ELSEVIER

Atherosclerosis 195 (2007) e217–e223

ATHEROSCLEROSIS

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Residual platelet reactivity is associated with clinical and laboratory characteristics in patients with ischemic heart disease undergoing PCI on dual antiplatelet therapy

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Received 5 February 2007; received in revised form 17 April 2007; accepted 27 April 2007
Available online 6 June 2007

Abstract

A residual platelet reactivity (RPR) on antiplatelet therapy in patients with ischemic heart disease (IHD) has been reported to be associated with adverse clinical events by some Authors. However, scarce data are present on the clinical parameters associated with this phenomenon. No study, at our knowledge, was designed with the specific aim to examine the relationship between clinical characteristics and RPR. We sought to evaluate the clinical and laboratory characteristics associated with RPR in patients with IHD undergoing coronary revascularization on dual (aspirin plus clopidogrel) antiplatelet therapy. We included in the study 868 patients undergoing a coronary angiography: 386 with acute coronary syndromes undergoing a primary coronary revascularization and 482 IHD patients scheduled to undergo an elective coronary angiography. We measured platelet function by both platelet aggregation with two agonists [0.5 mg/mL arachidonic acid (AA); 2 and 10 μM adenosine 5′-diphosphate (ADP)] and a point-of-care assay (PFA-100) on venous blood samples collected within 24 h from the end of the procedure.

In patients with acute coronary syndromes and elective PCI diabetes is independently associated with RPR [group A: OR = 2.9 (1.5–5.7) by 10 μM ADP, OR = 5.3 (1.1–27.8) by PFA-100; group B: OR = 4.0 (1.6–10.0) by 10 μM ADP]; reduced left ventricular systolic function [OR = 3.7 (2.2–6.5) by AA-PA, OR = 2.7 (1.6–4.6) by PFA-100], chronic use of aspirin [OR = 0.2 (0.1–0.4) by AA-PA, OR = 0.3 (0.2–0.5) by PFA-100] and loading dose of clopidogrel [OR = 0.2 (0.06–0.5) by 10 μM ADP] were independent variables significantly associated with RPR in patients undergoing elective PCI. In addition, inflammatory status was found to be significantly associated with RPR in both groups of patients. These results provide indications for the selection of patients for whom the evaluation of platelet reactivity could be useful.

Keywords: Aspirin resistance; Clopidogrel resistance; Diabetes; Heart failure; Inflammation

1. Introduction

Antiplatelet therapy in cardiovascular medicine was reported to reduce the risk of cardiovascular events by about 25% in a broad category of patients with arterial vascular disease [1]. Over the last years, a great attention has been focused on the phenomenon of antiplatelet resistance which may be defined as a failure of therapy to prevent

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0021-9150/$ – see front matter © 2007 Published by Elsevier Ireland Ltd.
doi:10.1016/j.atherosclerosis.2007.04.048

clinical events (i.e. clinical antiplatelet resistance) or as a failure of aspirin to inhibit platelet function in 'in vitro' tests (i.e. laboratory antiplatelet resistance) [2]. A number of studies documented the prevalence of laboratory aspirin [3–7] or clopidogrel resistance in different groups of patients with ischemic heart disease (IHD) [8–10] and some studies reported the association with clinical end-points [11–20], but the clinical parameters associated with antiplatelet resistance have not yet been defined.

We sought to evaluate the clinical characteristics associated with a residual platelet reactivity (RPR) in patients with IHD undergoing percutaneous coronary intervention (PCI) on dual antiplatelet therapy.

Thus, we measured platelet function by both platelet aggregation with two agonists (0.5 mg/mL arachidonic acid; 2 and 10 μM ADP) and a point-of-care assay (PFA-100) on venous blood samples collected within 24 h from the end of the procedure.

We found that diabetes, left ventricular systolic function, chronic use of aspirin, loading dose of clopidogrel and inflammatory status were significantly associated with platelet reactivity in patients undergoing primary or elective PCI.

2. Material and methods

2.1. Study population

A total of 868 patients with coronary artery disease and admitted to the Division of Cardiology and the Coronary Care Units of the Azienda Ospedaliero-Universitaria Careggi were included: 386 with acute coronary syndromes (ACS) (GROUP A) undergoing a primary PCI (STEMI $n = 189$; NSTEMI $n = 197$) and 482 coronary artery disease patients (GROUP B) scheduled to undergo an elective PCI (385 with ACS in the previous 6–12 months; 97 with stable angina. Ninety-one patients had had a previous coronary bypass surgery). All patients with ACS undergoing primary PCI received a clopidogrel loading dose (93 received 600 mg and 293 received 300 mg) followed by a daily dose of 75 mg. One hundred and fifty-three out of 482 patients undergoing an elective PCI received a clopidogrel loading dose of 600 mg. All patients received unfractioned heparin 70 IU/kg during the procedure and acetylsalicylic acid (i.v. 500 mg) followed by a daily dose of 100–325 mg by oral route. Three-hundred and eighty-seven patients were on aspirin and 209 on clopidogrel for at least 1 month before the index procedure.

Acute MI was diagnosed as an increase in creatine kinase MB isoenzyme at least twice the upper normal limits (3.6 ng/mL), and/or elevated cardiac Troponin I (cTnI) (>0.15 ng/mL) levels with at least one of the following: acute onset of prolonged (≥20 min) typical ischemic chest pain; ST-segment elevation of at least 1 mm in two or more contiguous electrocardiographic leads or ST-depression of ≥0.5 mm, 0.08 s after the J point in ≥2 contiguous leads, or T waves inversion >1 mm in leads with predominant R waves. All patients underwent coronary angiography performed by the Judkins' technique and PCI if indicated.

Patients were considered to have hypertension if they had been diagnosed as hypertensives according to the guidelines of European Society of Hypertension/European Society of Cardiology [21] or were taking antihypertensive drugs. Dyslipidemia was defined according to the Third report of the National Cholesterol Education Program (NCEP III) [22] and diabetes in agreement with the American Diabetes Association [23].

The exclusion criteria included history of bleeding diathesis, platelet count ≤ 100,000/mm^3, hematocrit 30%, creatinine ≥ 4.0 mg/dL, and glycoprotein (Gp) IIb/IIIa inhibitors use.

Informed written consent was obtained from all patients and the study was approved by the local Ethical Review Board.

2.2. Experimental procedure

Venous blood samples anticoagulated with 0.129 M sodium citrate (ratio 9:1) were taken from each patient after PCI (15–24 h). The PFA-100 device (Dade-Behring®) was used to measure platelet function at high shear conditions on whole citrated blood. The method determines the time to occlusion of an aperture in a membrane coated with collagen and epinephrine (CT/EPI). Two hundred control samples were run to determine normal reference laboratory values [CT/EPI: mean ± S.D. 144.1 ± 29.5; median (range) 142 (89–204) s]. The coefficient of variation was 8.4% [7].

Whole-blood specimens were centrifuged for 10 min at 250 × *g* to obtain platelet-rich plasma (PRP). After transferring into plastic tubes, the remaining blood was centrifuged at 3000 × *g* for 3 min to obtain platelet poor plasma (PPP). PRP was stimulated with 2 and 10 μM ADP (Mascia Brunelli, Milan, Italy) and with 0.5 mg/mL arachidonic acid (AA) (Sigma–Aldrich, Milan, Italy) using a APACT 4 aggregometer (Helena Laboratories Italia s.p.a., Milan, Italy). The 100% line was set using PPP and the 0% baseline established with PRP (adjusted from 18×10^9/L up to 30×10^9/L). As previously described, platelet aggregation (PA) (according to Born's method) was evaluated considering the maximal percentage of platelet aggregation in response to different stimuli (ADP-PA and AA-PA) after 10 min. The coefficient of variation of AA-LTA and ADP-LTA were 5.8% and 6.8% respectively [7].

2.3. Residual platelet reactivity

We defined patients with 'residual platelet reactivity' (RPR) those with platelet aggregation by AA > 20% [11] and/or ADP (2 and 10 μmol) above 70% [24,25] and/or those with values of CT/EPI by PFA-100 below the 90th percentile of controls (203 s).

2.4. Statistical analysis

Statistical analysis was performed with SPSS (Statistical Package for Social Sciences, Chicago, USA) software for Windows (Version 11.5). Values are presented either as geometric mean (95% confidence intervals). As the parameters investigated had a non-gaussian distribution, log-transformed values for leukocyte count and erythrocyte sedimentation rate (ESR) were used in the analyses, and back transformed for data presentation. The chi-square test for unpaired data was used for comparisons between single groups. To perform the multivariate analysis, logistic regression was used with RPR as the dependent variable and with the parameters differently ($p < 0.20$) distributed between patients with and without RPR as the independent variables. Clinical characteristics were included in the logistic regression analysis in a model in which each routinely laboratory parameter (leukocytes, ESR) was added separately as continuous variable. All odds ratios (OR) are given with their 95% confidence interval. All probability values are two-tailed, with values of less than 0.05 considered statistically significant.

3. Results

3.1. Residual platelet reactivity by arachidonic acid induced aggregation (AA-PA)

GROUP A: RPR was found in 163/386 (42.2%) patients and was significantly ($p < 0.05$) more prevalent in STEMI (93/189 = 49.2%) patients with respect to NSTEMI (70/197 = 35.5%).

Age, diabetes, percentage of patients receiving 100 or 325 mg/die of aspirin, leukocyte count and ESR were significantly different between patients with RPR and patients without RPR (Table 1).

At the logistic regression analysis, adjusted for all potential confounders, leukocyte count and ESR were significant and independent predictors of RPR detected by AA-PA (Fig. 1).

GROUP B: RPR was found in 110/482 (22.8%) patients (Table 1).

Age, diabetes, chronic use of aspirin, percentage of patients receiving 100 or 325 mg/die, leukocyte count and ESR were significantly different between patients with RPR and patients without RPR (Table 1).

At the logistic regression analysis, adjusted for all potential confounders, reduced left ventricular systolic function, chronic use of aspirin and ESR were significant and independent predictors of RPR detected by AA-PA (Fig. 2).

3.2. Residual platelet reactivity by aggregation induced by ADP

As the 54 patients found with RPR by 2 μM ADP had RPR also by 10 μM ADP, we report below the results obtained with 10 μM ADP.

GROUP A: RPR was found in 99/386 (25.6%) patients and was significantly ($p < 0.001$) more prevalent in STEMI (61/189 = 32.2 %) patients with respect to NSTEMI (38/197 = 19.2%).

Diabetes, reduced left ventricular systolic function (EF ≤ 40%), percentage of patients treated with a loading dose of 600 mg, leukocyte count and ESR significantly differed according to RPR (Table 2).

At the logistic regression analysis, adjusted for all potential confounders, diabetes and leukocyte count were significant and independent predictors of RPR detected by 10 μM ADP-PA (Fig. 1).

GROUP B: RPR was found in 93/482 (19.2%) patients (Table 2). Diabetes, dyslipidemia, reduced left ventricular systolic function (EF ≤ 40%), percentage of patients on clopidogrel treatment prior to the index procedure, leukocyte count and ESR significantly differed according to RPR (Table 2).

Table 1
Clinical and laboratory characteristics according to platelet reactivity by AA-PA

	GROUP A ($n = 386$)		*p*-value	GROUP B ($n = 482$)		*p*-value
	With RPR ($n = 163$)	Without RPR ($n = 223$)		With RPR ($n = 110$)	Without RPR ($n = 372$)	
Age	70.2 (68.2–72.1)	66.8 (65.1–68.5)	*0.001*	69.5 (67.6–71.5)	67.2 (66.1–68.2)	*0.003*
Sex (M/F)	110/53	166/57	*ns*	87/23	281/91	*ns*
Smoking habit (%)	44.2	49.5	*ns*	47.1	45.6	*ns*
Hypertension (%)	62.0	67.1	*ns*	72.3	67.9	*ns*
Diabetes (%)	31.3	22.2	*0.03*	28.8	17.0	*0.007*
Dyslipidemia (%)	25.3	29.5	*ns*	15.6	22.2	*ns*
Peripheral artery disease (%)	12.5	7.6	*ns*	27.3	26.1	*ns*
Atrial fibrillation (%)	16.2	14.3	*ns*	15.7	14.5	*Ns*
Chronic use of aspirin (%)	20.7	26.6	*ns*	39.4	67.9	*0.0001*
Ejection fraction ≤ 40% (%)	47.8	43.3	*ns*	50	21.7	*0.0001*
100 mg aspirin (%)	11.6	25.7	*0.001*	65.2	85.5	*0.0001*
325 mg aspirin (%)	88.4	73.3	*0.001*	34.8	14.5	*0.0001*
Leukocytes ($\times 10^9$/L)	11749 (10965–12303)	10000 (9550–10471)	*0.0001*	8128 (7586–8317)	7244 (7079–7413)	*0.0001*
ESR (mm/h)	39.8 (35.5–44.7)	28.2 (25.7–30.9)	*0.006*	25.7 (22.9–29.5)	17.4 (16.2–29.5)	*0.0001*

Data are expressed as % or as geometric mean (95%CI).

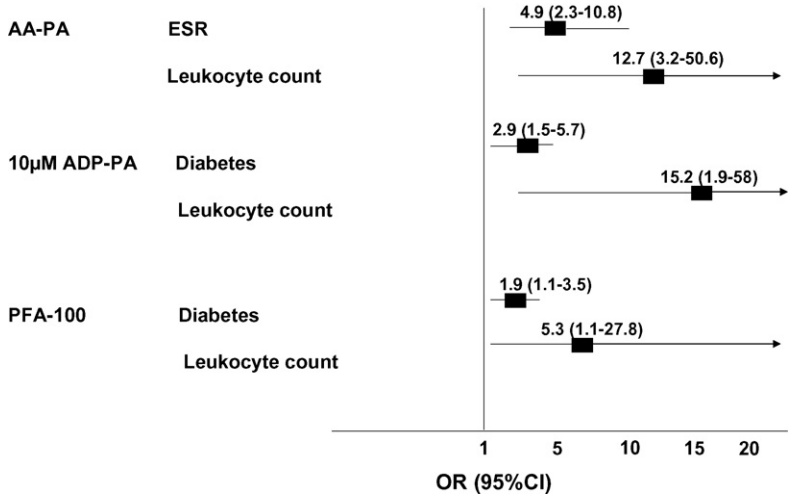

Fig. 1. Independent risk factors for RPR in ACS patients (GROUP A, $n = 386$). In the logistic regression analysis clinical characteristics differently ($p < 0.20$) distributed between patients with and without RPR were included as independent and dichotomous variables in a model in which each laboratory parameter (leukocyte count, ESR) was added separately as a continuous variable.

At the logistic regression analysis, adjusted for all potential confounders, diabetes and a 600 mg loading dose of clopidogrel were significant and independent predictors of RPR detected by 10 μM ADP-PA (Fig. 2).

Dual RPR (by AA-PA and 10 μM ADP-PA) was observed in 122/868 (14%) patients [77/386 (19.9%) of GROUP A and 45/482 (9.3%) of GROUP B].

At multiple logistic regression analysis, diabetes was the only clinical characteristic which significantly predicted the presence of dual RPR with respect to RPR only by AA or 10 μM ADP both in group A [OR: 2.8 (95%CI 1.3–6.3), $p < 0.01$] and group B [OR: 2.4 (95%CI 1.1–6.2), $p < 0.05$].

The prevalence of RPR by AA-PA was significantly ($p < 0.0001$) higher in patients with RPR by 10 μM ADP-PA [121/192 (63%)] with respect to those without RPR by 10 μM ADP-PA [154/670 (23%)]. The prevalence of RPR by 10 μM ADP-PA was significantly ($p < 0.0001$) higher in patients with RPR by AA-PA [114/271 (42%)] with respect to those without RPR by AA-PA [63/597 (10.6%)].

3.3. Residual platelet reactivity by PFA-100

GROUP A: RPR was found in 151/386 (39.1%) patients and was significantly ($p < 0.05$) more prevalent in STEMI (87/189 = 46%) patients with respect to NSTEMI (64/197 = 32.4%).

Hypertension, diabetes, reduced left ventricular systolic function (EF ≤ 40%), chronic use of aspirin, dose of aspirin, leukocyte count and ESR significantly differed in RPR patients with respect to patients without RPR (Table 3).

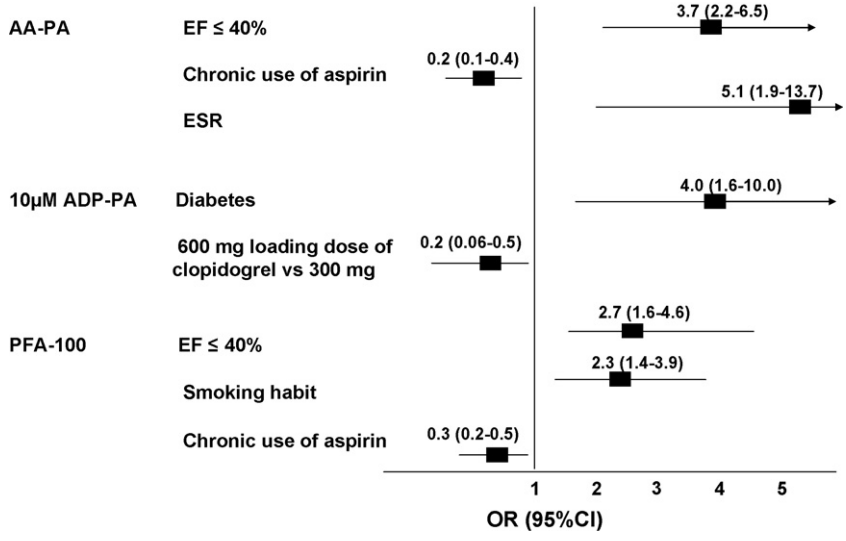

Fig. 2. Independent risk factors for RPR in patients undergoing elective PCI (GROUP B, $n = 482$). In the logistic regression analysis, clinical characteristics differently ($p < 0.20$) distributed between patients with and without RPR were included as independent and dichotomous variables in a model in which each laboratory parameter (leukocyte count, ESR) was added separately as a continuous variable.

Table 2
Clinical and laboratory characteristics according to platelet reactivity by 10 μM ADP-PA

	GROUP A ($n=386$)		p-value	GROUP B ($n=482$)		p-value
	With RPR ($n=99$)	Without RPR ($n=287$)		With RPR ($n=93$)	Without RPR ($n=389$)	
Age	68.3 (65.7–70.8)	68.3 (66.9–69.8)	*ns*	68.4 (66.2–70.6)	67.7 (66.6–68.8)	*ns*
Sex (M/F)	69/30	206/81	*ns*	70/23	294/95	*ns*
Smoking habit (%)	45.6	48.1	*ns*	40.5	47.3	*ns*
Hypertension (%)	63.6	64.7	*ns*	64.5	70.2	*ns*
Diabetes (%)	44.3	20.6	*0.0001*	34.2	16.6	*0.001*
Dyslipidemia (%)	25.0	28.5	*ns*	13.4	22.4	*0.04*
Peripheral artery disease (%)	13.9	7.8	*ns*	24.6	27.6	*ns*
Atrial fibrillation (%)	15.2	14.8	*ns*	14.4	15.8	*ns*
Previous use of clopidogrel (%)	4.4	7.7	*ns*	28.9	41.8	*0.02*
Ejection fraction $\leq 40\%$ (%)	61.2	41.0	*0.003*	38.3	26.0	*0.04*
Leukocytes ($\times 10^9$/L)	12882 (12023–13804)	10000 (9550–10471)	*0.0001*	8318 (7762–8709)	7244 (7079–7413)	*0.0001*
ESR (mm/h)	45.7 (39.8–52.5)	29.5 (26.9–31.6)	*0.0001*	23.9 (20.9–28.2)	18.2 (17.0–19.5)	*0.0001*

Data are expressed as % or as geometric mean (95%CI).

At the logistic regression analysis, adjusted for all potential confounders, diabetes and leukocyte count were significant and independent predictors of RPR detected by PFA-100 (Fig. 1).

GROUP B: RPR was found in 114/482 (23.6%) patients (Table 3 1). Smoking habit, reduced left ventricular systolic function (EF $\leq 40\%$), chronic use of aspirin, percentage of patients taking 100 mg/die, leukocyte count and ESR significantly differed according to RPR (Table 3).

At the logistic regression analysis, adjusted for all potential confounders, reduced left ventricular systolic function, chronic use of aspirin and smoking habit were significant and independent predictors of RPR detected by PFA-100 (Fig. 2).

4. Discussion

This study demonstrates that a number of clinical parameters are independent predictors of RPR on antiplatelet therapy in patients with coronary artery disease undergone primary or elective PCI. In particular, diabetes is independently associated with RPR (by ADP-PA and PFA-100) in both groups of patients; reduced left ventricular systolic function, diabetes, chronic use of aspirin and loading dose of clopidogrel, but not AF, were the independent variables significantly associated with RPR (by different methods and agonists) in patients undergoing elective PCI.

Diabetes is a condition known to be associated with an increased platelet function which may account for the persistent platelet reactivity despite dual antiplatelet therapy [26]. At present, it is still under debate whether enhanced platelet activation is merely a consequence of more prevalent atherosclerotic lesions or reflects the influence of the accompanying metabolic disturbances on platelet biochemistry and function. The present study does not allow to evaluate the role of metabolic alterations for RPR in high-risk ischemic heart disease patients. However, many in vitro and in vivo studies point to a role of platelets in the early phases of the diabetic disease [27].

Table 3
Clinical and laboratory characteristics according to platelet reactivity by PFA-100

	GROUP A ($n=386$)		p-value	GROUP B ($n=482$)		p-value
	With RPR ($n=151$)	Without RPR ($n=235$)		With RPR ($n=114$)	Without RPR ($n=368$)	
Age	68.1 (66.1–70.1)	67.7 (66.6–68.8)	*0.005*	68.7 (67.0–70.3)	67.8 (66.0–70.1)	*0.005*
Sex (M/F)	113/38	165/70	*ns*	89/25	275/93	*ns*
Smoking habit (%)	50	46.2	*ns*	56.4	41.9	*0.007*
Hypertension (%)	68.5	59.4	*0.05*	70.2	65.3	*ns*
Diabetes (%)	33.8	21.7	*0.009*	23.7	18.5	*ns*
Dyslipidemia (%)	29.3	25.6	*ns*	21.3	20.1	*ns*
Peripheral artery disease (%)	10.9	8.8	*ns*	22.6	28.1	*ns*
Atrial fibrillation (%)	16.0	14.3	*ns*	15.7	14.3	*ns*
Chronic use of aspirin (%)	18.1	28.2	*0.02*	40.7	66.4	*0.0001*
Ejection fraction $\leq 40\%$ (%)	53.0	40.7	*0.03*	43.9	23.3	*0.0001*
100 mg aspirin (%)	5.6	26.0	*0.0001*	63.0	84.5	*0.0001*
325 mg aspirin (%)	94.4	74.0	*0.0001*	37.0	15.5	*0.0001*
Leukocytes ($\times 10^9$/L)	11749 (11220–12589)	10000 (9550–10716)	*0.0001*	7762 (7586–8318)	7244 (7079–7586)	*0.006*
ESR (mm/h)	39.8 (35.5–44.6)	29.5 (26.9–32.3)	*0.0001*	22.4 (19.5–25.7)	18.2 (16.9–19.5)	*0.005*

Data are expressed as % or as geometric mean (95%CI).

A novel finding of the present study is related to the documented association between reduced left ventricular function and RPR. Platelet abnormalities in chronic heart failure have been well described: heart failure patients have increased whole blood aggregation, platelet-derived adhesion molecules, higher mean platelet volume and soluble P-selectin [28]. Therefore, also in this setting, an increased platelet reactivity may be hypothesized to be the mechanism leading to a higher prevalence of RPR. The frequent persistence of platelet activation in heart failure patients on antiplatelet agents may have a role for the results of two recent trials, the WASH [29] and WATCH [30] trials which did not show any difference in mortality in patients treated with aspirin.

In our patients, chronic use of aspirin and clopidogrel was associated with a lower prevalence of persistent hyperactivated platelets. This finding is apparently in contrast with data demonstrating that a long-term treatment with aspirin is associated with a progressive reduction in platelet sensitivity to antiplatelet therapy [31]. In our study, the majority of patients undergoing elective PCI were patients with a previous ACS scheduled to undergo a coronary angiography after 6 months or 1 year, all on antiplatelet therapy. Therefore, the 'apparent protective' effect of chronic use of aspirin and clopidogrel on the occurrence of RPR might have been determined by the fact that the majority of these patients were stable, i.e. with a reduced platelet reactivity with respect to ACS patients.

Furthermore, at our knowledge, this is the first study which documents an association between RPR and routinary inflammatory markers; interestingly they remained independently associated with RPR in ACS patients and in elective PCI, suggesting the possible role of other blood cells, such as leukocytes, in modulating the effect of antiplatelet therapy on platelet reactivity (as we previously demonstrated for red blood cells [32]).

Accumulating evidence supports a central role for inflammation in atherosclerosis, with ACS as the principle clinical expression [33]. Platelets represent an important linkage between inflammation, thrombosis, and atherogenesis. Inflammation is characterized by interactions among platelets, leukocytes and endothelial cells [34]. When activated, platelets coaggregate with circulating leukocytes [35]. Once adherent to the vascular wall, platelets also provide a sticky surface to recruit leukocytes to the vessel wall [36].

The measurement of circulating platelet monocyte aggregates would have provided a further insight into the mechanisms involved in the different response to antiplatelet drugs.

Another issue to be underlined is the possible influence of ex vivo platelet activation on platelet reactivity results obtained by platelet-rich plasma aggregometry. Validation of methods non-sensitive to ex vivo platelet activation is ongoing, even though a relationship between platelet reactivity index and ADP-induced PA has been reported [37].

A relevant finding of the present study is related to patients with RPR by both AA-PA and ADP-PA. We found that, according to a previous study on a small number of patients [38], RPR by AA-PA is associated with a significantly higher risk of RPR also by ADP-PA (42% versus 10.6%) and that RPR by ADP-PA is associated with a significantly higher risk of RPR by AA-PA (63.4% versus 23%). AA-PA changes are mainly influenced by the inhibition of the thromboxane synthesis and ADP-PA is sensitive to the inhibition of ADP receptors, both P2Y1 and P2Y12. However, it was demonstrated the role of the P2Y12 receptor, target of clopidogrel, as a functional regulator of thromboxane A2 generation consequent to protein-activated receptor stimulation [39] and, conversely, the fact that ADP- and collagen-induced platelet aggregation are affected to some extent by aspirin [40]. Therefore, the finding of an elevated prevalence of dual RPR may be result from a hyperstimulated state of platelets, not adequately blunted by aspirin and clopidogrel. Furthermore, the non negligible number of patients with a dual RPR (both by AA-PA and ADP-PA) identifies a group of patients with a relevant platelet reactivity for whom different antithrombotic strategies – higher dosage or a change in the pharmacological agent – might be indicated. We have identified diabetes as the characteristic significantly associated with the presence of dual RPR, underscoring that diabetic patients with IHD undergoing PCI are at higher risk of developing a prothrombotic condition. As in the last years, a number of studies have demonstrated an association between RPR and an adverse prognosis, these results underline the possible usefulness of evaluating platelet reactivity in high-risk patients with diabetes and reduced left ventricular systolic function for whom it may be indicated a carefully 'tailored' antithrombotic therapy.

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