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Full Length Article

Residual thrombin potential predicts cardiovascular death in acute coronary syndrome patients undergoing percutaneous coronary intervention

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ABSTRACT

Introduction: Thrombin generation (TG) is a central step of the coagulation system involved in hemostatic and thrombotic roles. Scarce data evaluating in the acute phase the association between TG and the risk of cardiovascular death of acute coronary syndrome (ACS) patients are available, in the era of percutaneous coronary intervention (PCI) and stenting with the use of dual antiplatelet treatment.

Materials and methods: We investigated TG in 292 ACS patients undergoing PCI with stent implantation on dual antiplatelet treatment. Venous samples were obtained 12–24 h after PCI. TG was assessed using the Calibrated Automated Thrombogram (CAT).

Results: At two years of follow-up, 57 out of 292 patients (19.5%) died from cardiovascular causes. Higher values of endogenous thrombin potential (ETP) [1115.9 (705–1441.3) *vs* 940.2 (666.0–1253.1), $p = 0.049$], peak [176.1 (80.5–259.4) *vs* 107.3 (59.9–181.1), $p = 0.002$] and velocity index [61.75 (21.03–97.88) *vs* 25.64 (11.95–50.90), $p < 0.001$] were observed in relation to survival patients. At the multivariate model adjusted for the Global Registry of Acute Coronary Events risk score, the association between TG and cardiovascular death remained significant for ETP [OR (95% CI): 2.58 (1.10–6.03), $p = 0.029$], peak [OR (95%CI): 3.27 (1.35–7.92), $p = 0.009$] and velocity index [OR (95% CI): 3.06 (1.27–7.39), $p = 0.013$]. This result was confirmed after adjustment for high on-treatment platelet reactivity [ETP: OR (95% CI) 2.35 (1.11–5.00), $p = 0.027$; peak: OR (95% CI) 2.42 (1.13–5.15), $p = 0.022$; velocity index: OR (95% CI) 2.43 (1.14–5.20), $p = 0.022$].

Conclusions: ACS patients with a residual TG after PCI and stent implantation have a significantly higher risk of long-term cardiovascular death. These results might be useful in improving risk stratification for ACS patients and support the need of a tailored antithrombotic therapy.

1. Introduction

The most common underlying mechanism of acute coronary syndrome (ACS) is thrombus development on a damaged atherosclerotic plaque. Thrombin is a serine proteinase and is involved in hemostatic and thrombotic roles, from promotion and inhibition of clotting, to fibrinolysis, platelet activation, and inflammation. In the past, biochemical signs of coagulation activation in the acute phase of unstable angina or acute myocardial infarction (AMI) were demonstrated [1,2] and blood clotting activation markers were found to be independent risk factors for recurrence and long-term mortality [3–6]. In the last years the diffuse use of PCI and stenting stimulated several studies investigating primarily the role of platelet hyper-reactivity in determining clinical outcome [7,8]. On the other hand, scarce data are available on the possible role of blood clotting activation on the prognosis of ACS patients, in the era of PCI and stenting on the top of dual antiplatelet therapy. In 2011 in a small group of AMI patients the risk of recurrent ischemic cardiovascular complications was evaluated in relation to thrombin

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generation, but the low percentage of primary endpoints did not allow to obtain clear results [9]. In 2014 Schneider et al. investigated TG in a non-homogeneous cohort of CAD patients (unstable and stable coronary artery disease), performing the biohumoral evaluation in a large time interval from the index event, found an inverse association between TG and cardiovascular death [10]. Aim of our study was to evaluate the possible association between cardiovascular death and the entity of thrombin generation measured in the acute phase of ACS in patients undergone PCI and stenting. We measured thrombin generation (TG) by the Calibrated Automated Thrombogram (CAT) assay, which offers a global assessment of coagulation [11]. Residual platelet reactivity was also evaluated in relation to the risk of cardiovascular death.

2. Methods

The study population comprised a group of 421 patients discharged from the Coronary Unit of the Careggi University Hospital (Florence, Italy) from April 2008 to April 2010 with a diagnosis of acute coronary syndrome (ACS) and enrolled in the frame of the Florence Acute Myocardial Infarction-2 (AMI-Florence 2) registry [12]. Fifty patients were excluded as they interrupted dual antiplatelet therapy for major surgery (due to cardiovascular or liver disease or cancer) during the follow-up in order to avoid the inclusion of ischemic events possibly occurring for the therapy interruption. Sixty-six patients were lost at follow-up and 13 patients were excluded as they were on oral anticoagulant treatment. Therefore, the final study population included 292 ACS patients undergone PCI with stent implantation (155 ST-elevation myocardial infarction [STEMI]; 137 NSTEMI/Unstable Angina). In all these patients dual antiplatelet therapy was continued for 12 months. ACS was diagnosed according to criteria established by the European Society of Cardiology [13]. Unstable angina was defined as a history of new-onset, more frequent, more persistent or rest episode of chest pain, without typical changes of myocardial enzymes and with electrocardiogram evidence of myocardial ischemia (transient ST segment displacement > 0.1 mV during chest pain).

All patients underwent coronary angiography performed by the Judkins' technique and percutaneous coronary intervention (PCI). Before PCI, all patients received a loading dose of 500 mg of acetylsalicylic acid (ASA) and 600 mg of clopidogrel, followed by 100/325 mg of ASA daily and 75 mg of clopidogrel daily. During PCI, standard activated clotting time (ACT)-guided doses of intravenous unfractioned heparin (UFH) were used (ACT: 250–300 s) after an 85 U/kg bolus of UFH.

Current smoking status was determined at the time of blood collection. The subjects were classified as having hypertension according to the guidelines of European Society of Hypertension/European Society of Cardiology [14] or if they reported taking antihypertensive medications, as verified by the physician. Diabetic subjects were defined in agreement with the American Diabetes Association [15] or on the basis of self-report data (if confirmed by medication or chart review). Dyslipidemia was defined according to the Third report of the National Cholesterol Education Program (NCEP-III) [16] or if they reported taking antidyslipidemic drugs, as verified by the physician. A positive family history was defined as the presence of at least one first-degree relative who had developed coronary artery disease (CAD) before the age of 55 years for men and 65 years for women. All subjects gave informed consent; the study complies with the Declaration of Helsinki and was approved by the local ethic committee.

Venous blood samples anticoagulated with 0.109 M sodium citrate were taken between 12 and 24 h after PCI. Platelet poor plasma for the assessment of thrombin generation was obtained by double centrifugation at 2000 × *g* for 15 min at room temperature and stored at − 80 °C until analysis.

As regard Calibrated Automated Thrombogram (CAT) assessment, we used the method described by Hemker and coworkers [17] and commercialized by Thrombinoscope BV, (Maastricht, the Netherlands). According to the manufacturer's instructions, measurements were conducted in 80 μl of platelet poor plasma (PPP) triggered by 20 μl PPP-reagent (tissue factor 5 pmol/l) in 96-well microtiter plates. Measurements were calibrated against the fluorescence curve obtained in the same plasma mixed with 20 μl Thrombin Calibrator (Thrombinoscope BV). Fluorogenic substrate (20 μl) were added to sample and calibrator wells and the fluorescence intensity was detected in a Fluoroskan Ascent reader (Thermo Labsystems OY, Helsinki, Finland) with a 390/460 filter set for 60 min and the TG curves were calculated with Thrombinoscope software (Thrombinoscope BV). TG was expressed as endogenous thrombin potential (ETP: the area under the curve that represents the total amount of thrombin generated), peak (the maximum concentration of thrombin produced), and velocity index (the slope between the start of thrombin formation and the peak). To rule out any "contamination" of unfractioned heparin (UFH), anti-Xa levels were determined in all samples. Only samples with undetectable anti-Xa (<0.05 U ml) were considered to be free of UFH and only these samples were included in the analysis.

To assess platelet reactivity, platelet-rich plasma, obtained by centrifuging whole blood for 10 min at 200*g*, was stimulated with 10 μM adenosine diphosphate (ADP; Mascia Brunelli, Milan, Italy), and with 1 mM arachidonic acid (AA; Sigma-Aldrich, Milan, Italy) and aggregation was assessed using a APACT 4 light transmission aggregometer (Helena Laboratories, Milan, Italy) as previously reported [18]. We defined patients with high platelet reactivity (HPR) those with platelet aggregation by AA ≥ 20% and/or by ADP ≥ 70% [19–22].

All data were prospectively collected and entered into a central database. Clinical follow-up information was obtained by contacting all the patients. All possible information derived also from hospital readmission or by referring physician, relatives, or municipality live registries were entered into the prospective database. All deaths were considered cardiac unless an unequivocal non cardiac cause could be documented.

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) software for Windows (Version 21.0). Values are presented as median and interquartile range (IQR). The Mann-Whitney test for unpaired data was used for comparison between two groups. Dichotomous variables were compared by Chi^2 test. A receiver operating characteristic curve (ROC) analysis was used to determine the ability of TG parameters in predicting cardiovascular death at 24 months. The optimal cut-off point was calculated by determining the TG parameters that provided the greatest sum of sensitivity and specificity. We defined patients with residual TG those with ETP ≥ 1027.2 nM/min and/or peak ≥ 139.28 nM and/or velocity index ≥ 35.7 nmol/min. Cumulative survival curves were constructed by the Kaplan Meier method, and the log-rank test was used to assess statistical differences between survival curves. In order to test the independent association between TG parameters and cardiovascular death, we performed a multiple logistic regression analysis adjusted for an established risk score which has been extensively validated such as the Global Registry of Acute Coronary Events (GRACE) risk score (which includes age, heart rate, systolic blood pressure, creatinine, Killip class, elevated cardiac enzymes, ST-segment deviation, cardiac arrest at admission). All odds ratios (OR) are given with their 95% confidence interval (CI). $p < 0.05$ was considered to be statistically significant.

3. Results

We investigated thrombin generation in 292 (217 men/75 women) ACS patients (155 ST-elevation myocardial infarction [STEMI]; 137 NSTEMI/Unstable Angina).

TG parameters did not significantly differ in relation to age, sex, smoking habit, hypertension, dyslipidemia, diabetes and leukocyte number (data not shown). Higher values of peak (192.21; IQR 141.73–249.35) and velocity index (59.58; IQR 42.22–100.89) were found in ACS patients with renal failure respect to those without renal failure [peak 119.33 (IQR 61.21–200.42); velocity index 30.62 (IQR 12.62–