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Full Length Article

High and low on-treatment platelet reactivity to $P2Y_{12}$ inhibitors in a contemporary cohort of acute coronary syndrome patients undergoing percutaneous coronary intervention

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ABSTRACT

Introduction: There is compelling evidence supporting the association between high on-treatment platelet reactivity (HPR) and low on-treatment platelet reactivity (LPR) to clopidogrel with atherothrombotic and bleeding events, respectively. However, it is uncertain if current cutoff values should be used in prasugrel- or ticagrelor-treated subjects. The objective of this analysis was to evaluate the pharmacodynamic (PD) efficacy of $P2Y_{12}$ antagonists in a contemporary real-world population.

Materials and methods: This PD study included 988 patients with acute coronary syndromes (ACS) undergoing percutaneous coronary intervention (PCI) and receiving dual therapy with aspirin and a $P2Y_{12}$ inhibitor (clopidogrel, prasugrel or ticagrelor). Platelet function was assessed at day 1 and day 30 post-PCI by VerifyNow P2Y12 assay, multiple electrode aggregometry and vasodilator-stimulated phosphoprotein (VASP) assay.

Results: Clopidogrel-treated patients ($n = 324$) had greater platelet reactivity than those receiving ticagrelor ($n = 469$) or prasugrel ($n = 195$) at both time points ($p < 0.001$ for all comparisons). No difference between ticagrelor and prasugrel was observed at day 1 with the VerifyNow P2Y12 assay (51.5 ± 2.8 vs. 42.7 ± 3.5 PRUs; $p = 0.298$), whereas ticagrelor achieved greater platelet inhibition at day 30 (48.1 ± 2.5 vs. 89.2 ± 4.2 PRUs; $p < 0.001$). Similar results were obtained with the VASP assay. Both prasugrel and ticagrelor had markedly lower HPR rates than clopidogrel and very high rates of LPR at both time points.

Conclusions: Prasugrel and ticagrelor displayed more potent and consistent PD effects than clopidogrel in ACS patients undergoing PCI, with a trend towards greater platelet inhibition with ticagrelor during the maintenance phase of therapy compared to prasugrel.

1. Introduction

The combination of aspirin and a $P2Y_{12}$ receptor inhibitor, known as dual antiplatelet therapy (DAPT), is currently the standard of care of oral antithrombotic therapy for secondary prevention in patients with acute coronary syndromes (ACS) and undergoing percutaneous coronary intervention (PCI) [1]. Numerous studies have shown a broad interindividual variability in the pharmacodynamic (PD) response to $P2Y_{12}$ inhibitors, in particular to clopidogrel, which has an impact on clinical outcomes [2]. In fact, there is strong evidence supporting the association between high on-treatment platelet reactivity (HPR) and low on-treatment platelet reactivity (LPR), using adenosine diphosphate (ADP) as stimulus, with ischemic and bleeding events, respectively, which will suggest a therapeutic window of a supposed optimal platelet reactivity flanked by HPR and LPR thresholds [3,4].

Randomized clinical trials, however, have failed to show a clear

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benefit of tailored antiplatelet therapy guided by platelet function testing (PFT) and, therefore, routine measurement of platelet reactivity has not been implemented in clinical practice [5–8]. Despite consensus-derived cutoff values for platelet function assays have been proposed [4,9], it is uncertain whether these thresholds may differ across different populations or if they could be used in patients on prasugrel or ticagrelor therapy [10]. Further, optimal timing to measure responsiveness to antiplatelet agents in the setting of ACS is also a matter of debate [11].

The aim of the present analysis was to evaluate the PD efficacy of available oral $P2Y_{12}$ blockers in a contemporary cohort of ACS patients undergoing PCI at 1 day and 30 days after the procedure, as well as to explore HPR and LPR rates to these agents with currently proposed cutoff values for platelet function assays.

2. Materials and methods

2.1. Subject population and study design

This is an interim PD analysis of a prospective national multicenter study (the "HIGH on-treatment PLAtelet reactivity to $P2Y_{12}$ inhibitors: finding the Cutoff valuE associated with clinical outcomes in Spanish population", HIGHPLACES study) with the aim of assessing HPR and LPR rates to clopidogrel, prasugrel and ticagrelor in a Spanish cohort of ACS patients undergoing PCI with stent implantation and treated with DAPT. The clinical follow-up of the main study is ongoing at the time of preparation of this manuscript and has the objective of evaluating the predictive ability of platelet function assays with currently used definitions as well as of finding the optimal cutoff values in the Spanish population.

All patients were ≥18 years of age and on DAPT with ASA and a $P2Y_{12}$ blocker, having received a loading dose (300–600 mg of clopidogrel, 60 mg of prasugrel or 180 mg of ticagrelor) during index admission or at the time of PCI, or being under chronic therapy (> 1 week) with a $P2Y_{12}$ blocker. Exclusion criteria were known allergies to aspirin, clopidogrel, prasugrel or ticagrelor, administration of glycoprotein IIb/IIIa inhibitors during PCI, chronic oral anticoagulation, cardiogenic shock, any active bleeding or malignancy, recent history (< 6 months) of gastrointestinal bleeding, platelet count < 100×10^6/μl, hemoglobin < 10 g/dl, recent history (< 6 months) of stroke, severe chronic kidney disease (creatinine clearance < 30 ml/min) and pregnant females. Technical procedures during PCI and choice of medications were left at treating physicians' criteria according to standard clinical practice.

Patients were screened at the interventional cardiology units from the tertiary care hospitals participating in the trial. The study complied with the Declaration of Helsinki and received ethics committee approval at every participating site. All subjects provided written informed consent to the study.

2.2. Sample collection and platelet function assays

Blood samples for PFT were collected at two time points: day 1 and 30 ± 5 days after PCI; samples were drawn in the morning before intake of the corresponding maintenance doses of antiplatelet agents. Samples were processed by trained laboratory personnel and treating physicians were unaware of the results. Platelet function assessments included VerifyNow P2Y12 assay, flow cytometric analysis of phosphorylation status of the vasodilator-stimulated phosphoprotein and multiple electrode aggregometry.

2.3. VerifyNow (VN) P2Y12 assay

The VN assay is a rapid whole blood point-of-care device and was utilized according to the instructions of the manufacturer (Accumetrics, San Diego, CA, USA) as previously described [12]. In brief, VN-P2Y12 assay mimics turbidometric aggregation and utilizes disposable cartridges containing 20 μM ADP and 22 nM prostaglandin E_1 (PGE_1). Aggregation testing using ADP as a sole agonist activates $P2Y_1$ and $P2Y_{12}$ purinergic signaling, whereas adding PGE_1 increases the specificity of the test for $P2Y_{12}$ signaling. In a separate channel of the cartridge in which iso-thrombin receptor-activating peptide is used as an agonist, a baseline value for platelet function is obtained, enabling assessment of platelet inhibition without having to wean the patient off antiplatelet treatment. Results are reported as $P2Y_{12}$ reaction units (PRU), which decrease with increasing platelet inhibition. The cutoff values used to define HPR and LPR were > 208 and < 85 PRUs, respectively [3,4].

2.4. Vasodilator-stimulated phosphoprotein (VASP) assay

The $P2Y_{12}$ reactivity index (PRI) is calculated as a measure of the functional status of the $P2Y_{12}$ signaling pathway, and is determined through assessment of phosphorylation status of VASP, a key and specific intraplatelet mediator of $P2Y_{12}$ signaling, according to standard protocols [13]. VASP phosphorylation (VASP-P) was measured by quantitative flow cytometry using commercially available labelled monoclonal antibodies (Biocytex Inc., Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels following challenge with PGE_1 and PGE_1 + ADP. PGE_1 increases VASP-P levels through stimulation of adenylate cyclase (AC); ADP binding to purinergic receptors leads to inhibition of AC; thus, the addition of ADP to PGE_1-stimulated platelets reduces levels of PGE_1-induced VASP-P. The PRI was calculated as follows: ([MFI PGE1]-[MFI PGE1 + ADP] / [MFI PGE1]) × 100%. A reduced PRI is indicative of greater inhibition of the $P2Y_{12}$ signaling pathway. The cutoff values used to define HPR and LPR were ≥50% and < 16% PRI, respectively [3,4].

2.5. Multiple electrode aggregometry (MEA)

MEA was assessed in whole blood with the Multiplate® analyzer (Roche Diagnostics, Basel, Switzerland) as previously described [14]. This instrument can perform up to five parallel aggregometry measurements assessing the change in impedance caused by the adhesion of platelets onto sensor units formed by silver-covered electrodes. Curves were recorded for 6 min and platelet aggregation was determined as area under the curve of arbitrary aggregation units (AU∗min). In the present investigation, 6.4 μmol/l ADP was used as agonist to evaluate $P2Y_{12}$ inhibitors responsiveness. The cutoff values used to define HPR and LPR were > 468 and < 188 AU∗min, respectively [4,14].

2.6. Statistical analyses

For baseline characteristics, continuous variables are expressed as mean ± SD and categorical variables as frequencies and percentages. Qualitative variables were compared with chi-square test or Fisher's exact test if application conditions were not fulfilled, whereas comparisons of quantitative variables between groups according to the $P2Y_{12}$ antagonist used were made with the ANOVA method. An ANCOVA method with a general linear model was used to evaluate all between-groups comparisons of platelet function parameters (Bonferroni correction for multiple testing), using as covariates unbalanced baseline characteristics in the univariate analysis ($p < 0.10$) and demographic or clinical variables known to have an effect on responsiveness to P2Y12 inhibitors (age, gender, obesity, smoking habit, hypertension, diabetes mellitus, dyslipidemia, peripheral artery disease, prior myocardial infarction, prior percutaneous coronary intervention, prior coronary artery bypass grafting, chronic renal disease, stroke, chronic obstructive pulmonary disease, type of ACS, number of vessels affected, use of proton-pump inhibitors, use of statins, use of dihydropyridines). Results presented in the manuscript for these

comparisons of platelet function measurements correspond to the multivariate analyses and are reported as least squares mean (LSM) ± standard error of the mean (SEM). For the exploratory analyses of HPR and LPR rates, platelet function measurements were divided using present consensus-derived cutoff values in three categories: HPR, suggested optimal platelet reactivity (SOPR) and LOPR. A p value < 0.05 was considered statistically significant for all comparisons. Statistical analysis was performed using SPSSv18.0 software (SPSS Inc., Chicago, IL). There was no formal specific sample size calculation for the present investigation since this is a substudy of the population (1000 subjects) enrolled in the HIGHPLACES study.

3. Results

Between March 2015 and June 2018, a total of 1000 patients agreed to participate and were enrolled, of whom 12 had to be excluded due to failure to measure platelet function at day 1 (e.g. insufficient volume or inaccurate processing of blood samples, hemolysis…); therefore, 988 patients were included in the present analysis. Among these, 324 (32.8%) were treated with clopidogrel at day 1, whereas 195 (19.7%) and 469 (47.4%) received prasugrel and ticagrelor, respectively; most patients (98.8%) received a loading dose of the $P2Y_{12}$ inhibitor. Baseline demographics, procedural and angiographic characteristics of the overall population and according to the $P2Y_{12}$ antagonist are summarized in Table 1.

Numerous differences were observed in baseline characteristics according to the antiplatelet agent used. In brief, subjects receiving clopidogrel were older and had a greater burden of cardiovascular risk factors than those receiving prasugrel or ticagrelor. ST-elevation myocardial infarction (STEMI) was the most frequent type of ACS among prasugrel-treated patients and non-ST-elevation ACS was the most common diagnosis in ticagrelor-treated subjects. Between day 1 and day 30, 100 patients underwent switching between $P2Y_{12}$ inhibitors as per their physicians indication (11 and 40 upgraded from clopidogrel to prasugrel and ticagrelor, respectively; 16 and 27 downgraded from prasugrel and ticagrelor to clopidogrel; and 6 switched between the more potent agents). In these patients, the 30-day platelet function assessment was performed under steady-state conditions of the $P2Y_{12}$ inhibitor, since the switch was performed at least 10 days before PFT. At day 30, 890 patients were included in the analysis (losses due to patients' negative or unfeasibility to undergo a visit in the scheduled time or to failure to obtain a correct blood sample to measure platelet function); among them, 275 (30.9%), 169 (19.0%) and 446 (50.1%) were treated with clopidogrel, prasugrel and ticagrelor, respectively.

At day 1, patients receiving clopidogrel had greater platelet reactivity measured with the VN-P2Y12 assay (174.2 ± 3.5 PRUs) than those receiving prasugrel or ticagrelor (p < 0.001 for both comparisons), with a small numerical but not statistically significant trend towards greater platelet inhibition achieved with prasugrel (42.7 ± 3.5 vs. 51.5 ± 2.8 PRUs; p = 0.298) (Fig. 1). Similar results were obtained with the VASP assay and the MEA ADP test (Table 2). At day 30, platelet reactivity was higher in clopidogrel-treated patients (191.8 ± 3.3) than in those receiving prasugrel or ticagrelor (p < 0.001 for both comparisons), whereas prasugrel reached significantly lower platelet inhibition than ticagrelor (89.2 ± 4.2 vs. 48.1 ± 2.5 PRUs; p < 0.001) (Fig. 1). For the latter comparison, consistent results were observed with the VASP assay, but not with the MEA ADPtest (Table 2).

HPR rates were markedly lower with both prasugrel and ticagrelor compared with clopidogrel at both time points. At day 1, LPR rates of prasugrel and ticagrelor were similar, and both were prominently higher compared to clopidogrel. At day 30, there was a trend towards higher LPR with ticagrelor (17.8% to 92.1%) compared to prasugrel (24.1% to 65.7%), whereas LPR rates with clopidogrel remained much lower (2.7% to 10.0%). HPR and LPR rates with all platelet function measurements are portrayed in Fig. 2.

4. Discussion

The findings of this PD investigation can be summarized as follows: 1) larger variability and higher rates of HPR were observed among clopidogrel-treated patients compared to prasugrel or ticagrelor with all platelet function assessments; 2) prasugrel and ticagrelor had very low HPR rates and high LPR rates at both the acute and the maintenance phase of therapy; and 3) ticagrelor achieved greater platelet inhibition than prasugrel at 30 days.

As expected, larger interindividual variability in response was found among patients receiving clopidogrel, with very low rates of HPR among prasugrel and ticagrelor-treated patients, although this is subject to variability depending on the assay used. Even though prasugrel and ticagrelor, two more potent $P2Y_{12}$ antagonists, have been proven superior to clopidogrel in reducing atherothrombotic events in ACS patients [15,16], clopidogrel is still widely used in daily clinical practice due to its lower cost and a more favorable safety profile in terms of bleeding risk [17]. Despite multiple studies have demonstrated a clear association between HPR to clopidogrel and adverse atherothrombotic events [2], routine evaluation of platelet reactivity is currently not recommended as randomized clinical trials to date have failed to show a superiority of a tailored strategy based on PFT [5–8]. However, these trials have several remarkable limitations and, therefore, they do not provide enough evidence to completely refute the potential utility of PFT, particularly in high-risk ACS patients [10].

To date, it has not been fully elucidated which is the optimal assay with the highest predictive ability and, more importantly, whether thresholds to define HPR or LPR may differ across different populations or ethnicities. In addition, it is unclear if the same cutoff values may be used for prasugrel or ticagrelor therapy, since the prognostic value of consensus-defined HPR and LPR has not been determined for these agents [10,18]. In particular, prasugrel and ticagrelor provide strong platelet inhibition with very low rates of HPR, but a percentage of patients treated with these drugs still present recurrent atherothrombotic events [15,16]. Further, prasugrel and ticagrelor have very high rates of LPR, as observed in the results of the present investigation, and yet these agents only slightly increase the risk of bleeding compared to clopidogrel [15,16], which questions the validity of currently used LPR definitions to predict hemorrhagic outcomes with these more potent drugs. Of note, our results showed greater platelet inhibition with ticagrelor compared to prasugrel in the steady-state phase of therapy, which is consistent with some prior PD studies [19,20].

There is limited data on the optimal timing to measure responsiveness to oral antiplatelet agents in the setting of ACS. Most studies showing an association between HPR to clopidogrel and clinical outcomes in ACS patients have performed a single measurement of platelet function in the peri-PCI period [2], but this strategy could potentially overestimate HPR rates due to increased platelet reactivity during the early phase of an ACS [21]. In the present investigation, for instance, the time from the loading dose of the antiplatelet drug to the collection of the blood sample for PFT was much lower in the prasugrel group (1 day in average) than in the clopidogrel or ticagrelor arms, which was clearly due to the greater percentage of STEMI among patients receiving prasugrel compared to the other agents. This might have affected the PD efficacy of prasugrel measured at day 1 after PCI, since it would be subject to greater variability due to being almost exclusively dependent of the effect of the loading dose. On the contrary, this issue will most certainly not affect platelet reactivity measurements at day 30 with patients at the steady-state phase of therapy will all three $P2Y_{12}$ inhibitors. In line with this, some investigations have suggested that platelet function assays performed remote from PCI might provide greater prognostic accuracy than those obtained in the periprocedural period [11]. This matter will be analyzed when the clinical follow-up of the present study (currently ongoing) is finished.

It must be noted that the greater platelet inhibition achieved with ticagrelor compared to prasugrel at 30 days was consistently observed

Table 1
Clinical, angiographic and procedural characteristics of the overall population and according to $P2Y_{12}$ inhibitor at day 1 post-PCI.

	Overall (n = 988)	Clopidogrel (n = 324)	Prasugrel (n = 195)	Ticagrelor (n = 469)	P value
Age (years), mean ± SD	64.2 ± 11.8	67.4 ± 15.1	57.2 ± 7.1	64.5 ± 13.7	< 0.001
Male gender, n (%)	771 (78.0)	229 (70.7)	161 (82.6)	381 (81.2)	< 0.001
Caucasian ethnicity, n (%)	960 (97.2)	312 (96.3)	191 (97.9)	457 (97.4)	0.484
Body mass index (kg/m^2), mean ± SD	28.3 ± 4.4	30.4 ± 3.4	25.8 ± 4.1	27.8 ± 3.6	0.695
Obesity (BMI > 30)	300 (30.4)	106 (32.7)	49 (25.1)	145 (30.9)	0.179
Cardiovascular risk factor, n (%)					
Active smokers	372 (37.7)	83 (25.6)	102 (52.3)	187 (39.9)	< 0.001
Hypertension	619 (62.7)	232 (71.6)	97 (49.7)	290 (61.8)	< 0.001
Diabetes mellitus	294 (29.8)	110 (34.0)	59 (30.3)	125 (26.7)	0.086
Dyslipidemia	624 (63.2)	211 (65.1)	108 (55.4)	305 (65.0)	0.043
Other medical history, n (%)					
Peripheral arterial disease	94 (9.5)	43 (13.3)	13 (6.7)	38 (8.1)	0.016
Prior MI	149 (15.1)	65 (20.1)	19 (9.7)	65 (13.9)	0.004
Prior PCI	159 (16.1)	69 (21.3)	23 (11.8)	67 (14.3)	0.006
Prior CABG	36 (3.6)	18 (5.6)	4 (2.1)	14 (3.0)	0.069
Chronic renal disease	140 (14.2)	91 (28.1)	10 (5.1)	39 (8.3)	< 0.001
Stroke	42 (4.3)	17 (5.2)	0 (0.0)	23 (4.9)	0.006
Chronic obstructive pulmonary disease	110 (11.1)	52 (16.0)	14 (7.2)	44 (9.4)	0.002
ACS diagnosis, n (%)					< 0.001
STEMI	372 (37.7)	111 (34.3)	135 (69.2)	126 (26.9)	
NSTEACS	395 (40.0)	109 (33.6)	34 (17.4)	252 (53.7)	
Unstable angina	198 (20.0)	97 (29.9)	23 (11.8)	78 (16.6)	
Other	23 (2.3)	7 (2.2)	3 (1.5)	13 (2.8)	
Baseline labs, mean ± SD					
Creatinine (μmol/l)	78.8 ± 32.6	90.6 ± 31.0	76.3 ± 19.6	80.5 ± 18.9	0.040
Hemoglobin (g/dl)	13.6 ± 1.9	13.7 ± 1.1	14.2 ± 1.0	14.1 ± 1.3	< 0.001
Platelets ($\times 10^9$/l)	226.2 ± 69.4	191.3 ± 35.0	216.7 ± 55.0	196.4 ± 41.5	0.883
Leukocytes ($\times 10^9$/l)	9.7 ± 3.1	8.4 ± 1.8	11.3 ± 3.0	9.0 ± 2.8	< 0.001
LDL cholesterol (mmol/l)	2.8 ± 6.2	2.3 ± 0.7	3.2 ± 2.1	2.8 ± 1.0	0.036
LD of $P2Y_{12}$ inhibitor, n (%)	976 (98.8)	317 (97.8)	193 (99.0)	466 (99.4)	0.152
Time from LD to PFT (days), mean ± SD	2.7 ± 2.9	3.1 ± 3.3	1.0 ± 0.3	3.2 ± 2.9	< 0.001
Procedural characteristics					
Number of vessels affected	1.63 ± 0.73	1.72 ± 0.77	1.58 ± 0.68	1.59 ± 0.72	0.040
Multivessel disease	474 (48.0)	169 (52.2)	93 (47.7)	212 (45.2)	0.155
Number of stents per patient	1.24 ± 0.80	1.27 ± 1.13	1.21 ± 0.54	1.22 ± 0.60	0.683
DES only	699 (70.8)	213 (65.9)	143 (73.3)	343 (73.1)	0.063
Anticoagulant during PCI, n (%)					< 0.001
Unfractionated heparin	823 (83.2)	281 (86.7)	178 (91.3)	360 (76.8)	
Other	165 (16.8)	43 (13.3)	17 (8.7)	109 (23.2)	
Other medications during hospitalization					
β-blocker	846 (85.6)	268 (82.7)	168 (86.2)	410 (87.4)	0.174
ACE inhibitors/ARBs	751 (76.1)	241 (74.4)	154 (79.0)	356 (76.1)	0.494
Nitrates	222 (22.5)	84 (25.9)	31 (15.9)	107 (22.8)	0.029
Proton-pump inhibitors	917 (92.8)	304 (93.8)	178 (91.3)	435 (92.8)	0.552
Statins	971 (98.3)	317 (97.8)	193 (99.0)	461 (98.3)	0.629
Dihydropyridines	139 (14.1)	67 (20.7)	13 (6.7)	59 (12.6)	< 0.001

ACE: angiotensin-converting enzyme; ARB: angiotensin receptor blocker; BMI: body mass index; CABG: coronary artery bypass grafting; DES: drug-eluting stent; MI: myocardial infarction; LD: loading dose; PCI: percutaneous coronary intervention; PFT: platelet function testing; SD: standard deviation.

with the VN-P2Y12 and the VASP assays, but not with the MEA ADPtest that displayed a similar efficacy for both agents. This discrepancy between tests cannot be considered entirely surprising because it is known that agreement between platelet function assays is moderate at its best with currently used thresholds to define HPR and LPR [22]. In addition, there are several factors (e.g. age, gender, diabetes mellitus, hemoglobin level, platelet count…) that have been proposed to differentially influence individual PFT methods and, therefore, modify test-to-test platelet reactivity comparisons and subsequently agreement between assays [22,23]. We actually believe this issue suggests the usefulness of performing dedicated studies to compare platelet function assays in specific populations and find the one with the optimal predictive ability.

We acknowledge the inherent limitations of this investigation due to being an interim PD analysis of a study of observational nature. In particular, since the clinical follow-up of this registry is still ongoing, no data is reported in the present manuscript regarding the association between platelet function measurements and clinical outcomes, which can be considered a limitation of the results presented. However, this is, to the best of our knowledge, the largest contemporary study evaluating the PD effect of the three most used oral $P2Y_{12}$ inhibitors in both the acute and maintenance phase of therapy in a real-life ACS population undergoing PCI. Baseline characteristics were quite different among groups; in particular, clopidogrel-treated patients had a greater burden of risk factors and comorbidities. The multivariate analysis was performed in order to minimize the effects of unbalanced baseline variables, but an impact of these characteristics on some between-agents differences in platelet reactivity measurements cannot be completely ruled out. Of note, the use of clopidogrel instead of more potent antiplatelet agents in patients with a higher risk profile has been observed previously and may represent physicians' intention to minimize the bleeding risk in real-life clinical practice [24].

In conclusion, our results, although not particularly novel, overall confirm the more potent and consistent PD effects of prasugrel and ticagrelor compared to clopidogrel and demonstrate that both prasugrel and ticagrelor had very low rates of HPR as well as very high rates of LPR during the acute and the maintenance phase of therapy after an ACS undergoing PCI with current consensus-defined thresholds. Further

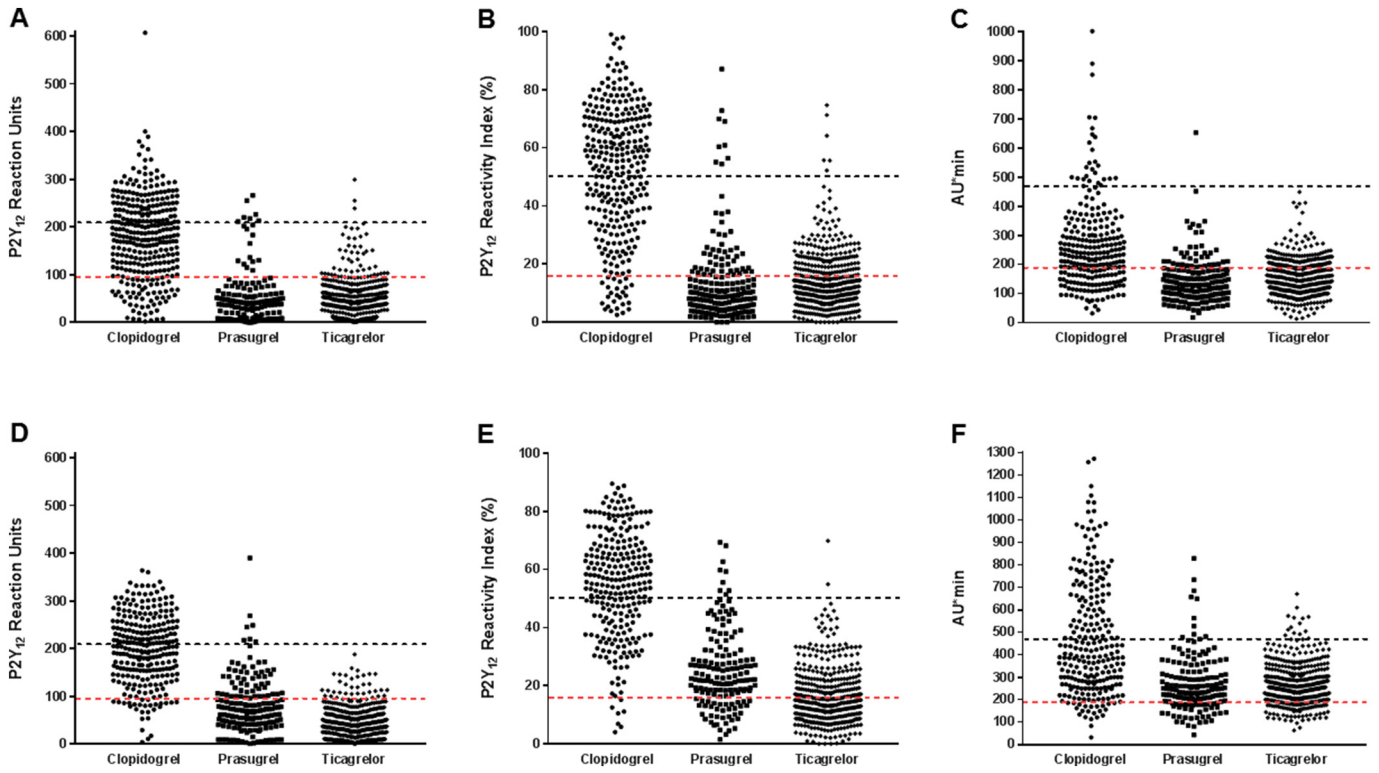

Fig. 1. Pharmacodynamic assessments according to $P2Y_{12}$ inhibitor.
A) $P2Y_{12}$ Reaction Units measured by the VerifyNow P2Y12 assay at day 1. B) Platelet reactivity index measured by the vasodilator-stimulated phosphoprotein assay at day 1. C) Platelet aggregation measured by multiple electrode aggregometry at day 1. D) $P2Y_{12}$ Reaction Units measured by the VerifyNow P2Y12 assay at day 30. E) Platelet reactivity index measured by the vasodilator-stimulated phosphoprotein assay at day 30. F) Platelet aggregation measured by multiple electrode aggregometry at day 30.
Black-dotted lines indicate cutoff values for high on-treatment platelet reactivity. Red-dotted lines indicate cutoff values for low on-treatment platelet reactivity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

investigation and dedicated studies are needed to understand the true usefulness of PFT in contemporary real-world clinical practice, especially when more potent antiplatelet agents such as prasugrel and ticagrelor are being used more and more frequently, which may increase the risk of bleeding in certain population subsets. Determining the specific cutoff values to define LPR to prasugrel and ticagrelor, which could be different from those established for clopidogrel, may undoubtedly help identifying these patients. In addition, newer clinical strategies such as de-escalation of $P2Y_{12}$ inhibitors [25,26] or monotherapy with a potent $P2Y_{12}$ agent are currently being investigated [27,28], and platelet function monitoring might help understanding what patients could benefit from these strategies.

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Role of the funding sources

The funding sources had no involvement in study design; in the

Table 2
Platelet function measurements at day 1 and day 30 post-PCI according to $P2Y_{12}$ inhibitor.

Day 1	Clopidogrel (n = 324)	Prasugrel (n = 195)	Ticagrelor (*n* = 469)	P value (CLO vs. PRA)	P value (CLO vs. TIC)	P value (PRA vs. TIC)
VN-P2Y12 assay (PRUs)	174.2 ± 3.5	42.7 ± 4.6	51.5 ± 2.8	< 0.001	< 0.001	0.298
VASP assay (PRI)	51.8 ± 0.9	12.5 ± 1.2	15.2 ± 0.8	< 0.001	< 0.001	0.189
MEA ADPtest (AU∗min)	269.5 ± 6.5	134.9 ± 7.8	160.4 ± 4.8	< 0.001	< 0.001	0.017
Day 30	**Clopidogrel (*n* = 275)**	**Prasugrel (*n* = 169)**	**Ticagrelor (*n* = 446)**	**P value (CLO vs. PRA)**	**P value (CLO vs. TIC)**	**P value (PRA vs. TIC)**
VN-P2Y12 assay (PRUs)	191.8 ± 3.3	89.2 ± 4.2	48.1 ± 2.5	< 0.001	< 0.001	< 0.001
VASP assay (PRI)	54.6 ± 0.9	25.2 ± 1.1	16.8 ± 0.7	< 0.001	< 0.001	< 0.001
MEA ADPtest (AU∗min)	458.4 ± 11.0	267.6 ± 13.5	276.7 ± 8.1	< 0.001	< 0.001	~1

Values are expressed as LSM ± SEM.
CLO: clopidogrel; MEA: multiple electrode aggregometry; PRA: prasugrel; TIC: ticagrelor; VN: VerifyNow; VASP: vasodilator-stimulated phosphoprotein.

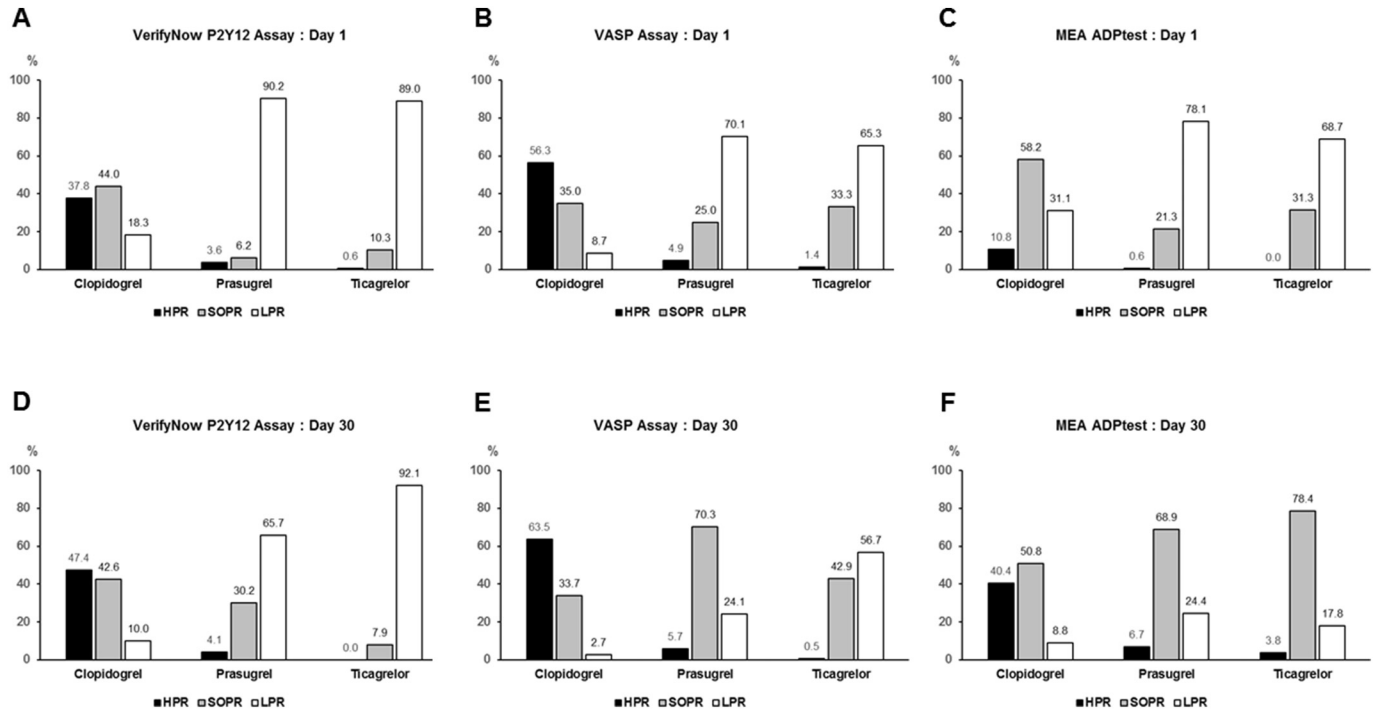

Fig. 2. High and low on-treatment platelet reactivity rates according to $P2Y_{12}$ inhibitor.
A) VerifyNow P2Y12 assay at day 1. B) Vasodilator-stimulated phosphoprotein assay at day 1. C) Multiple electrode aggregometry at day 1. D) VerifyNow P2Y12 assay at day 30. E) Vasodilator-stimulated phosphoprotein assay at day 30. F) Multiple electrode aggregometry at day 30.
HPR: high on-treatment platelet reactivity; LPR: low on-treatment platelet reactivity; MEA: multiple electrode aggregometry; SOPR: supposed optimal platelet reactivity; VASP: vasodilator-stimulated phosphoprotein.

collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Declaration of interest

José Luis Ferreiro (corresponding author) reports a) honoraria for lectures from Eli Lilly Co, Daiichi Sankyo, Inc., AstraZeneca, Roche Diagnostics, Pfizer, and Boehringer Ingelheim; b) consulting fees from AstraZeneca, Eli Lilly Co., and Ferrer; c) research grants from AstraZeneca.

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