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Full Length Article

A time course study of high on treatment platelet reactivity in acute coronary syndrome male patients on dual antiplatelet therapy

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ABSTRACT

Introduction: Limited data are available on the natural history of high on treatment platelet reactivity (HPR) by arachidonic acid and ADP - markers of unfavorable prognosis in acute coronary syndrome patients -.
Material and methods: In a cohort of acute coronary syndrome male patients (n = 101), we evaluated the time-course of HPR by ADP (platelet aggregation by 10 μM ADP ≥ 70%) and arachidonic acid (platelet aggregation by 1 mmol arachidonic acid ≥ 20%) measuring platelet function in the acute phase (T0), at 6 months (T1) and 1 year (T2).
Results: We identified *persistent* (HPR at T0,T1 and T2), *acute non persistent* (HPR only at T0), and *late* (HPR only at T1 or T2). Patients with *persistent* HPR by ADP were more frequently with higher values of BMI. Patients carrying CYP2C19*2 variant were more prevalent in the group of *persistent* HPR (33%). Significant higher values of immature platelet fraction and high immature platelet fraction at 6 and 12 months and mean platelet volume were present in patients with *late* HPR. Immature platelet fraction was the only variable significantly associated with late HPR by ADP at multivariate analysis (OR = 1.6 (1.08-2.3), p = 0.016). Patients with *persistent* HPR by arachidonic acid were more frequently diabetics. Immature platelet fraction at 6 months and high immature platelet fraction at 6 and 12 months were the parameters associated with late HPR by AA (OR = 1.4 (1.0-1.9), p = 0.036; OR = 1.5 (1.08-2.4), p = 0.05; OR = 4.9 (1.3-18.8), p = 0.018, respectively).
Conclusions: About 25% of 101 patients has *persistent* HPR; they are more frequently diabetics, overweight or carriers of CYP2C19*2. The occurrence of an inflammatory state, indicated by the increase of immature platelet fraction, is associated with the occurrence of *late* HPR.

1. Introduction

During the last years, a number of studies described the crucial role of platelet activation and aggregation in thrombus formation in patients with acute coronary syndromes (ACS) [1].

In large clinical trials, adverse vascular events remain a serious clinical problem that occurs in a significant proportion of ACS patients despite of, the wide use of the antiplatelet therapy and the significant benefits reported with combined antiplatelet treatment (clopidogrel and aspirin) [2–4].

During the acute phase of coronary artery disease, a growing body of evidence has demonstrated a higher prevalence of high on-treatment platelet reactivity (HPR) on antiplatelet therapy and several mechanisms leading to HPR involve genetic, clinical and cellular factors [5].

It is well documented that HPR, in ACS patients on dual antiplatelet treatment, has a relevant clinical role because of it is significantly associated with an increased risk of cardiovascular events [6].

Limited data, in unstable and stable patients are available in the literature, on the prevalence of HPR by ADP - which reflects the so-called clopidogrel non –responsiveness, during the follow-up period after percutaneous coronary intervention (PCI) [7,8]. Whereas no data are available on the prevalence of HPR by arachidonic acid (AA) in both stable and unstable patients after PCI and on the factors associated with the HPR throughout the late phase of ACS.

To provide this lack of information we evaluated the time-course of HPR by ADP and AA and its association with clinical and laboratory characteristics during 1 year of follow-up from the acute event of a cohort of ACS male patients receiving drug-eluting stent or bare-metal stent on dual antiplatelet treatment.

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2. Methods

2.1. Study Population

The study population consisted of a group of 157 male patients discharged from the Coronary Unit of Careggi University Hospital, Florence, Italy from April 2008 to April 2009 and enrolled in the frame of the Florence Acute Myocardial Infarction-2 (AMI-Florence 2) registry. Twenty-two patients were excluded as they interrupted dual antiplatelet therapy for major surgery. Thirty-four patients dropped out from the program. Therefore the final study population included 101 patients.

The AMI-Florence 2 registry is a second-wave survey of the AMI-Florence registry; it included all patients who arrived alive, between April 2008 and April 2009, to the emergency departments of one of the six participating hospitals in the Florence health district with a suspected ACS [9]. Acute coronary syndromes was diagnosed according to criteria established by the European Society of Cardiology [10]. Briefly: acute myocardial infarction (MI) was defined as typical rise and gradual fall of troponin, or more rapid rise and fall of CK-MB, defined as > 99% of normal levels (troponin T >0.05 ng/ml; CK-MB >10 ng/ml), with at least one of the following: acute onset of typical ischaemic chest pain; some Q waves in V1-V3, 30 ms Q waves ≥1 mm in two contiguous leads; ST- segment elevation or depression in ≥ 2 leads, ≥ 0.2 mV in V1-V3, >0.1 mV in other leads. 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 ECG evidence of myocardial ischaemia (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 300 mg of clopidogrel, followed by 100 (15.9%) or 325 mg (84.1%) of ASA daily and 75 mg of clopidogrel daily. Unfractioned heparin 70 IU/kg was used in all patients during PCI as anticoagulant.

A clinical follow-up was performed in all patients at 6 and 12 months. 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 [11] or if they reported taking antihypertensive medications, as verified by the physician. Diabetic subjects were defined in agreement with the American Diabetes Association [12] or on the basis of self-report data if confirmed by medication or chart review. Dyslipidaemia was defined according to the Third report of the National Cholesterol Education Program (NCEP-III) [13] or if they reported taking antidyslipidaemic 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.

On the day of examination patients took their clopidogrel medication in the morning before blood sampling and their acetylsalicylic acid medication in the afternoon. Antiplatelet therapy with aspirin and clopidogrel remained unmodified during the entire follow-up for all patients.

All subjects gave informed consent; the study complies with the Declaration of Helsinki and was approved by the local ethic committee.

2.2. Blood Collection

Venous blood samples anticoagulated with 0.109 M sodium citrate for platelet aggregometry were taken at baseline within 24–48 hours (h) from clopidogrel and acetylsalicylic acid loading dose (T0), and after 6 (T1) and 12 (T2) months. To minimize the effect of anticoagulant on platelets, all measurements were performed within 2 h after blood collection.

2.3. Light Transmission Aggregometry Measurement

Turbidimetric platelet aggregation (PA) was used to measure agonist-induced PA. Citrated whole blood samples were centrifuged for 10 minutes (min) at 250 x g to obtain platelet-rich plasma (PRP). Platelet-rich plasma was stimulated with 10 μM ADP (Mascia Brunelli, Milan,Italy) and with 1 mmol AA (Sigma-Aldrich,Milan, Italy) using an APACT 4 aggregometer (Helena Laboratories Italia S.P.A, Milan, Italy). Platelet aggregation was evaluated, according to Born's method, considering the maximal percentage of platelet aggregation in response to different stimuli (ADP-PA and AA-PA) after 10 min. High on-treatment platelet reactivity by ADP or AA was diagnosed in the presence of ADP-PA ≥ 70% and AA-PA ≥ 20% respectively. We defined patients with dual HPR those patients with PA induced by AA ≥ 20% and by ADP ≥ 70% according to the literature [14] and studies from our group [15,16].

2.4. Reticulated Platelets

A fully automated method has been developed that uses blood cell counter for the quantification of reticulated platelets (RP) [17]. Reticulated platelets were measured by using the Sysmex XE-2100 haematology analyser (Sysmex, Kobe, Japan). Briefly, the flow cytometric determination of RP uses a proprietary fluorescent dye containing polymethine and oxazine. These two dyes penetrate the cell membrane staining the mRNA in RP. The stained cells were passed through a semiconductor diode laser and the resulting forward scatter light and fluorescent intensity were measured. A computer algorithm (Sysmex IPF Master) applies a pre-set gate to separate mature platelets (blue dots) and RP (green dots). Reticulated platelets were expressed as a percentage (%) of the total optical platelet count (immature platelet fraction; IPF). The immature platelet fraction indicates the rate of platelet production. Reticulated platelets were also expressed as the percentage of platelets, within the immature platelet fraction, with a major amount of highly fluorescent m-RNA (highly fluorescent immature platelet fraction; H-IPF). The average coefficients of variation (CV) for IPF and H-IPF were 10.6% and 18.8%, respectively.

Table 1
Characteristics of the Study Population.

	Baseline (T0) Male, (n = 101)
Age, median and IQR (y)	67 (58–74)
BMI, median and IQR (Kg/m^2)	26 (24–26)
Diabetes, n (%)	21 (21)
Hypertension, n (%)	64 (63)
Hypercholesterolemia, n (%)	59 (58)
Hypertriglyceridemia, n (%)	33 (33)
Cigarette smoking, n (%)	41 (41)
Familiary history for CAD, n (%)	48 (48)
Prior PCI, n (%)	17 (17)
Prior CABG, n (%)	8 (8)
Prior ACS, n (%)	24 (24)
Admission for NSTEMI/UA, n (%)	45 (45)
Admission for STEMI, n (%)	56 (55)
PCI, n (%)	87 (86)
DES, n (%)	63 (62)
BMS, n (%)	21 (21)
CABG, n (%)	9 (9)
CYP2C19*2 +/−, n (%)	25 (25)
PA-ADP 10 μM, median and IQR (%)	52 (36–64)
PA-AA 1 mmol, median and IQR (%)	13 (9–16)

IQR = interquartile range, BMI = body mass index, CAD = coronary artery disease, PCI = percutaneous coronary intervention, DES = drug eluting stent, BMS = bare metal stent, CABG = coronary artery bypass graft, ACS = acute coronary syndrome, NSTEMI = non-ST-segment elevation myocardial infarction, STEMI = ST-segment elevation myocardial infarction, UA = unstable angina, CYP2C19*2 = Cytochrome P450 2C19, PA-ADP 10 μM = platelet aggregation by ADP 10 μM, PA-AA 1 mM = platelet aggregation by arachidonic acid.