Hign on treatment platelet reactivity by more than one agonist predicts 12-month follow-up cardiovascular death and non-fatal

High on-treatment platelet reactivity by more than one agonist predicts 12-month follow-up cardiovascular death and non-fatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting

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Summary

There is some data available on the role of high on-treatment platelet reactivity by ADP whereas, as regards arachidonic acid or other agonists, there is no proof of the best cut-off for identifying populations with a different cardiovascular outcome by the construction of appropriate receiver-operator characteristics (ROC) curves. We enrolled 1,108 acute coronary syndrome patients undergoing percutaneous coronary intervention (PCI) with stent implantation and followed them up for 12 months. Platelet reactivity was assessed by light transmission aggregometry (LTA) using 10 μM ADP, 1 mM arachidonic acid (AA) and 2 μg/ml collagen. At a 12-month follow-up, we found 37 cardiovascular deaths (3.3%), 54 non-fatal myocardial infarctions (MI) (4.8%) and 154 target vessel revascularisations (TVR) (13.8%). ROC analysis demonstrated that 10 μM ADP LTA, 1 mM AA and 2 μg/ml collagen LTA were able to distinguish between patients with and without subsequent cardiovascular death and non-fatal MI (area under the curve for 10 μM ADP 0.63 (0.55–0.71), p<0.001; for 1 mM AA 0.68 (0.61–0.76), p<0.0001; for 2 μg/ml collagen 0.62 (0.52–0.73), p<0.0111), whereas no association was demonstrated with the occurrence of TVR. Ten μM ADP LTA ≥55%, 1 mM AA LTA ≥15% and 2 μg/ml collagen LTA ≥31% were identified as the optimal cut-off to predict cardiovascular death and non-fatal MI at 12-month follow-up. The contemporary platelet hyperreactivity to more than one agonist was associated with a higher risk of 12-month cardiovascular death and MI, whereas isolated platelet hyperreactivity to only one agonist had not a predictive value [10 μM ADP LTA ≥55% + 1 mM AA LTA ≥15%: odds ratio [OR]=3.6(2.4–6.1), p<0.0001; ADP LTA ≥55% + 1 mM AA LTA ≥15% + 2 μg/ml collagen LTA ≥31%: OR=4.7(2.9–7.7), p<0.0001]. In this prospective study on a large number of acute coronary syndrome patients undergoing stent implantation, we have found that high on-treatment platelet reactivity measured by LTA induced by more than one agonist – AA, ADP, collagen – is an independent risk factor for 12-month cardiovascular death and non-fatal MI. Isolated platelet hyperreactivity to only one agonist has not a predictive value for clinical recurrences.

Keywords

Acute myocardial infarction, aspirin resistance, atherothrombosis

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Introduction

A growing body of evidence is demonstrating that the biological entity of high on-treatment platelet reactivity is associated with an increased risk of adverse cardiovascular events, such as stent thrombosis, cardiovascular (CV) death and non-fatal myocardial infarction (MI) (1–4). The majority of available studies has been performed on a limited number of patients and has restricted the analysis on the responsiveness to a single agonist. Several data are available on the role of high on-treatment platelet reactivity by adenosine diphosphate (ADP) (5–11) whereas, as regards arachidonic acid (AA) or other agonists, there is no proof of the best cut-off for identifying populations with a different cardiovascular outcome by the construction of appropriate receiver-operating characteristics (ROC) curves (12–19). Furthermore, several data obtained on chronic, stable cardiovascular patients are sapped by the postulate that non compliance may be the major practical reason for high on-treatment platelet reactivity. With this regards, it is

critical to separate the available evidence in acute and chronic settings.

This is a large prospective study planned to evaluate the clinical impact of high on-treatment platelet reactivity measured by light transmittance aggregometry (LTA) induced by AA, ADP and collagen on the occurrence of major adverse coronary events in the setting of acute coronary syndrome (ACS) patients undergoing stent implantation. We have found, by the construction of appropriate ROC analysis, the optimal cut-off points for the detection of patients at risk of clinical recurrences.

Materials and methods

Study population

Patients with ACS who underwent PCI and with an anticipated compliance to dual antiplatelet treatment for 12 months were considered eligible for the study. Informed written consent was obtained from all patients, and the study was approved by the local Ethical Review Board. Six hundred and eighty-three patients were included in a previous prospective study for the evaluation of the clinical impact of platelet reactivity measured by a point-of-care system (11).

PCI and antiplatelet management

All interventions were performed according to current standard guidelines, and the type of stent implanted and the use of IIb/IIIa inhibitors were at discretion of the operator. All patients received one clopidogrel loading dose of 600 mg followed by a daily dose of 75 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 according to the current guidelines for PCI (20).

Platelet reactivity assessment

Venous blood samples anticoagulated with 0.109 M sodium citrate (ratio 9:1) were taken from each patient within 24 hours from 600 mg clopidogrel loading. For patients receiving in the catheterisation laboratory both the loading dose of clopidogrel and a IIb/IIIa inhibitor, blood samples were obtained six days later while the patient was on the 75 mg maintenance dose of clopidogrel.

Platelet-rich plasma, obtained by centrifuging whole blood for 10 minutes (min) at 200 g, was stimulated with 10 μM ADP (Mascia Brunelli, Milan, Italy), with 1 mM AA (Sigma-Aldrich, Milan, Italy) and with 2 μg/ml collagen and aggregation was assessed using a APACT 4 light transmission aggregometer (Helena Laboratories, Milan, Italy) as previously reported (21).

Data collection and follow-up

All data were prospectively collected and entered into a central database. Clinical follow-up information was obtained by contacting all patients at 12 months and source documents of potential events were obtained.

Endpoints: 1) CV death, defined as death in the presence of acute coronary syndrome, significant cardiac arrhythmia or refractory chronic heart failure (CHF); 2)non-fatal MI (a rise in serum troponin I and/or an increase in creatine kinase MB isoenzyme at least twice the upper normal limits with at least one of the following: acute onset of prolonged (≥20 min) typical ischaemic chest pain; ST-segment elevation of at least 1 mm in two or more contiguous electrocardiographic leads or ST-depression of ≥0.5 mm in ≥2 contiguous leads; 3) target vessel revascularisation (TVR) (need for repeat PCI or coronary artery bypass grafting).

Statistical analysis

Continuous variables are presented as median (range). Categorical data are reported as frequencies. Differences in continuous variables were compared by Student's t test or Mann-Whitney U test, as appropriate. Dichotomous variables were compared by chi-square test or Fisher's Exact test, as appropriate.

A ROC analysis was used to determine the ability of 10 μM ADP, 1 mM AA and 2 μg/ml collagen LTA to distinguish between patients with and without post-discharge events after PCI. The optimal cut-off point was calculated by determining the post-treatment LTA value that provided the greatest sum of sensitivity and specificity. Cumulative survival curves for both patients with and without high on-treatment platelet reactivity (as defined by 10 μM ADP LTA ≥55%, 1 mM AA LTA ≥15% and 2 μg/ml collagen LTA ≥31%, cut-off calculated by ROC analysis) were constructed by the Kaplan-Meier method, and the log-rank sum test was used to assess the statistical differences between these two survival curves. Univariate and multivariate regression analyses were used, respectively, to identify risk factors for clinical end points and to adjust for potential confounders [cardiovascular risk factors, renal failure, left ventricular ejection fraction <40%, multivessel disease, total stent length, bifurcation lesions, number of lesions treated and type of stent (bare-metal stent [BMS] or drug-eluting stent [DES]), use of GpIIb/IIIa inhibitors, aspirin dosage]. McNamer test was used to compare specificity between different aggregometry tests. A significant level was defined when $p<0.05$. All analysis was performed using SPSS 10.0 (SPSS Inc., Chicago, IL, USA).

Results

From January 2005 to February 2007, a total of 1,108 patients were enrolled in the present study. Baseline characteristics are shown in

Table 1: Clinical characteristics of patients investigated.

	Overall group (n=1108)	12 month follow-up MACE (n=91)	12 month follow up no -MACE (n=1017)	P-value°
Age, years	69 (32–94)	69 (42–90)	69 (32–94)	0.006
Male gender, n (%)	836 (75.4)	27 (29.7)	245 (24.1)	0.1
Diabetes, n (%)	266 (24.0)	25 (27.5)	241 (23.7)	0.3
Smoking, n (%)	390 (35.2)	35 (38.5)	355 (34.9)	0.3
Hypertension, n (%)	730 (65.9)	59 (64.8)	671 (66)	0.4
Dyslipidaemia, n (%)	613 (55.3)	49 (53.8)	564 (55.5)	0.4
Family history of CAD, n (%)	121 (10.9)	5 (5.5)	116 (11.4)	0.005
LVEF<40%, n (%)	281 (25.4)	29 (31.9)	252 (24.8)	0.01
Renal failure*, n (%)	102 (9.2)	9 (9.9)	93 (9.1)	0.4
STEMI, n (%)	413 (37.3)	35 (38.5)	378 (37.2)	0.4
ACE-inhibitors, n (%)	753 (67.9)	62 (68.1)	691 (67.9)	0.9
Beta-blockers, n (%)	454 (40.9)	37 (40.6)	417 (41)	0.8
Statins, n (%)	698 (62.9)	57 (62.6)	641 (63)	0.8
Pump inhibitors, n (%)	1030 (92.9)	84 (92.3)	946 (93)	0.8
Glycoprotein IIb/IIIa, n (%)	443 (39.9)	35 (38.4)	408 (40.1)	0.5
Lesions treated, n	2032	166	1866	…
Vessels treated, n	1639	179	1460	…
Drug-eluting stents, n (%)	181 (16.3)	20 (22)	161 (15.8)	0.2
Bifurcation lesions, n (%)	467 (42.1)	39 (42.8)	428 (42)	0.6
Total stent length, mm	35 ± 23.4	37.6 ± 28.4	34.8 ± 22.9	0.4
LTA by 10 µM ADP	47.1 ± 21.4	55.6 ± 21.0	46.4 ± 21.3	0.001
LTA by 1 mM AA	20.3 ± 21.7	32.4 ± 27.6	19.2 ± 20.8	0.001
LTA by 2 µg/ml collagen	29.2 ± 22.5	39.6 ± 26.6	28.3 ± 21.9	0.001

Renal insufficiency defined by creatinine levels above 2.0 mg/dl. °= MACE (CV death and nonfatal MI) vs. no MACE. For abbreviations see text.

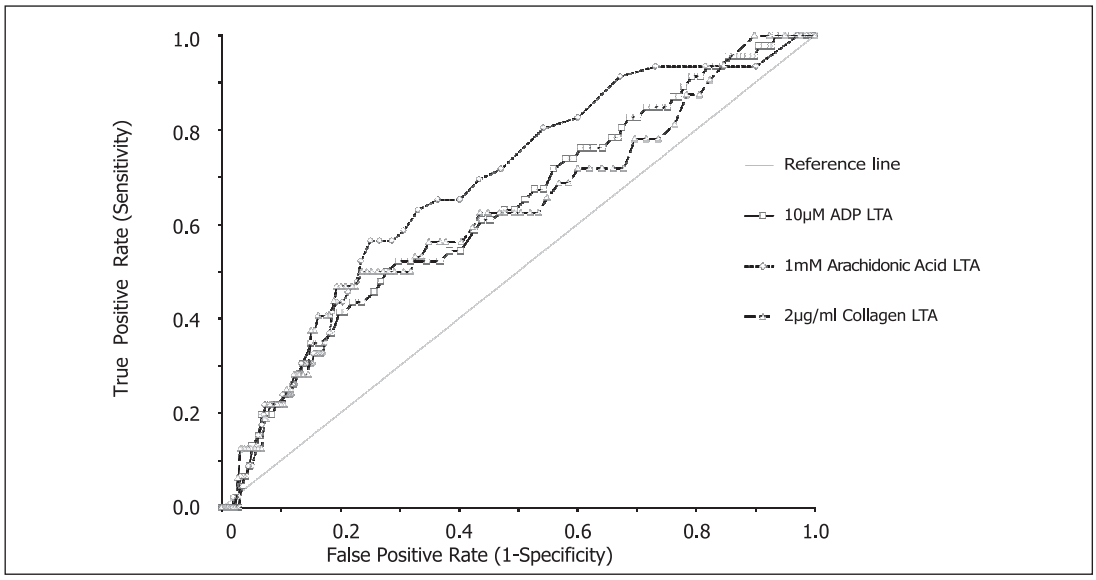

Figure 1: Receiver-operating characteristic (ROC) curve for light transmission aggregometry (LTA) induced by AA, ADP and collagen.

	10 μM ADP	1 mM AA	2 μg/ml collagen	10 μM ADP + 2 μg/ml collagen	10 μM ADP + 1 mM AA	10 μM ADP + 1 mM AA + 2 μg/ml collagen
Sensitivity	59(49–69)	62(51–71)	67(57–77)	53(42–63)	49(39–60)	45(35–55)
Specificity	63(51–66)	68(65–71)	68(65–70)	78(76–81)	80*(77–82)	85*^(83–87)
Positive predictive value	13(9–15)	15(11–18)	16(12–19)	18(13–22)	18(13–23)	21(15–27)
Negative predictive value	95(93–96)	95(94–97)	96(94–97)	95(93–96)	95(93–96)	95(93–96)

°p<0.001 vs. 10 μM ADP. *p<.0001 vs. 10 μM ADP, vs. 1 mM AA and vs. 2 μg/ml collagen; ^ p<0.001 vs. 10 μM ADP + 1 mM AA. For abbreviations see text.

Table 2: Sensitivity, specificity, positive and negative predictive values of light transmission aggregometry (LTA) in predicting cardiovascular (CV) death and non-fatal myocardial infarction (MI).

Table 1. The 12-month follow-up rate was 100%. A total of 254 clinical recurrences were recorded: 37 CV deaths (3.3%), 54 non-fatal MI (4.8%) and 154 TVR (13.8%). 10 μM ADP LTA, 1 mM AA and 2 μg/ml collagen LTA values were significantly higher in patients with the subsequent occurrence of CV death and non-fatal MI (Table 1) whereas no differences were detected among patients with or without TVR (10 μM ADP LTA: 45.5 ± 20.7% vs. 47.4 ± 21.5%, p=0.1;1 mM AA: 20.8 ± 24% vs. 20.1 ± 21.3%, p=0.1; 2 μg/ml collagen: 28.2 ± 23.2% vs. 29.4 ± 22.4%, p=0.2). Clinical characteristics according to the occurrence of CV deaths and non-fatal MI are reported in Table 1.

ROC curve analysis demonstrated that 10 μM ADP LTA, 1 mM AA and 2 μg/ml collagen LTA were able to distinguish between patients with and without subsequent CV death and non-fatal MI at 12-month follow-up (area under the curve [AUC] for 10 μM ADP 0.63 (0.55–0.71), p<0.001; for 1 mM AA 0.68 (0.61–0.76), p<0.0001; for 2 μg/ml collagen 0.62 (0.52–0.73), p<0.0111) (Fig. 1), whereas no association was demonstrated with the occurrence of TVR (AUC for 10 μM ADP 0.47 (0.42–0.52), p=0.09; for 1 mM AA 0.48 (0.43–0.53), p=0.09; for 2 μg/ml collagen 0.48 (0.43–0.53), p=0.08).

Table 3: Clinical characteristics according to platelet reactivity.

	10 μM ADP LTA ≥55% (n=432)	10 μM ADP LTA <55% (n=676)	P-value	1 mM AA LTA ≥15% (n=379)	1 mM AA LTA <15% (n=729)	P-value	2 μg/ml collagen LTA ≥31% (n=441)	2 μg/ml collagen LTA <31% (n=667)	P-value
Age, years	69 (46–93)	68 (32–94)	<0.01	70 (32–93)	68 (33–94)	<0.01	69 (32–93)	68 (33–94)	<0.01
Male gender, n (%)	315 (73)	521 (78.1)	<0.01	272 (71.7)	564 (77.3)	<0.01	313 (71)	523 (78.4)	<0.01
Diabetes, n (%)	118 (27.3)	148 (21.8)	<0.001	107 (28.2)	159 (21.8)	<0.001	123 (27.9)	143 (21.4)	<0.001
Smoking, n (%)	147 (34)	244 (36)	0.4	130 (34.3)	260 (35.6)	0.3	150 (34)	2408369	0.4
Hypertension, n (%)	306 (70.8)	424 (62.7)	0.5	261 (68.8)	469 (64.3)	0.2	308 (69.8)	422 (63.3)	0.6
Dyslipidaemia, n (%)	228 (52.7)	385 (56.9)	0.2	216 (56.9)	397 (54.4)	0.5	239 (54.2)	374 (56.1)	0.5
Family history of CAD, n (%)	47 (10.8)	74 (10.9)	0.1	31 (8.2)	9 (12.3)	0.4	40 (9.1)	81 (12.6)	0.3
LVEF<40%, n (%)	118 (27.3)	163 (24.1)	<0.001	112 (29.5)	169 (23.2)	<0.001	118 (26.7)	163 (24.4)	<0.001
Renal failure*, n (%)	34 (7.8)	68 (10)	0.4	28 (7.4)	74 (10.1)	0.5	39 (8.8)	63 (9.4)	0.2
STEMI, n (%)	197 (45.6)	216 (31.9)	<0.001	168 (44.3)	245 (33.6)	<0.001	182 (41.2)	231 (34.2)	<0.001
ACE-inhibitors, n (%)	293 (67.8)	460 (68)	0.3	260 (68.6)	493 (67.6)	0.4	297 (67.3)	456 (68.3)	0.1
Beta-blockers, n (%)	177 (40.9)	277 (40.9)	0.3	153 (40.3)	301 (41.2)	0.3	183 (41.5)	271 (40.6)	0.1
Statins, n (%)	274 (63.4)	424 (62.7)	0.1	240 (63.39	458 (62.8)	0.2	280 (63.5)	418 (62.6)	0.2
Pump inhibitors, n (%)	403 (93.3)	627 (92.7)	0.2	350 (92.3)	680 (93.2)	0.2	412 (93.4)	618 (92.6)	0.3
Glycoprotein IIb/IIIa, n (%)	176 (40.7)	267 (39.4)	0.9	153 (40.3)	290 (39.7)	0.2	174 (39.4)	269 (40.3)	0.4
Lesion treated, n	823	1209	…	735	1297	…	828	1204	…
Vessels treated, n	659	980	…	593	1046	…	668	971	…
Drug-eluting stents, n (%)	94 (21.7)	87 (12.8)	<0.01	69 (18.2)	112 (15.4)	<0.01	80 (18.1)	101 (15.1)	<0.01
Bifurcation lesions, n (%)	182 (42.1)	285 (42.1)	0.2	165 (43.5)	302 (41.4)	0.3	198 (44.9)	269 (40.3)	0.2
Total stent length, mm	37.9 ± 25.6	33.4 ± 21.7	<0.01	36.5 ± 23.2	34.0 ± 23.4	<0.01	38.4 ± 26.8	33.6 ± 22	<0.01

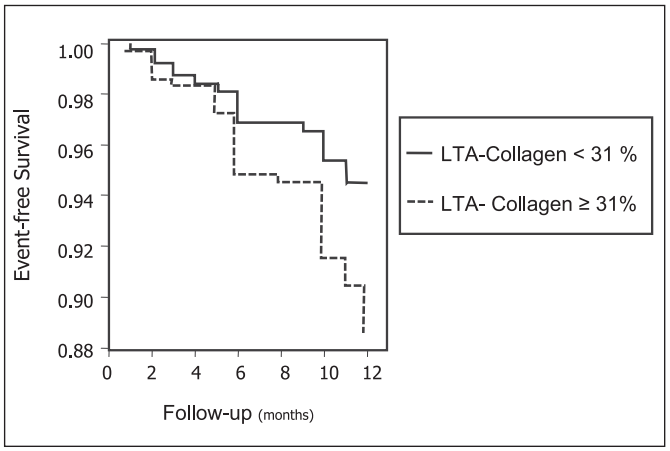

Figure 2: Survival free from cardiovascular (CV) death and non-fatal myocardial infarction (MI) in patients with and without AA LTA ≥15%.

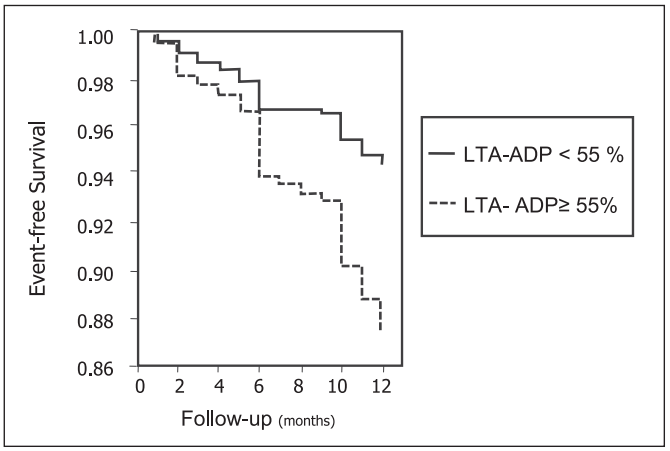

Figure 3: Survival free from cardiovascular death and non-fatal myocardial infarction (MI) in patients with and without ADP-LTA ≥55%.

Ten µM ADP LTA ≥55%, 1 mM AA LTA ≥15% and 2 µg/ml collagen LTA ≥31% were identified as the optimal cut-off to predict CV death and non-fatal MI at 12-month follow-up. The contemporary hyperreactivity to both AA and ADP LTA (AA LTA ≥15% + ADP LTA ≥55%) and the hyperreactivity to three agonists (AA LTA ≥15% + ADP LTA ≥55% + collagen LTA ≥31%) resulted in a higher specificity and positive predictive values for detection of 12-month CV death and non-fatal MI whereas negative predictive values were similar. Sensitivity, specificity, positive and negative predictive values for the different aggregometry tests are reported in Table 2.

Clinical characteristics according to platelet reactivity are shown in Table 3. Patients with high on-treatment platelet reactivity (defined according to ROC-based cut-off) were significantly older, more likely to be female, diabetics, with a diagnosis of ST-elevation myocardial infarction (STEMI), with a reduced ejection fraction than patients without hyperreactivity. Interestingly, we have observed that the total stent length and the number of DES were significantly higher in patients with high on-treatment platelet reactivity. No differences were detected among these clinical and procedural characteristics according to the type of agonist used.

The event-free survival curves for CV death and non-fatal MI according to the presence of residual platelet reactivity (RPR) by different agonists are shown in Figures 2, 3 and 4.

At univariate analysis, 10 µM ADP LTA ≥55%, 1 mM AA LTA ≥15% and 2 µg/ml collagen LTA ≥31% were associated with a significant higher risk of CV death and non-fatal MI, whereas no significant associations were detected with TVR (Table 4). The contemporary presence of platelet hyperreactivity by AA, ADP and collagen was associated with a higher risk for CV death and non-fatal MI (Table 4). These results were confirmed after adjustment for confounding variables (cardiovascular risk factors, renal failure, reduced ejection fraction, multivessel disease, total stent length, bifurcation lesions, number of lesions treated, type of stent used, use of glycoprotein IIb/IIIa inhibitors and aspirin dosage)

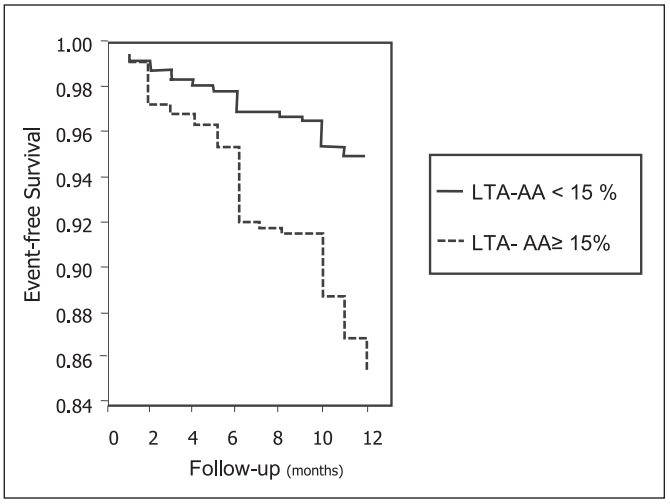

Figure 4: Survival free from cardiovascular (CV) death and non-fatal myocardial infarction (MI) in patients with and without % collagen LTA ≥31%.

[10 µM ADP LTA ≥55%: odds ratio [OR]=2.4 (1.6–3.8), p<0.0001; 1 mM AA LTA ≥15%: OR=3.0 (1.8–5.3), p<0.0001; 2 µg/ml collagen LTA ≥31%: OR=2.4 (1.5–3.9), p<0.0001; 10 µM ADP LTA ≥55% + 1 mM AA LTA ≥15%: OR=3.6 (2.4–6.1), p<0.0001; ADP LTA ≥55% + 1 mM AA LTA ≥15% + 2 µg/ml collagen LTA ≥31%: OR=4.7 (2.9–7.7), p<0.0001].

Discussion

In this prospective study on a large number of ACS patients undergoing stent implantation, we have found that high on-treatment platelet reactivity measured by LTA induced by more than one agonist – AA, ADP, collagen – is an independent risk factor for

Table 4: Clinical outcome at 12-month follow-up according to platelet reactivity.

	CV death + non-fatal MI (n=91)	OR (95%CI)*	P-value	TVR (n=154)	OR (95%CI)*	P-value
10 μM ADP LTA ≥55% (n=432)	54(12.5)	2.4(1.6–3.8)	0.001	52(12)	0.8(0.5–1.1)	0.3
1 mM AA LTA ≥15% (n=379)	56(14.7)	3.4(2.2–5.3)	0.001	49(12.9)	0.9(0.6–1.3)	0.4
2 μg/ml collagen LTA ≥31% (n=441)	42(9.5)	2.4(1.5–3.9)	0.001	51(11.6)	0.9(0.6–1.2)	0.4
10 μM ADP LTA ≥55% + 2 μg/ml collagen LTA ≥31% (n=269)	48(17.8)	4.0(2.6–6.2)	0.001	31(11.5)	0.9(0.6–1.3)	0.4
10 μM ADP LTA ≥55% + 1 mM AA LTA ≥15% (n=251)	45(17.9)	3.8(2.5–5.9)	0.001	31(12.3)	0.8(0.5–1.3)	0.3
10 μM ADP LTA ≥55% + 1 mM AA LTA ≥15% +2 μg/ml collagen LTA ≥31% (n=194)	41(21.1)	4.6(2.9–7.2)	0.001	26(13.4)	0.9(0.6–1.5)	0.4
Isolated 10 μM ADP LTA ≥55% (n=113)	9(7.9)	0.9(0.5–1.9)	0.5	14(12.4)	0.9(0.5–1.5)	0.4
Isolated 1 mM AA LTA ≥15% (n=57)	5(8.8)	1.1(0.4–2.7)	0.4	8(14)	1.0(0.5–2.2)	0.5
Isolated 2 μg/ml collagen LTA ≥31% (n=44)	0	-	-	7(15.9)	1.2(0.5–2.6)	0.5

Patients with LTA values equal or above the reported cut-off vs. patients with LTA values less than the reported cut-off. For abbreviations see text.

12-month CV death and non-fatal MI, and that isolated platelet hyperreactivity to only one agonist has not a predictive value for clinical recurrences.

The strength of our results is based on: 1) the definition of the cut-off points on the basis of the clinical recurrences by the construction of ROC curves; 2) the large number of patients enrolled; 3) the length of follow-up.

Furthermore, with respect to data available in the literature, as regards AA or other agonists than ADP, there is no proof of the best cut-off for identifying populations with a different cardiovascular outcome by the construction of appropriate ROC curves. Indeed, published studies have investigated platelet function induced by a single agonist (5–11, 13–15, 17–19), on a limited number of patients (14, 17, 19) and using an '*a priori*' cut-off calculated on the distribution in controls (13–15, 17–19). No study has calculated a cut-off of AA- or collagen-induced platelet function based on a ROC curve (i.e. on the basis of cardiovascular recurrences) or has contemporaneously investigated platelet function induced by different agonists.

By using different agonists which reflect different pathways of platelet function, we have demonstrated that ACS patients with a 12-month recurrence have an '*aggressive*' platelet, independently of the responsiveness to a single antiplatelet drug. We evaluated platelet function by using three agonists – AA, ADP and collagen. AA-induced platelet aggregation is mainly influenced by the inhibition of the thromboxane synthesis and ADP-induced platelet aggregation is mainly sensitive to the inhibition of ADP receptors, both P2Y1 and P2Y12. However, evidence exists of the role of the P2Y12 receptor, target of clopidogrel, as a functional regulator of TxA2 generation consequent to protein-activated receptor stimulation (22) and/or, conversely, the fact that ADP- and collagen- induced platelet aggregation are affected to some extent by aspirin (23). Collagen interacts with integrin α2β1 and glycoprotein VI (GPVI) and triggers intracellular signals that shift platelet integrins to a high-affinity state and induce the release of the secondary mediators ADP and TXA2 (24). Therefore, even if a single agonist may mainly reflect a single pathway of platelet function, there is an important overlap between the different pathways activated by the three agonists. The consequence is that, beyond the activation of a single pathway and the responsiveness to a single drug, the finding of a high on treatment platelet reactivity using different agonists identifies a *hyperactive* platelet which is not adequately inhibited by the dual antiplatelet treatment. The translation of this finding into the clinical practice remains to be evaluated. The history of the antiplatelet therapy in acute coronary syndrome patients undergoing PCI moves from the use of a single drug – aspirin -, to the dual antiplatelet therapy – aspirin and clopidogrel – and finally to the loading dose of clopidogrel (firstly 300 mg and secondly 600 mg) followed by a dual daily antiplatelet therapy. This history is yet ongoing as new antiplatelet drugs, such as prasugrel which has a more intense and rapid antiplatelet effect, have been found to reduce thrombotic risk in this setting even if at the price of an increased bleeding risk (25). Our data demonstrate that ACS patients who die or experience a MI after the percutaneous revascularisation are characterised by the presence of an hyperactive platelet, on top of the optimal standard antiplatelet therapy. It is conceivable that these patients might take advantage from a more intense antiplatelet therapy – higher dosage of the current drugs or the addition of a third antiplatelet agent (10, 26, 27, 28) – without an increased bleeding risk. Recent data obtained by the oral PAR-1 antagonist SCH 530348 are in line with this hypothesis [29]. On the other hand, retrospective analyses of trials such as PCI-CURE or

CHARISMA suggest that daily aspirin doses higher than 100 mg are not associated with clear benefit and may cause harm compared with lower doses (75 or 81 mg/day). This datum is not against our findings of a correlation between worse outcome and residual platelet reactivity. We have found that a global high on-treatment platelet reactivity is associated with a worse clinical outcome; subsequently, we will have to evaluate the better treatment, if any, to overcome this hyperreactivity.

The results of this study, obtained in patients with ACS and with a 12-month follow-up, are in keeping with those obtained in the RECLOSE trial (30), underlying the role of high on-treatment platelet reactivity in the setting of ACS patients undergoing PCI.

We are aware that platelet reactivity at the time of ACS may be influenced by a number of clinical and laboratory parameters, including the high level of the inflammatory state and the increased platelet turnover. Consequently a percentage of patients with platelet hypereactivity in the acute state might subsequently return to an adequate platelet inhibition. The issue of the durability of high on-treatment platelet reactivity remains to be solved by *ad hoc* studies. Nevertheless, our results underline the concept that an 'aggressive' platelet characterises patients at higher risk of clinical recurrences, even if this laboratory finding might not be stable over time. In other words, an aggressive platelet at the time of the acute event identifies, together with procedural and clinical characteristics, high-risk patients for which a tailored antiplatelet therapy might be useful.

Furthermore, we have to take into account the possible confounding role that patient non-adherence has on individual antiplatelet response as evidence indicates its impact in the real-world setting.

What is known about this topic?

- High on-clopidogrel platelet reactivity by ADP has been associated with an increased risk of cardiovascular events in patients undergoing dual antiplatelet treatment.
- As regards arachidonic acid (AA) or other agonists, there is no proof of the best cut-off for identifying populations with a different cardiovascular outcome by the construction of appropriate receiver-operating curves.

What does this paper add?

- In this prospective study on a large number of acute coronary syndrome patients undergoing stent implantation, we have found that high on-treatment platelet reactivity measured by light transmission aggregometry induced by more than one agonist – AA, ADP, collagen – is an independent risk factor for 12-month cardiovascular death and non-fatal myocardial infarction, and that isolated platelet hyperreactivity to only one agonist has not a predictive value for clinical recurrences.
- The strenght of our results depends on: 1) the definition of the cut-off points on the basis of the clinical recurrences by the construction of receiver-operating curves; 2) the large number of patients enrolled; 3) the length of follow-up.

LTA, the standard method of platelet function assessment, has logistic problems which make its routine use difficult. For this reason, a number of assays for platelet reactivity by different methods and agonists are under laboratory and clinical evaluation, also by our group. Most importantly, our findings stress the need of systems for the evaluation of platelet function based on the use of multiple agonists able to explore different pathways of platelet function.

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