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ORIGINAL ARTICLE

Evaluation of the role of the new INNOVANCE PFA P2Y test cartridge in detection of clopidogrel resistance

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Abstract

Light transmittance aggregometry (LTA) has been extensively used in monitoring clopidogrel therapy. However, the availability of simple and rapid point-of-care platelet function assays is of great clinical importance. Thus, the manufacturer of the Platelet Function Analyzer (PFA)-100 System has recently produced the INNOVANCE PFA P2Y test cartridge. We assessed the ability of this new test to reliably detect clopidogrel resistance. We enrolled 90 consecutive patients with coronary artery disease receiving chronic clopidogrel maintenance therapy in combination with aspirin. Twenty healthy volunteers served as controls. Clopidogrel resistance was simultaneously analysed by the INNOVANCE PFA P2Y test cartridge, ADP-induced LTA, the flow-cytometric vasodilator-stimulated phosphoprotein (VASP)-phosphorylation assay and the multiple electrode aggregometry (Multiplate). Agreement among the four platelet function methods by two was assessed using Cohen's kappa coefficient. According to the cut-off points for clopidogrel resistance proposed by the literature, agreement was fair between INNOVANCE PFA-100 P2Y and LTA (74.4%) and Multiplate (75.6%), while poor agreement was noticed in VASP assay (63.3%). Based on cut-off points indicating a higher thrombotic risk, agreement between the PFA-100 System and the other three methods did not significantly differ compared to the previous cut-offs (72.2%, 71.1% and 55.1%, respectively). The INNOVANCE PFA-100 P2Y test seems to be comparable to other established platelet function assays in detecting clopidogrel resistance. However, the modest agreement among platelet function methods makes the performance of platelet function testing crucial with more than one technique in order to reliably identify poor responders to clopidogrel treatment.

Keywords: *Clopidogrel resistance, antiplatelet treatment, INNOVANCE PFA-100 P2Y*

Introduction

Clopidogrel has proved to be an important agent for the management of patients with cardiovascular disease. It is a pro-drug converted to an active metabolite by the hepatic cytochrome P450 system. The anti-aggregatory effect of the active metabolite is mediated by irreversibly binding and antagonizing the P2Y12 receptor for the life of the platelet [1] and can be measured *ex vivo* by light transmittance aggregometry (LTA). Although LTA is considered the gold standard for platelet function testing [2], it requires large sample volumes and skilled technicians to perform the tests and interpret the results. Additionally, in case aggregometry is used to define the response to anti-aggregating agents, agreement

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on the kind of inducer, its concentrations and the selection of cut-off points is essential [3]. Therefore, it is of great clinical importance to develop simple, rapid, point-of-care platelet function assays that are easier to use and more reproducible [4].

For the measurement of thienopyridines' effects, the manufacturer of the Platelet Function Analyzer (PFA)-100 System (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) has recently produced the INNOVANCE PFA P2Y test cartridge. The PFA-100 is an instrument for the evaluation of high-shear stress-dependent platelet function based on a cartridge system in which the process of platelet adhesion and aggregation following a vascular injury is simulated *in vitro* [5]. The instrument estimates the ability of platelets activated in a high-shear environment to occlude an aperture in a membrane treated with collagen and epinephrine (CEPI) or collagen and ADP (CADP). The time taken for flow across the membrane to stop (closure time, CT) is recorded. The CADP cartridge is usually not very sensitive to clopidogrel maintenance doses [6–8].

On the contrary, the new cartridge, the membrane of which is coated with 20 μg ADP, 5 ng prostaglandin E1, and 459 μg calcium chloride, has been provided as more suitable for assessing the effect of clopidogrel on platelet function. In a recently performed *in vitro* study, the INNOVANCE PFA P2Y test cartridge proved to be sensitive to P2Y(12) inhibition and comparable to other currently available platelet function tests [9].

Taking into consideration that insufficient response to antiplatelet therapy has been reported to contribute to a higher risk of recurrent cardiovascular events [10, 11] and that available comparative clinical studies evaluating the validity of the new method are scarce [12, 13], we attempted to assess the capacity of the INNOVANCE PFA P2Y test cartridge to detect clopidogrel resistance in relation with two other well-established methods, the LTA and the flow-cytometric vasodilator-stimulated phosphoprotein (VASP)-phosphorylation assay [14], and with a point-of-care device, the multiple electrode aggregometry (Multiplate, MEA) [15] in a group of patients with coronary artery disease (CAD).

Methods

The study population consisted of 90 consecutive patients with documented – by coronary angiography – CAD who were enrolled during a 12-month period (June 2010–June 2011). Patients were hospitalized after an acute coronary syndrome (ST-elevation myocardial infarction, STEMI; Non-ST-elevation myocardial infarction, NSTEMI, and unstable angina) and underwent an elective coronary angiogram in the Second University Department of Cardiology of the ''Attikon'' University Hospital in Athens, Greece.

All patients were on chronic clopidogrel maintenance therapy of 75 mg/day for >5 days in combination with daily per os aspirin therapy (100 mg). The study was performed in accordance with the Declaration of Helsinki and was approved by the hospital's institutional review board. Informed consent was obtained from all patients. We also enrolled 20 healthy blood donors as controls in order to confirm normal ranges proposed by the literature. Exclusion criteria were renal or hepatic insufficiency, malignant disease, use of drugs known to affect platelet function, history of bleeding diathesis, platelet count $<120 \times 10^9$/l and a hematocrit <28%.

The last dose of antithrombotic medication was administered 1–24 hours before blood sampling. All platelet function tests were performed on the same day, within 2 hours of sampling, which was performed in the mid-morning hours [16]. Baseline assessment included recording of demographic data, medical history, hematological parameters, von Willebrand factor (VWF) activity, fibrinogen levels and concomitant medications. Full blood counts were performed on Sysmex XE-2100 analyzer (Roche, IL, USA) while ristocetin cofactor activity of VWF activity in patients' citrated plasma was estimated using a platelet agglutination method (Von Willebrand Reagent Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) on the BCS® XP System Hemostasis analyzer (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). Fibrinogen was measured by the Clauss method on the BCS system, using the Multifibren U reagent (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). EDTA and citrate (3.2 and 3.8%) blood collection tubes (BD Vacutainer, Belliver Industrial Estate, Plymouth, UK) were used for blood sampling.

In statistical calculations, for all platelet function assays we used reference ranges as they are provided by the literature of manufacturers. Furthermore, a second agreement analysis was performed with cut-offs indicating a higher thrombotic risk [17]. Eight control samples were doubled-measured with the four methods in order to estimate their reliability.

Light transmittance aggregometry

The whole blood specimen was collected in 3.8% trisodium citrate and centrifuged at 200 *g* for 10 minutes to obtain platelet-rich plasma (PRP). The remaining specimen was re-centrifuged at 2000*g* for 15 minutes to obtain platelet-poor plasma (PPP). The platelet count was adjusted to between 200,000

and 300,000/μl with PPP. Aggregation was performed using a Biodata-PAP-4 aggregometer (Bio/Data Corporation, Horsham, PA, USA). The 100% line was set using PPP and a 0% baseline established with PRP before addition of the agonist. The agonist used was ADP 2.0×10^{-5} M (Bio/Data Corporation). Test procedure was performed as previously described [18]. In brief, 0.45 ml PRP were transferred into a cuvette incubated at 37°C for 3 minutes. Then 0.05 ml of the agonist was added into the PRP and the aggregation pattern was allowed to generate for 6 minutes. Typical normal platelet aggregation responses were considered 63–89% according to the reference values reported in package insert. Results are given as the percentage change in light transmittance from baseline 6 minutes after the addition of the agonist. Since it has been suggested that the focus of interpretation of the results from LTA in clopidogrel-treated patients should be the absolute level of late aggregation instead of ''peak'' aggregation [19], we have measured both the maximum extent of peak, and late aggregation (at 6 min) after the addition of ADP. Another tested parameter was the degree of disaggregation. Non-responsiveness to clopidogrel was defined as peak aggregation responses within the reference range despite clopidogrel medication. The potential association of late aggregation and disaggregation with INNOVANCE PFA P2Y CTs was also investigated.

PFA-100

For this test, whole blood collected in 3.8% trisodium citrate. 0.8 ml of the mixed whole blood was pipetted into the sample reservoir of the new test cartridge (prewarmed to room temperature) and then loaded into the PFA-100. CT was recorded. The maximal CT was 300 s, and values higher than 300 s were reported as non-closure. For non-closures, a CT value of 300 s was used in calculations. INNOVANCE PFA P2Y CTs $\leq$106 s were considered normal, while those exceeding 106 seconds were viewed as abnormal, as mentioned in the manufacturer's reagent instruction booklet. A CT within the reference range was considered to indicate non-responsiveness to clopidogrel.

Multiple electrode aggregometry

Platelet aggregation in whole blood was assessed by MEA using an impedance aggregometer (Multiplate, Dynabyte, Munich, Germany). Samples were collected into 3.2% citrate tubes and analysed within the period of 0.5 to 2 hours after blood collection according to manufacturer's instructions. Due to the use of citrated tubes, our effort was to analyse samples as soon as possible [20, 21]. Platelet aggregation was induced by ADP in final concentration 6.5 μM. Each disposable test cell contains two pairs of electrodes, thus enabling two simultaneous measurements. Aggregation was reported as area under the curve (AUC), an integrated measure of aggregation velocity and maximal aggregation. Values within the reference ranges (38–85 AUC, Units) were indicative of resistance to clopidogrel.

Flow-cytometric VASP-phosphorylation assay

Flow-cytometric analysis of VASP-phosphorylation was performed by a CE marked diagnostic kit from Biocytex (Marseille, France), as described previously [22]. In brief, whole-blood into 3.2% citrate tubes was incubated with PGE1 alone or PGE1 + ADP. After cellular permeabilization, VASP under its phosphorylated state was labeled by indirect no wash immunofluorescence using a specific monoclonal antibody (clone 16C2). Dual color flow cytometry analysis allows to compare the two tested conditions and to evaluate for each sample the capacity of ADP to inhibit VASP phosphorylation. A platelet reactivity index (PRI) was calculated using corrected mean fluorescence intensities in the presence of PGE1 alone or PGE1 and ADP simultaneously. Samples were run on a Partec CyFlow ML (Partec GmbH, Münster, Germany) with a 488 nm argon laser, with front-scatter (FSS) and side-scatter (SSC) set on logarithmic scale. According to the publication of Aleil et al. [14] the PRI of the patients with ischemic cardiovascular disease, not receiving clopidogrel was $79.0 \pm 4.1\%$ (express as mean $\pm$ SD). Poor responders were defined by a PRI greater than this value.

Statistical methods

Mean $\pm$ SD or median (Q_1–Q_3) values for normally or non-normally distributed continuous variables or *n* (%) for categorical values are presented.-VASP assay PRI is given as geometric mean [23, 24]. Reliability of the four methods was estimated using intra-class correlation coefficient (ICC) and coefficient of variation (CV) [25]. Agreement among the four platelet function methods by two was assessed then using Cohen's kappa coefficient. Spearman correlation coefficients were also used to test for associations between INNOVANCE PFA-100 P2Y CTs and ADP-induced LTA values or between the PFA-100 System measurements and other haematological parameters for cases and controls separately. All tests of significance were two-sided and we used an alpha level of 0.05 for all statistical tests. All statistical analyses were performed using the package STATA 11.0.

Table I. Descriptive statistics.

Characteristics	Cases ($n = 90$)
Age (years)	61.7 ± 12.2
Males (%)	78 (87.6)
Hyperlipidemia (%)	74.4
Hypertension (%)	61.2
Smoking (%)	54.4
Diabetes (%)	35.5
PLT (10^3/μl)	240 ± 64
Ht (%)	40.6 ± 4.8
WBC (10^3/μl)	8.17 (7.01–9.96)
VWF activity (IU/dl)	150.4 (110.8–150.4)
Multiplate AUC (units)	24.3 ± 12.4
PFA-100 P2Y CT (s)	300 (77–300)
VASP PRI (%) (cases = 49)	58.5 {52.4, 65.4}*
LTA ADP (%)	39.3 ± 17.5
Medications	
Beta-blockers (%)	93.3
Nitrates (%)	84.4
Calcium channel blockers (%)	15.5
ACE/ARBs (%)	83.3
Statins (%)	94.4

Notes: Data are presented as mean ± SD, percentages, or median (Q_1–Q_3).
*Geometric mean, 95% confidence intervals (CI). *Abbreviations*: PLT, platelets; Ht, hematocrit; WBC, white blood cells; VWF, von Willebrand factor; AUC, area under the curve; CT, closure time; VASP, vasodilator-stimulated phosphoprotein; PRI, platelet reactivity index; LTA, light transmittance aggregometry; and ACE/ARBs, angiotensin converting enzyme inhibitors/angiotensin receptor blockers.

Results

The descriptive characteristics, hematological parameters, platelet function assays measurements and VWF activity levels of cases are presented in Table I. The control group consisted of 12 males and 8 females, with a mean ± SD age of 38.8 ± 11.8. The mean ± SD values for haematocrit (%) and platelet count ($\times 10^3$/μl) were 45.6 ± 3.6 and 226 ± 47, respectively, while the median (Q_1–Q_3) value of VWF activity (IU/dl) was 67.2 (56.4–140.7).

ICCs for VASP-assay, Multiplate, INNOVANCE PFA-100 P2Y and LTA were 0.84 (95% CI: 0.15–0.97), 0.94 (95% CI: 0.68–0.99), 0.89 (95% CI: 0.46–0.98), and 0.90 (95% CI: 0.47–0.98), respectively, showing high reliability of the methods (even though lower 95% CI limits cannot be considered satisfactory). CVs were 2.0%, 7.4%, 11.9%, and 3.3% for VASP-assay, Multiplate, INNOVANCE PFA-100 P2Y and LTA, respectively. The flow-cytometric VASP-phosphorylation assay was performed only in 49 patients due to transient reagent insufficiency. The cut-off values based on the reference range derived from the 5th–95th percentiles of measurements from the group of healthy volunteers were 113 seconds, 49.5%, 76.5%, and 35 AUC units for INNOVANCE PFA-100 P2Y, LTA, flow-cytometric VASP assay and Multiplate, respectively, which are comparable to those proposed by the literature. Based on cut-off points derived from the literature, the NNOVANCE PFA-100 P2Y cartridge, LTA, Multiplate and VASP-assay detected 34.4%, 8.8%, 14.4%, and 8.8% of clopidogrel poor responders, respectively.

Table II gives Cohen's kappa for the four methods according to the cut-off points proposed by the literature. Agreement was fair between INNOVANCE PFA-100 P2Y and ADP-induced aggregometry or Multiplate, while poor agreement was noticed between the PFA-100 System and VASP-phosphorylation assay. Table II also presents Cohen's kappa for the four methods according to the cut-off points, indicating a higher thrombotic risk. Agreement between the PFA-100 System and the other three methods was similar compared to the previous cut-offs (Figures 1–3).

There was a statistically significant negative correlation of INNOVANCE PFA-100 P2Y CTs with peak and late aggregation, Multiplate and VASP-assay values, a weak positive correlation with disaggregation values and weakly but statistically significant association with hematocrit in cases (Table III). The INNOVANCE PFA-100 P2Ycartridge CTs were moderately negatively correlated with VWF activity values in controls (r: –0.20, p: 0.07).

Discussion

The agreement between INNOVANCE PFA-100 P2Y and ADP-induced LTA or Multiplate was about 75%. Cohen's kappa coefficients, as a measure of agreement between the methods, indicated fair agreement regardless the cut-off points used. On the contrary, the agreement between INNOVANCE PFA-100 P2Y and the flow-cytometric VASP assay was poor.

The phenomenon of ''clopidogrel resistance'' has been estimated to range between 4% and 30%. This great variation is probably due to its complex definition. Although LTA has been extensively used in monitoring clopidogrel therapy, the physiological extent of inhibition detected by ADP-induced LTA can vary widely, especially as ADP can also activate platelets via a second receptor, P2Y1. Based on our findings, INNOVANCE PFA-100 P2Y cartridge revealed the higher percentage of poor responders to clopidogrel (34%) compared to the other three methods, while flow-cytometric VASP assay showed the lower percentage (8.8%). This is in keeping with the finding of Koessler et al. [13] that INNOVANCE PFA-100 P2Y is more sensitive than flow-cytometric VASP assay in the detection of P2Y12 receptor

Table II. Cohen's kappa coefficient for the four methods according to cut-off points derived from the literature (in normal fonts) and those indicating a higher thrombotic risk (in *italics*).

	INNOVANCE PFA-100 P2Y CT (s)		LTA with ADP (%)		Flow-cytometric VASP assay PRI (%)	
Cut-off points	<106	≥106	>63	≤63	≥79	≤79
	% Agreement	Kappa (SE)	% Agreement	Kappa (SE)	% Agreement	Kappa (SE)
LTA with ADP (%)	74.4%	0.31 (0.08)*				
	72.2%	*0.24 (0.07)**				
≥63	8	0				
≥64.5	6	0				
≤63	23	59				
≤64.5	25	59				
Flow-cytometric VASP assay PRI (%)	63.3%	0.16 (0.11)	83.7%	0.25 (0.13)*		
	55.1%	*0.18 (0.11)**	*28.6%*	*0.04 (0.04)*		
>79	5	3	2	6		
>50	18	20	3	35		
≤79	15	26	2	39		
≤50	2	9	0	11		
Multiplate (AUC, units)	75.6%	0.37 (0.09)*	85.6%	0.30 (0.10)*	73.5%	–0.02 (0.14)
	71.1%	*0.20 (0.06)**	*90.0%*	*0.13 (0.10)*	*28.6%*	*0.04 (0.04)*
≥38	11	2	4	9	1	6
≥46.8	5	0	1	4	3	0
≤38	20	57	4	73	7	35
≤46.8	26	59	5	80	35	11

Notes: Abbreviations: CT, closure time; PRI, platelet reactivity index; SE, standard error; LTA, light transmittance aggregometry; VASP, vasodilator-stimulated phosphoprotein; and PRI, platelet reactivity index.
*Statistically significant value.

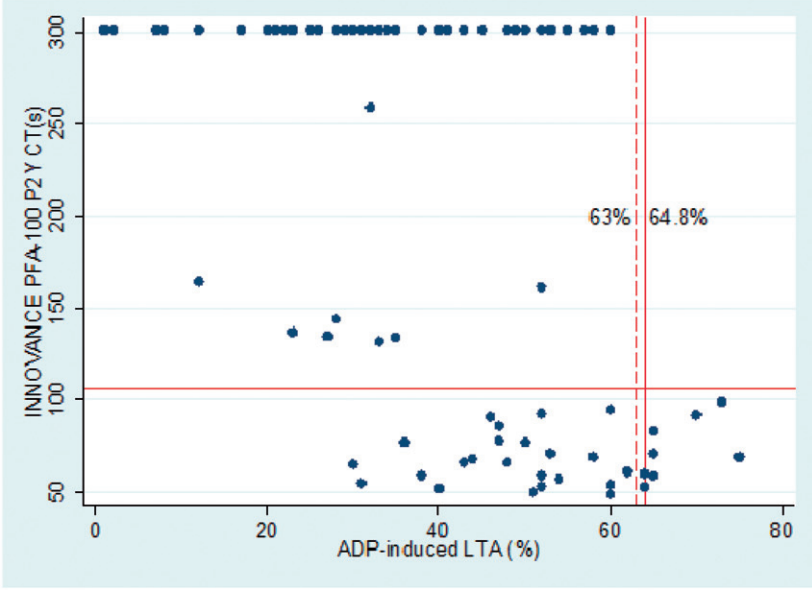

Figure 1. Distribution of values derived from measurements performed in patient samples ($n = 90$) by the INNOVANCE PFA-100 P2Y cartridge and ADP-induced LTA. Points in the right lower quadrant are those detected to be resistant with both methods, while points in the right upper and left lower quadrant are those found to be resistant with only one method at the time. Cut-off points are indicated by the lines on the graph (dashed line = literature-supported cut-off; continuous line = cut-off indicating a higher thrombotic risk).

inhibition in patients under dual antiplatelet therapy. Similarly, the increased sensitivity of INNOVANCE PFA-100 P2Y compared to LTA noted in our study, has also been reported by Linnemann et al. [12], supporting the usefulness of this system for differentiating between clopidogrel responders and non-responders in patients on combination therapy of aspirin and clopidogrel. It seems that the impact of aspirin on ADP-induced aggregometry renders this method less suitable for monitoring clopidogrel

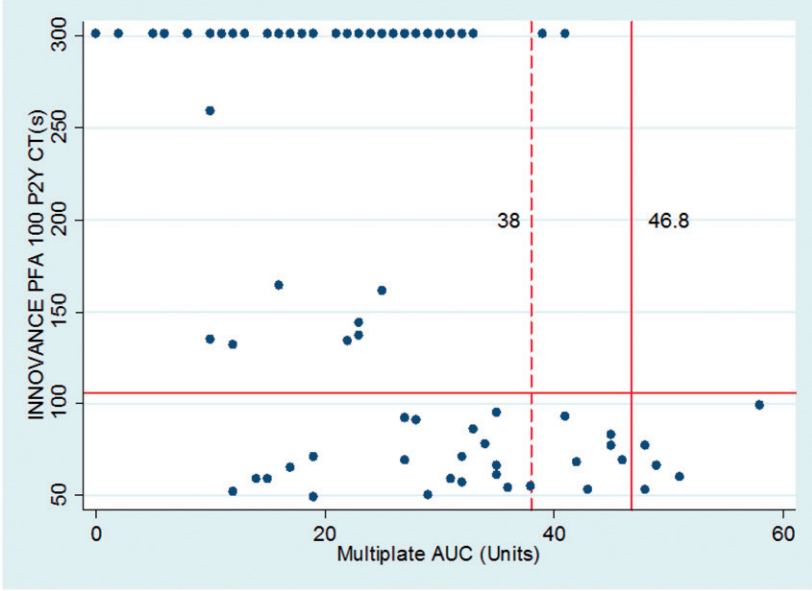

Figure 2. Distribution of values derived from measurements performed in patient samples ($n = 90$) by the INNOVANCE PFA-100 P2Y cartridge and Multiplate device. Points in the right lower quadrant are those detected to be resistant with both methods, while points in the right upper and left lower quadrant are those found to be resistant with only one method at the time. Cut-off points are indicated by the lines on the graph (dashed line = literature-supported cut-off; continuous line = cut-off indicating a higher thrombotic risk).

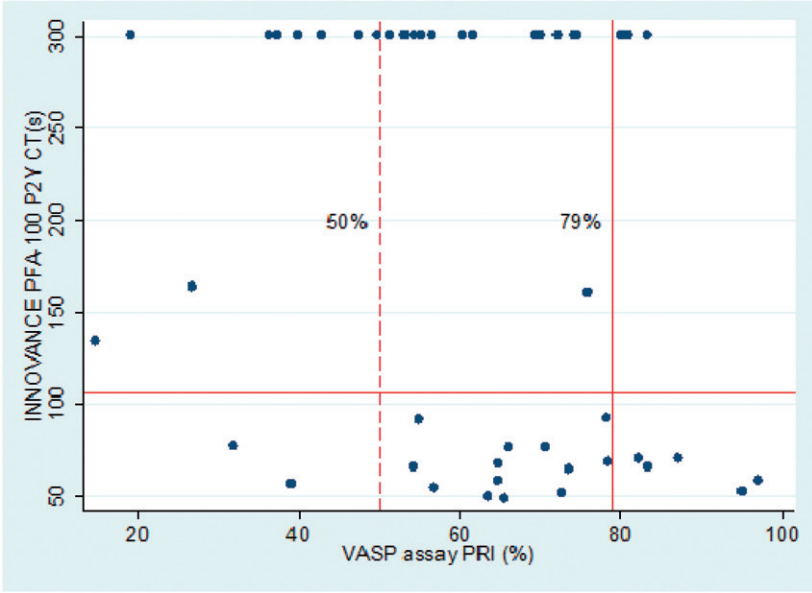

Figure 3. Distribution of values derived from measurements performed in patient samples ($n = 49$) by the INNOVANCE PFA-100 P2Y cartridge and flow-cytometric VASP assay. Points in the right lower quadrant are those detected to be resistant with both methods, while points in the right upper and left lower quadrant are those found to be resistant with only one method at the time. Cut-off points are indicated by the lines on the graph (dashed line = literature-supported cut-off; continuous line = cut-off indicating a higher thrombotic risk).

treatment in patients receiving dual antiplatelet therapy.

On the other hand, it remains unclear whether peak or late aggregation represents the best pharmacodynamic evaluation of the platelet response to clopidogrel, although the latter has been reported as more suitable [19]. It has been hypothesized that P2Y1 is mainly responsible for the maximal amplitude of platelet aggregation, whereas P2Y12 is responsible for the stabilization of aggregation [26, 27]. Therefore, the absolute level of platelet aggregation after a fixed period of time is considered a better representation of the inhibitory effects of clopidogrel treatment [28]. However, in our study both peak and

Table III. Spearman correlation coefficients between INNOVANCE PFA-100 P2Y (CT) and other laboratory parameters.

	Cases	
	r	*p*-value
Peak aggregation (%)	–0.51	<0.001
Late aggregation (%)	–0.55	<0.001
Disaggregation (%)	0.39	0.0003
Multiplate (AUC, units)	–0.47	<0.001
VASP PRI (%)	–0.41	0.003
Plt ($\times 10^3/\mu L$)	0.14	0.21
Ht (%)	0.26	0.02
WBC (/μL)	0.05	0.69
VWF activity (IU/dL)	*–0.20*	*0.07*
Fibrinogen (mg/dL)	–0.17	0.17

Notes: Abbreviations: CT, closure time; AUC, area under the curve; PRI, platelet reactivity index; Plt, platelets; Ht, Hematocrit; WBC, white blood cells; and VWF, von Willebrand factor.

late aggregation were found to be equally correlated with INNOVANCE PFA-100 P2Y CT values. Another parameter tested was disaggregation, which is often observed in patients under clopidogrel treatment. On the contrary, in healthy subjects disaggregation is rarely seen. A statistically significant positive correlation was found between INNOVANCE PFA-100 P2Y CTs and disaggregation corroborating the significant association between the PFA-100 system and LTA parameters. Generally, these two methods seem to exhibit satisfactory agreement in detection of several platelet function disorders [18, 29], despite the different methodology they use.

As residual P2Y1 function can potentially widely vary despite P2Y12 inhibition, this probably suggests that ADP alone may not be specific enough to measure the effect of clopidogrel. That is why, some assays use prostaglandin E1 in addition to ADP to increase intracellular cyclic adenosine monophosphate, which theoretically enhances the sensitivity and specificity of the test for ADP-induced activation of platelets via P2Y12 [30]. The PGE1 should suppress the activation of platelets by P2Y1. The combination of ADP and PGE1 is used in the flow-cytometric-based VASP assay [31] and INNOVANCE PFA-100 P2Y. Despite this common characteristic in their performance, the two methods exhibited poor agreement. As far as it concerns the other point-of-care device used in our study, Multiplate, which measures the change in resistance between two electrodes as platelets adhere and aggregate in response to the agonist, showed similar extent of agreement with the PFA-100 System in relation with LTA. The degree of agreement between Multiplate and LTA suggests that this point-of-care platelet function assay is also a valid method of measuring the response to clopidogrel.

Estimated CVs were consistent with high reliability of the instruments used, and specifically for the new INNOVANCE PFA-100 P2Y cartridge were similar with CVs of duplicates estimated on normal subjects not receiving any antiplatelet medication for the CEPI and CADP cartridges [32, 33]. The four platelet function assays used in this study, assess different aspects, pathways and characteristics of primary hemostasis. This might partially account for the wide range of agreement observed among the different testing modalities. Furthermore, factors such haematocrit and platelet count affect several platelet function methods to a different extent [34]. For instance, it has been reported that the two initially commercially available PFA-100 cartridges are influenced by several parameters like platelet count, haematocrit, and plasma levels of VWF [35]. In controls the significant effect (inverse correlation) of the VWF activity levels on the new INNOVANCE PFA-100 P2Y cartridge CTs was corroborated [36], while this influence was not observed in patients treated with clopidogrel, which seems to be the major determinant of the INNOVANCE PFA-100 P2Y CT values. A possible limitation in our study is the limited number of healthy blood donors recruited to confirm the cut-off points for platelet function assays. But reference ranges derived from our control group were similar with those proposed by the literature. It has to be noted that values within normal range does not always mean ''clopidogrel resistance'', since the exact definition of ''resistance'' to antiplatelet therapy on the basis of physiology has not been established. On the other hand, the prevalence of hyporesponsiveness may be an aberration of the methodology. Although evidence suggests that poor responders to clopidogrel experience more frequent cardiovascular events than do responders [37], it should be noted that this correlation does not indicate that treatment failure (the clinical outcome of a recurrence of ischemic events) is always associated with clopidogrel resistance (failure to inhibit platelets' activity).

Conclusively, the pharmacodynamic effect of thienopyridines can be demonstrated reliably by several platelet function testing modalities based on different principles and affected by different factors, but they display a wide range of agreement. The INNOVANCE PFA-100 P2Y seems to overcome the limitations of the LTA and be comparable to other established platelet function assays in identification of patients with clopidogrel resistance, especially in patients under dual antiplatelet therapy. However, due to the lack of high agreement among platelet function tests, the limited experience in their use, and their unknown clinical relevance,

establishment of the appropriate testing cut-offs for several methods, and performance of platelet function testing with more than one techniques are of great importance in order to facilitate inter-hospital comparisons and identify reliably clopidogrel poor responders.

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Notice of Correction

The version of this article published online ahead of print on 30 May 2012 contained an error on page 4, Table II. Some of the numbers were not italicized that should have been. The corrected version is shown in this issue.