
[b] Department of Surgical Sciences, Dentistry, Gynecology and Pediatrics – Division of Cardiac Surgery, University of Verona, Italy

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ABSTRACT

Introduction: Coagulation plays a crucial role in coronary artery disease (CAD) contributing to both atherosclerotic plaque development and acute thrombotic complications, like myocardial infarction (MI). Coagulation biomarkers have been linked with ischemic heart disease, but results are still controversial.
Materials and methods: D-dimer and thrombin generation, two "overall" coagulation assays, were evaluated in 775 subjects with or without angiographically-proven CAD (170 CAD-free and 605 CAD, 355 of whom with history of previous MI). Subjects taking anticoagulant drugs or with any acute illness were excluded. D-dimer plasma concentration was determined by an immuno-turbidimetric assay. Thrombin generation was assessed as the ability of plasma to generate thrombin triggered by the addition of tissue factor *ex-vivo* by means of a chromogenic method.
Results: Both D-dimer and thrombin generation parameters were associated with several traditional cardiovascular risk factors. Lag-time, time-to-peak, peak height, and Endogenous Thrombin Potential (ETP), as well as D-dimer levels, were higher in CAD patients than in CAD-free subjects. After adjustment for all the traditional risk factors, only ETP levels remained significantly associated with CAD (the highest *versus* the lowest tertile: OR 2.61 with 95%CI 1.14–5.99), but without improvement of C-statistic. The association of D-dimer vanished after adjustment for inflammatory markers. No difference of either D-dimer or thrombin generation parameters was found between CAD patients with or without previous MI history.
Conclusions: Our results suggest that an increased plasma thrombin potential is characteristic in patients with clinically stable CAD, irrespective of previous MI history and independent of traditional cardiovascular risk factors.

1. Introduction

Hypercoagulability can predispose to cardiovascular events. Thrombosis superimposed on ruptured or unstable atherosclerotic plaques is the leading cause of myocardial infarction (MI) in coronary artery disease (CAD), which represents one of the major causes of death worldwide [1]. More than a half century ago, the Norwegian physiologist Dedichen coined the suggestive medical aphorism: "Man lives with atherosclerosis but dies of the complicating thrombosis" [2], thus emphasizing the importance of coagulation pathway in the most dramatic and potentially lethal cardiovascular events. But coagulation mechanisms play a role also in the development and progression of atherosclerotic lesions and not only in their acute thrombotic complications. Coagulation factors can trigger non-haemostatic proatherogenic effects (e.g. by activating protease-activated receptors (PARs), which are widely expressed on several vascular cells) [3]. In apolipoprotein E (ApoE)-deficient mice, a well-recognized atherosclerosis-prone model, hypocoagulability due to the lack of coagulation factor VIII ameliorates early-stage atherosclerotic vascular lesions [4]. Mice homozygous for factor V (FV) Leiden crossed with ApoE-deficient strain develop more aortic atherosclerosis compared with FV wild-type/ApoE-deficient mice [5].

On the other hand, studies investigating coagulation biomarkers in ischemic heart disease have led to controversial results so far [6,7]. Plasma levels of coagulation factors appear to have a highly variable and inconsistent predictive value in CAD, especially after adjustment for traditional risk factors [8,9]. Moreover, single factor assays allow a

* Corresponding author at: Department of Medicine, University of Verona, Medicina Interna B, Policlinico "G.B. Rossi", piazzale L.A. Scuro, 37134 Verona, Italy.
E-mail address: nicola.martinelli@univr.it (N. Martinelli).
[1] Equally contributing authors.

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very limited view on the whole and complex pathway of coagulation, in which several different molecules play synergic or opposing roles. Hence there is a growing interest in cardiovascular research on biomarkers reflecting the global activation/potential of coagulation.

D-dimer is one of the most extensively investigated coagulation biomarkers and its role in the diagnostic algorithm of venous thromboembolism is universally recognized. D-dimer is a reliable and sensitive index of fibrin deposition and stabilization, thus being indicative of in vivo thrombus formation at one point in time. However, its plasma concentration may be high in a number of other conditions unrelated to thrombosis, including inflammatory disorders [10]. High plasma concentrations of D-dimer have been shown in patients with CAD, but the clinical significance of such association remains still debated [8].

Thrombin generation is a global coagulation assay, which explores the individual's overall plasma propensity to form a blood clot when triggered by tissue factor exposure ex-vivo. Thrombin generation assays have been tested in both thrombotic conditions and bleeding disorders [11]. Earlier studies showed that parameters derived by thrombin generation analysis may be altered in patients with CAD [12].

The aim of this study was to evaluate both D-dimer plasma concentration and thrombin generation parameters in subjects with or without CAD in the setting of an angiographically-controlled population.

2. Materials and methods

2.1. Study population

This study was performed within the framework of the Verona Heart Study (VHS), a regional survey aimed to search for new risk factors for CAD in subjects with angiographic documentation of their coronary vessels. Details about enrolment criteria have been described elsewhere [13,14]. In brief, adult patients of both sexes who were recruited from those referred to the Institute of Internal Medicine and to the Institute of Cardiovascular Surgery of the University of Verona in Italy between June 1998 and June 2005 and undergoing to a coronary angiography examination. All the subjects in the VHS are required to have no history of any acute illness in the month preceding the enrolment. CAD patients with acute coronary syndrome were excluded from this study. Subjects with severe renal failure (estimated glomerular filtration rate (eGFR) < 30 mL/min) and those with severe hepatic impairment (clinically defined diagnosis of liver cirrhosis) were also excluded from this study. A total of 775 subjects, who were not taking anticoagulant drugs and for whom −80 °C frozen, platelet-poor, citrated plasma samples were available, were included in the present study. One-hundred seventy subjects had completely normal coronary arteries, being submitted to coronary angiography for reasons other than CAD, mainly valvular heart disease (CAD-free group), and serve as controls. These subjects were also required to have neither history nor clinical or instrumental evidence of atherosclerosis in vascular districts beyond the coronary bed. Six-hundred five subjects had angiographically-proven CAD (the majority of them being candidates for coronary artery bypass grafting) with at least one of major epicardial coronary arteries (left anterior descending, circumflex, and right) affected with ≥1 significant stenosis (≥50% lumen reduction). According to the hypothesis to be tested, subjects with non-advanced CAD (i.e. coronary stenosis <50%) were not included in the study. The angiograms were assessed by cardiologists who were unaware that the patients were to be included in the study.

Subjects with CAD were classified into MI and non-MI subgroups by combining data from history with a thorough review of medical records showing diagnostic electrocardiogram and enzyme changes, and/or the typical sequelae of MI on ventricular angiography. A clear-cut definition of previous MI history was obtained for 603/605 (99.7%) CAD patients.

All participants came from the same geographical area (Northern Italy). The study was approved by the Ethic Committee of our Institution (Azienda Ospedaliera Universitaria Integrata, Verona, Italy) and has been carried out according to the Declaration of Helsinki. A written informed consent was obtained from all the participants after a full explanation of the study.

2.2. Biochemical analysis

Samples of venous blood were drawn from each subject, after an overnight fast, at time of enrolment before coronary angiography. Serum lipids, as well as other CAD risk factors, including high sensitivity-C reactive protein (hs-CRP), were determined at time of enrolment as previously described [13,14]. The four variable version of the Modification of Diet in Renal Disease (MDRD) equation was used to estimate the glomerular filtration rate (eGFR) from serum creatinine levels [15].

2.3. D-dimer and thrombin generation assays

D-dimer and thrombin generation were assessed on −80 °C frozen citrated plasma samples, never thawed before this study.

Venous blood samples collected at the time of enrolment were centrifuged for 15 min at 2500*g*, stored in 0.5 mL aliquots and frozen at −80 °C within 1 h of sample collection. Subsequently, when thawed out, plasma samples were centrifuged once more for 15 min at 1500*g*.

D-dimer was assessed by means of a particle-enhanced, immunoturbidimetric assay, using the monoclonal antibody 8D3 (INNOVANCE D-Dimer assay, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany).

Thrombin generation assay was determined using the INNOVANCE Endogenous Thrombin Potential (ETP) Assay (*For Research use only*, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) according to the manufacturer's instructions. As compared with the classical fluorogenic method of calibrated automated thrombogram (CAT) developed by Hemker, INNOVANCE ETP is an automated chromogenic assay. The conversion kinetic of a synthetic thrombin substrate was measured by the release of a chromophore in platelet-poor plasma sample at a wavelength of 405 nm. The thrombin formation was started by the addition of recombinant tissue factor and undefined phospholipids (Dade® Innovin® Reagent, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) and calcium chloride. The tissue factor concentrations are treated confidentially by the manufacturer. However, the final tissue factor concentration during the measurements was 5 pM as previously described [16]. Four parameters of thrombin generation have been derived from the thrombin generation curve: i) lag time, ii) time to peak, iii) peak height, and iv) Endogenous Thrombin Potential (ETP), whose value was derived from the area under the thrombin generation curve. For calibration a ETP Standard was used, i.e. a normal plasma pool from selected healthy donors, which has been delipidated and subsequently calibrated for % of norm and U/mL units of factor II (these refer to fresh human citrated pool plasma that exhibits 100% of the norm or 1 U/mL for all factors by definition).

All testing was performed in duplicate. The intra-assay and inter-assay coefficients of variations were <5%.

2.4. Statistics

All calculations were performed using the IBM SPSS 20.0 (IBM Inc., Armonk, NY), Stata 13 (StataCorp. 2013. Stata Statistical Software: Release 13. StataCorp LP, College Station, TX), and R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria, 2015. https://www.R-project.org/ - accessed at January 2016) statistical packages.

Distributions of continuous variables in groups were expressed as means ± standard deviation. Skewed variables (i.e. triglyceride, hs-CRP, fibrinogen, D-dimer, lag time, and time to peak) have been logarithmically transformed and geometric mean with 95% confidence interval (95%CI) were reported. Quantitative data were assessed using the Student's *t*-test. Correlations between quantitative variables were assessed using Pearson's correlation test. Qualitative data were analysed with the χ^2-test and with χ^2 for linear trend analysis when indicated.