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Multi-parameter assessment of platelet inhibition and its stability during aspirin and clopidogrel therapy

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Abstract

Introduction—Poor response to antiplatelet drugs is associated with adverse outcomes. We assessed platelet inhibition and its stability and tested correlation and agreement between platelet function assays.

Methods—Peripheral blood from 58 patients on both aspirin and clopidogrel who underwent percutaneous coronary intervention (PCI) was collected at hospital discharge (visit-1) and at 30–90 days (visit-2). Platelet function was measured using light transmission aggregometry (LTA-AA and LTA-ADP), VerifyNow (Aspirin; ARU and P2Y12; PRU), *ex vivo* TxB₂, urinary 11dhTxB₂, and VASP (PRI) assays. Data were analyzed as continuous, quartiles and binary. Patients were defined as aspirin poor responder (PR) with ARU > 550, LTA-AA maximum > 20%, TxB₂ > 1 ng/mL or 11dhTxB₂ > 1,500 pg/mg of creatinine and as clopidogrel PR with PRU > 240, PRU > 208, LTA-ADP maximum > 40%, PRI > 50%, or PRI > 66%.

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Disclosure of Conflict of Interests

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Results—Aspirin PR was 3–33% and clopidogrel PR was 10–35% in visit-1. LTA-AA, 11dhTxB₂, and all clopidogrel-response measures showed correlation and agreement between visit-1 and visit-2. The highest agreement between two visits was revealed by PRU 240 and PRI 66% (PRU- κ =0.7, 95% CI=0.47, 0.93; PRI- κ =0.69, 95% CI=0.42, 0.95, p-values<0.001). Comparison of platelet function assays in a single visit (Visit-1) revealed a poor correlation between LTA-AA and 11dhTxB₂ assays and no agreement among aspirin-response assays. The highest correlation and agreement were obtained between VerifyNow P2Y12 and VASP assays (ρ =0.7, p-value<0.001 and PRU 208-PRI- κ =0.41–0.42, 95% CI=0.13, 0.69, p-values<0.001).

Conclusions—Platelet inhibition is stable during aspirin and clopidogrel treatment. Clopidogrel-response assays correlate and agree with each other better than aspirin-response assays.

Keywords

Aspirin; cardiovascular diseases; clopidogrel; drug resistance; percutaneous coronary intervention; platelet function assay

Introduction

Patients with stable or acute coronary syndromes and those undergoing percutaneous coronary intervention (PCI) receive pharmacotherapy targeting the inhibition of platelet activation and coagulation cascade [1–4]. The established and commonly used antiplatelet medications include aspirin and the thienopyridine clopidogrel. Aspirin irreversibly inhibits cyclooxygenase-1 (COX-1) and thereby prevents the conversion of arachidonic acid (AA) to thromboxane A₂ (TxA₂), while clopidogrel irreversibly blocks adenosine diphosphate (ADP) receptor P2RY12 [5]. However, platelet inhibition is still inadequate in 5.5–60% of the patients receiving aspirin and in 4–30% of the patients on clopidogrel [6]. High platelet reactivity against both drugs, dual non-responsiveness, was reported in 6% of the patients of whom one-half were not responsive to aspirin and were also non-responsive to clopidogrel [7,8].

Poor drug response has been associated with adverse clinical outcomes. Patients who were poor responders to aspirin were reported to have 4-fold risk of fatal and non-fatal cardiovascular, cerebrovascular, or vascular events compared to aspirin responsive patients [9]. Similarly, clopidogrel poor responders were shown to have 3-5-fold risk of myocardial infarction (MI), stent thrombosis, recurrent ischemic events, and cardiovascular death [10]. Therefore, to assess platelet inhibition and its stability is crucial for evaluation of antiplatelet treatment. On the other hand, identification of inadequate platelet inhibition is challenging due to the lack of correlation between currently available platelet function assays [11]. In this study, we aimed to assess the variability and durability of platelet inhibition in patients on combined aspirin and clopidogrel therapy over a 30-90-day period following PCI. Our secondary aim was to test the correlation and agreement between various platelet function assays.

Materials and methods

Patient population, study design, and sampling

ASCLOGEN (ASpirin and CLOpidogrel: Genotype vs. Platelet Function Phenotype in Clinical Response) is a prospective observational study which enrolled male and female patients undergoing PCI at the Cleveland Clinic (Cleveland, Ohio). Dual antiplatelet therapy with aspirin and clopidogrel was administered to these patients, with dosages determined by standard clinical practice. All patients originating from Northeast Ohio were sequentially approached to enroll in the substudy to determine the stability of platelet function with time. The first 62 patients that met the geographic requirements and consented were enrolled in the substudy. Exclusion criteria for the ASCLOGEN enrollment included the use of ticlopidine, dipyridamole, steroidal drugs, and COX-2 inhibitors during two weeks prior to enrolment, GPIIb/IIIa inhibitor within 7 days prior to enrolment or during PCI, pregnancy, known history of platelet disorders, thrombocytopenia ($<125 \times 10^3/\mu\text{L}$), severe anemia (hemoglobin <10 g/dL and hematocrit $<30\%$), allergy to aspirin or clopidogrel, major surgical procedures in the past week, interruption in aspirin and/or clopidogrel therapy for more than two weeks for any event in the 30–90 days following the catheterization. The study was approved by the Institutional Review Board.

Two visits were arranged for each patient to collect samples. Visit-1 was at patient discharge from the hospital following PCI and visit-2 was at 30–90 days after PCI. Daily dosage of aspirin was 81 mg, 162 mg, and 325 mg in 47%, 5%, and 48% of the patients, respectively. Almost half of the patients were on 75 mg/day clopidogrel. The rest was clopidogrel naive and received a 150–600 mg clopidogrel loading dose before PCI. Twenty patients also received 150–325 mg periprocedural clopidogrel. At visit-2, 53% of the patients were found switched from the initial dose of aspirin to one of three doses administered. All patients but two were taking 75 mg clopidogrel daily; two patients were taking 150 mg/day clopidogrel.

For each visit, peripheral venous blood was drawn into 1.8 ml and 3.15 ml vacutainer tubes containing 3.2% trisodium citrate and processed to measure platelet function within two hours of sampling. A urine sample >20 ml was also collected from each patient and stored at -80°C . Complete blood count (CBC) was carried on Beckman Coulter AC^C •T diffTM Analyzer (Beckman Coulter, Miami, Florida).

Platelet function assays

VerifyNow[®] assay—VerifyNow[®] assay (Accumetrics, Inc., San Diego, California) measures platelet-induced aggregation in whole blood. The assay cartridges VerifyNow[®] Aspirin and VerifyNow[®] P2Y₁₂ use AA and ADP as the platelet agonists, respectively, and contain a lyophilized preparation of human fibrinogen-coated beads. Two 1.8 ml-citrated whole blood samples were used for the assay. The instrument measures light transmittance as aggregation occurs and data are converted into Aspirin Reaction Unit (ARU) or P2Y₁₂ Reaction Unit (PRU).

Light transmission aggregometry (LTA)—Platelet rich plasma (PRP) was prepared by centrifuging the citrated whole blood at 180g for 15 minutes at room temperature. The

platelet count was adjusted to $200\text{--}300 \times 10^3/\mu\text{l}$ with platelet poor plasma (PPP) obtained by centrifuging the remaining blood at 2,880g for 15 minutes. Platelet aggregation was stimulated with 0.5 mg/mL (1.6 mmol/L) AA and 10 μM ADP and measured for 10 minutes using BioData PAP-4 platelet aggregometer (BioData Corp., Horsham, Pennsylvania). A 50 μl of Indomethacin/EDTA solution (1:20 mixture of 0.04 M Indomethacin and 0.05 M EDTA) was then added to prevent further platelet aggregation and samples were centrifuged at 2,880g for 15 minutes. Obtained supernatants were kept frozen at -80°C for TxB_2 assay. Aggregation was expressed as the maximal per cent change in light transmittance from baseline which was set with PPP. Two measures of aggregation, maximum and late aggregation at 10 minutes, were analyzed.

Thromboxane B_2 (TxB_2) assay— TxB_2 , a stable metabolite of thromboxane A_2 , was measured in plasma following *ex vivo* activation of platelets for LTA assay. The supernatants of platelet aggregates were first purified using Bond Elute C18 column (96-well plate, Varian, Inc., Walnut Creek, California). TxB_2 in these purified samples was then measured using TxB_2 ELISA Kit (Enzo[®] Life Sciences, Inc., Plymouth Meeting, Pennsylvania) according to the manufacturer's protocol.

Urinary 11-dehydro thromboxane B_2 (11dh TxB_2) assay—Urinary 11dh TxB_2 levels were measured using AspirinWorks[®] Test Kit (Corgenix, Inc., Broomfield, Colorado), normalized with urine creatinine level of the same sample and reported as pg/mg of creatinine.

Vasodilator stimulated phosphoprotein (VASP) phosphorylation assay—Phosphorylation of VASP was assessed by flow cytometry using PLT VASP/P2Y12 kit (Biocytex, Marseille, France). Briefly, citrated blood was incubated with phosphorylation activator prostaglandin E1 (PGE1) alone or PGE1 and phosphorylation inhibitor ADP simultaneously. Phosphorylated VASP was then labeled fluorescently using specific primary and secondary antibodies and analyzed according to the manufacturer's protocol. Results were converted to platelet reactivity index (PRI %) which is calculated using the corrected mean fluorescence intensities (MFIc) and the formula $\text{PRI} = [(\text{MFIc}_{\text{PGE1}} - \text{MFIc}_{\text{PGE1+ADP}}) / \text{MFIc}_{\text{PGE1}}] \times 100$. Negative values were imputed to zero.

Statistical analysis

Statistical analysis was performed using R statistical package (<http://www.r-project.org>). Continuous variables were summarized by median (25th, 75th percentile) and mean (SD, standard deviation) and compared between visit-1 and visit-2 by paired t-test. Data were categorized as quartiles and binary; responder and poor responder (PR). Patients were defined as aspirin PR based on ARU ≤ 550 , LTA-AA maximum $\leq 20\%$, $\text{TxB}_2 \leq 1 \text{ ng/mL}$ or 11dh $\text{TxB}_2 \leq 1,500 \text{ pg/mg}$ of creatinine [12–14] and as clopidogrel PR with PRU ≤ 240 , LTA-ADP maximum $\leq 40\%$ or PRI $\leq 50\%$ [15–18]. A recently published PRU cut-off ≤ 208 [19,20] and PRI $\leq 66\%$ based on 40 normal controls from our lab (mean minus 2SD) were included in defining PR. Pearson's rank correlation (ρ) and kappa (κ) statistics were used to determine the correlation and agreement between two visits and between the related platelet function assays. A ρ or kappa (κ) statistic < 0.2 translates into no correlation or

agreement, 0.2–0.4 into poor correlation or agreement, 0.4–0.6 into moderate correlation or agreement, and 0.6–0.8 strong correlation or agreement. Ad hoc power analyses were done separately for correlation coefficient and kappa, which shows that our analysis had sufficient power (>80%) with the sample size of 58 at significance level of 0.05.

Results

Characteristics of the study patients

Four patients were not eligible for the study due to compliance, sampling, and technical issues. Therefore, a total of 58 patients were involved in the study. Characteristics of this cohort including gender, age, clinical history and current medications are shown in Table 1. A majority of the patients were male (81%) and either current or past smokers (78%). Hypertension was the most encountered (74%) clinical condition in the study population.

Comparison of visit-1 and visit-2

Platelet function measures produced by VerifyNow[®], LTA, *ex vivo* TxB₂, urinary 11dhTxB₂, and VASP assays and blood measures are presented for each visit as median (25th, 75th percentile) and mean (SD) in Table 2. Although some patients switched to a different dose of aspirin prior to visit-2, the difference in aspirin dosages and platelet function measures between two visits did not show a correlation. Paired t-test indicated a significant difference between visit-1 and visit-2 for PRU, LTA-ADP, and PRI which were higher in the second visit (p-value<0.05) (Table 2). White blood cell (WBC) count and PRP concentration were also significantly different in visit-2 where WBC count was lower and PRP concentration was higher (p-value<0.001). Data from other measurements did not differ between two visits. Differences in a platelet function parameter between visit-1 and visit-2 were plotted versus mean of that parameter from visit-1 and visit-2 (Bland Altman plots, Fig. 1). Random distribution of scatters in PRU, LTA-ADP, and PRI plots indicated that difference between two visits was not influenced by the value of measurement.

Responders and poor responders (PR) of aspirin and clopidogrel

Each platelet function assay identified a variable number of PR defined by published cut-off levels. Aspirin PR based on VerifyNow[®] Aspirin, LTA-AA, TxB₂ and 11dhTxB₂ assays was 9 (16%), 2 (3%), 19 (33%), and 7 (12%) in visit-1 and 12 (21%), 3 (5%), 16 (28%), and 8 (14%) in visit-2, respectively (Table 3). The stability of the phenotype was low, as only 3 (5%) patients were persistent poor responder (PPR) (PR in both visits) with VerifyNow[®] Aspirin. Similarly, there were 1 (2%), 5 (9%), and 3 (5%) PPR according to LTA-AA, TxB₂ and 11dhTxB₂ assays, respectively.

Based on VerifyNow[®] P2Y₁₂ (PRU >240), 13 (24%) patients in visit-1 and 17 (30%) in visit-2 were defined as clopidogrel PR while 11 (20%) of these patients were PPR. LTA-ADP assay identified 6 (10%) PR in visit-1 and 10 (17%) PR in visit-2 of which 5 (9%) were PPR. By using VASP (PRI >50%), 18 (31%) and 26 (45%) patients were classified as clopidogrel PR in visit-1 and visit-2, respectively, of whom 16 (28%) were PPR. Poor response to both aspirin and clopidogrel (dual poor response) could not be assessed due to the small number of dual PR.

Correlation between visit-1 and visit-2

Correlation of measures related to platelet function and blood between visit-1 and visit-2 was tested by Pearson's rank correlation (Table 4 and Fig. 2). Among aspirin-response measures, ARU and TxB₂ did not correlate between two visits, whereas LTA-AA showed a moderate correlation and 11dhTxB₂ revealed a poor but significant correlation. Clopidogrel-response measures showed moderate to good correlation between two visits. There was also a moderate correlation between blood parameters of visit-1 and visit-2 (Table 4).

Agreement between visit-1 and visit-2

Agreement between visit-1 and visit-2 was tested with kappa (κ) statistic. Data were analyzed as quartiles (Table 4, middle column; κ^*) and binary traits (Responder and PR) (Table 4, last column; $\kappa^\dagger$). Among aspirin-response measures, only LTA-AA maximum showed a poor but significant agreement on quartiles between two visits. Agreement on binary traits of aspirin-response measures was also poor and only revealed by 11dhTxB₂ assay. Clopidogrel response defined by quartiles of PRU and PRI showed a moderate agreement between the visits. PRU ≥ 240 and PRI $\geq 66\%$ -defined PR showed a good and better agreement between two visits compared to PRU ≥ 208 and PRI $\geq 50\%$ cut-offs, respectively. A moderate agreement between the visits was present for PR defined by LTA-ADP maximum $\geq 40\%$. Quartiles of blood measures showed poor to moderate agreement between visit-1 and visit-2.

Correlation and agreement between platelet function assays

Correlation and agreement between the related platelet function assays were tested by Pearson's rank correlation and kappa statistic (Table 5). For simplicity, only visit-1 results are presented here. The only significant correlation among the aspirin-response assays was poor and between ARU and 11dhTxB₂ measures (Table 5a). On the other hand, there was no agreement between the aspirin-response assays for quartiles and binary definitions. A good correlation was evident among the clopidogrel-response measures (Table 5b). Kappa statistics indicated a poor agreement for quartiles between PRU and LTA-ADP maximum and between PRU and PRI (Table 5b). Agreement between PRU and LTA-ADP on binary definition was poor and evident only based on PRU ≥ 240 . On the other hand, the highest agreement between PRU and PRI was moderate and obtained using PRU $\geq 208\%$. PR definitions of LTA-ADP and PRI did not show agreement.

An overview of agreement between the assays defining PR was illustrated as Venn diagrams in Figure 4. There were 29 PR in visit-1 and 27 PR in visit-2 according to aspirin-response assays. None in each visit was identified as PR by all aspirin-response assays, while only one in visit-1 was identified as PR by VerifyNow[®] Aspirin, TxB₂, and 11dhTxB₂ assays. (Fig. 3A). Clopidogrel-response assays identified 23 PR in visit-1 and 31 PR in visit-2. Three PR in visit-1 and 8 PR in visit-2 were identified by all clopidogrel-response assays (Fig. 3B). Consistency in identification of initial PR in visit-2 was 26–50% for aspirin-response and 64–89% for clopidogrel-response assays (Table 3). Consistency was the lowest (26%) for TxB₂ and the highest (89%) for VASP (PRI $\geq 50\%$) assays.

Discussion

Changes in platelet response to antiplatelet drugs over a time period can result from compliance issue or dynamics in platelet function [21]. We found that stability of platelet response varied from assay to assay since it was reflected by repeatability or reproducibility of the platelet function assays. Moreover, its assessment was influenced by the way of data analysis. Data analysis based on continuous data from all assays, except *VerifyNow*[®] Aspirin and TxB₂ assays, showed stable inhibition of platelets with aspirin and clopidogrel. When data were analyzed as quartiles, stability in platelet response was observed only with LTA-AA (maximum), *VerifyNow*[®] P2Y₁₂, and VASP assays. Binary categorization revealed that platelet inhibition was stable according to 11dhTxB₂ and all clopidogrel-response assays. The influence of data categorization on the assessment of stability originates from binary categorization which is based on arbitrary cut-off levels of the assays.

There are limited studies on stability of platelet response to aspirin and clopidogrel. Consistent with our findings, these studies also showed that stability varies with assays employed to measure platelet response [22–25]. In a study that assessed platelet function in patients with lower extremity peripheral arterial disease (PAD) on aspirin at baseline, 6 months and 1 year, a poor to moderate degree of correlation and agreement was found between the repetitive measurements of LTA-AA and LTA-ADP [22]. Similarly, comparisons of baseline and 1 year platelet function profiles in patients with a recent transient ischemic attack (TIA) or ischemic stroke on aspirin revealed a poor correlation and poor to moderate agreement for *VerifyNow*[®] Aspirin, LTA-AA, and LTA-ADP assays [23]. The durability of platelet inhibition with both aspirin and clopidogrel was investigated in patients following PCI over a 30-day period. While a strong correlation was found among the measures of LTA carried out with 5 μ M ADP at 24 hours, 5 and 30 days of PCI, only, a moderate correlation was observed between the results of LTA using 20 μ M ADP at 24 hour and 5 days [24]. Another study that measured platelet function with LTA-ADP (5 μ M) once for three weeks in patients on long term dual antiplatelet therapy indicated stable platelet inhibition in 68% and 58% of patients based on cut-offs >53% and >42%, respectively [25].

In our study, in visit-1 only, aspirin PR varied between 3% and 33% and clopidogrel PR varied between 10% and 35% depending on the assay utilized. Moreover, 50% of patients were aspirin PR and 40% were clopidogrel PR counting all related assays. This wide range and high prevalence of PR is due to poor correlation and agreement between different platelet function assays and poor specificity of the assays [11,26]. Particularly, we observed poor correlation and lack of agreement between aspirin-response assays. In a study comparing six different aspirin-response tests, poor correlation was also found between *VerifyNow*[®] Aspirin, LTA-AA, and 11dhTxB₂ assays [27]. Although a poor agreement was revealed between *VerifyNow*[®] Aspirin and LTA-AA assays in the same study, there was no agreement between LTA-AA and 11dhTxB₂ assays. All these results indicate that measurement of platelet response to aspirin is highly variable, likely due to differing dependence of the arachidonic acid pathway between techniques. Since all of the functional tests measure platelet function, techniques that specifically measure cyclooxygenase inhibition or acetylation might increase sensitivity and specificity. On the other hand, based

on our results, a combination of VerifyNow® P2Y12 (especially PRU cut-off 208) and VASP assays can be used to follow clopidogrel response in addition to LTA-ADP.

There are several limitations to this study including small size of the patient population, lack of baseline specimens and compliance status for visit 2. As the sampling of visit-1 was done following PCI, results might not represent steady-state levels. However, the study utilized a larger variety of platelet function tests than other similar studies on patients receiving both aspirin and clopidogrel. Therefore, it provides information on the concordance of these tests. The TxB₂ assay was also performed differently than other studies – we measured the TxB₂ released by platelets following *ex vivo* activation during aggregation. Moreover, two different cut-off levels of VerifyNow® P2Y12 (PRU 208 and 240) and VASP (PRI 50 and 60) assays were analyzed simultaneously in this study. Our data analysis includes analysis as continuous, quartile and binary ranges, so it provides a more comprehensive assessment of differences between the platelet function assays.

In summary, our conclusions are: (1) Determination of platelet inhibition and its stability varies with platelet function assay, (2) Assessment of stability in platelet inhibition and of correlation and agreement between platelet function assays are influenced by data analysis as continuous, quartile or binary, (3) Platelet inhibition with aspirin is stable based on LTA-AA and 11dhTxB₂ measures, (4) Platelet response to clopidogrel is stable based on all clopidogrel-response measures, (5) There is a poor correlation and no agreement between aspirin-response assays, (6) Clopidogrel-response assays correlate well and show poor to moderate agreement. Therefore, individual platelet function tests may provide conflicting results, and unless future clinical trials demonstrate their value, treatment decisions should not be based solely on these test results.

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Abbreviations

AA	arachidonic acid
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ARU	aspirin reaction unit
CABG	previous coronary artery bypass graft
CBC	complete blood count
COX-1	cyclooxygenase-1
11dhTxB₂	11-dehydro thromboxane B ₂
HDL	high density lipoprotein

κ	kappa statistic
LDL	low density lipoprotein
LTA	light transmission aggregometry
MI	myocardial infarction
PAD	peripheral arterial disease
PCI	percutaneous coronary intervention
PGE1	prostaglandin E1
PPP	platelet poor plasma
PPR	persistent poor responder
PR	poor responder
PRI	platelet reactivity index
PRP	platelet rich plasma
PRU	P2Y12 reaction unit
SD	standard deviation
TxA₂	thromboxane A ₂
TxB₂	thromboxane B ₂
VASP	vasodilator stimulated phosphoprotein
WBC	white blood cell

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Fig. 1. Bland Altman plots. Difference of a parameter between two visits was plotted against mean of that parameter from visit-1 and visit-2. V1= Visit-1; V2= Visit-2.

Fig. 2.

Correlation of platelet function measures between visit-1 and visit-2. Correlation was illustrated by a solid line on each graph. Cut-off points ARU 550, LTA-AA maximum 20%, TxB₂ 1 ng/mL, 11dhTxB₂ 1,500 pg/mg of creatinine, PRU 240, LTA-ADP maximum 40% and PRI 50% were indicated by dashed lines.

Fig. 3.

Venn diagrams illustrating agreement between the assays in classification of poor responders. (A), Aspirin PR. (B), Clopidogrel PR. PRU and PRI cut-off levels were 240 and 50%, respectively.

Table 1

Characteristics of the study cohort.

Characteristic	n (%) ^I or Mean±SD
Male	47 (81)
Female	11 (19)
Age	63±9
Current Smoking	8 (14)
Past Smoking	37 (64)
Hypertension	43 (74)
Diabetes mellitus	15 (26)
Peripheral artery disease (PAD)	7 (12)
Myocardial infarction (MI)	25 (43%)
Stroke	2 (3)
Previous percutaneous coronary intervention (PCI)	27 (47)
Previous coronary artery bypass graft (CABG)	17 (29)
Total cholesterol (mg/dL)	160±46
HDL (mg/dL)	42±13
LDL (mg/dL)	90±40
Medications	
Aspirin	58 (100)
Clopidogrel	58 (100)
Statins	53 (91)
Thrombin inhibitors	48 (83)
β-blocker	47 (81)
ACE inhibitors	26 (46)
Proton pump inhibitors	19 (35)
Angiotensin receptor blockers	6 (10)

HDL = high density lipoprotein, LDL = low density lipoprotein, ACE = angiotensin converting enzyme, SD = standard deviation.

^ITotal number of subjects may vary depending on the availability of data.

Table 2

Platelet function and blood measures from each visit.

Parameter	Visit-1					Visit-2					p-value ^I
	N	25%	Median	75%	Mean (SD)	N	25%	Median	75%	Mean (SD)	
Aspirin-response parameters											
ARU	58	394	448	531	458 (75)	58	397	461	537	461 (73)	0.811
LTA-AA, maximum (%)	58	4	6	8	7 (4)	58	5	6	8	8 (9)	0.358
LTA-AA, late (%)	58	1	3	5	4 (3)	58	1	3	6	5 (9)	0.212
TxB ₂ (ng/mL)	58	122	470	1950	1691 (3539)	58	294	472	1034	2312 (5910)	0.513
11dhTxB ₂ (pg/mg of creatinine)	58	598	779	966	1047 (860)	57	620	847	1070	1005 (592)	0.69
Clopidogrel-response parameters											
PRU	55	87	152	239	159 (91)	57	120	181	241	178 (83)	0.04
LTA-ADP, maximum (%)	58	15	23	30	24 (11)	58	20	28	34	28 (12)	0.001
LTA-ADP, late (%)	58	1	5	11	7 (9)	58	3	9	13	11 (11)	0.006
PRI (%)	58	24	39	58	40 (23)	58	27	46	62	45 (22)	0.03
Blood parameters											
WBC count (K/ μ L)	58	5.2	6.1	6.9	6.1 (1.7)	58	4.2	4.9	6.0	5.3 (1.7)	<0.001
Hemoglobin (g/dL)	58	11.4	12.1	13.5	12.4 (1.5)	58	11.9	12.9	13.4	12.6 (1.5)	0.296
Hematocrit (%)	58	34.5	37.2	40.1	37.2 (4.1)	58	35.9	38.1	40.0	38.1 (5.0)	0.113
Platelet count (K/ μ L)	58	132	165	200	174 (58)	58	138	164	205	177 (55)	0.51
PRP (K/ μ L)	58	222	305	349	307 (96)	58	251	340	412	345 (114)	<0.001

ARU = aspirin reaction unit, AA = arachidonic acid, TxB₂ = thromboxane B₂, 11dhTxB₂ = 11-dehydro thromboxane B₂, PRU = P2Y₁₂ reaction unit, ADP = adenosine diphosphate, PRI = platelet reactivity index, WBC = white blood cell; PRP = platelet rich plasma, SD = standard deviation.

^I p-value was obtained by paired t-test and defined as significant if it was <0.05 (indicated as bold). Significantly different variables were shaded in the table.

Table 3

Responders and poor responders (PR) of aspirin and clopidogrel.

Assay Cut-off	N	Responder N (%)		Poor responder (PR) N (%)		Persistent poor responder (PPR) N (%)	Consistency ^I (%)
		Visit-1	Visit-2	Visit-1	Visit-2		
Aspirin-response assays							
VerifyNow® Aspirin;							
ARU 550	58	58	49 (84)	46 (79)	9 (16)	12 (21)	33
LTA-AA, maximum;							
20%	58	58	56 (97)	55 (95)	2 (3)	3 (5)	50
TxB ₂ ;							
1 ng/mL	58	58	39 (67)	42 (72)	19 (33)	16 (28)	26
11dhTxB ₂ ;	58	57	51 (88)	49 (86)	7 (12)	8 (14)	43
1,500 pg /mg of creatinine							
Clopidogrel-response assays							
VerifyNow® P2Y12;							
PRU 240	55	57	42 (76)	40 (70)	13 (24)	17 (30)	85
PRU 208			36 (65)	33 (58)	19 (35)	24 (42)	74
LTA-ADP, maximum;							
40%	58	58	52 (90)	48 (83)	6 (10)	10 (17)	83
VASP;							
PRI 50%	58	58	40 (69%)	32 (55%)	18 (31%)	26 (45%)	89
PRI 66%			47 (81%)	50 (86%)	11 (19%)	8 (14%)	64

Abbreviations are as in Table 2.

^I Consistency refers to re-identification of a visit-1 PR as PR in visit-2.

Table 4

Correlation and agreement between visit-1 and visit-2.

Parameter	Pearson's rank correlation		κ statistics ¹ (Quartiles)		κ statistics ² (Binary)	
	ρ ³	p-value ⁴	κ (95% CI)	p-value	κ (95% CI)	p-value
<i>Aspirin-response parameters</i>						
ARU	0.13	0.344	0.04 (−0.12, 0.19)	0.637	0.13 (−0.25, 0.51)	0.308
LTA-AA, maximum (%)	0.49	<0.001	0.27 (0.1, 0.45)	<0.001	−0.02 (−1.15, 1.1)	0.849
LTA-AA, late (%)	0.49	<0.001	0.19 (0.02, 0.36)	0.011	–	–
TxB ₂ (ng/mL)	−0.11	0.412	0.05 (−0.1, 0.2)	0.504	−0.02 (−0.32, 0.28)	0.88
11dhTxB ₂ (pg/ng of creatinine)	0.31	0.021	0.04 (−0.11, 0.2)	0.559	0.31 (−0.1, 0.72)	0.019
<i>Clopidogrel-response parameters</i>						
PRU						
Cut-off: 240	0.77	<0.001	0.46 (0.29, 0.63)	<0.001	0.70 (0.47, 0.93)	<0.001
Cut-off: 208					0.46 (0.21, 0.7)	<0.001
LTA-ADP, maximum (%)	0.5	<0.001	0.14 (−0.03, 0.3)	0.064	0.57 (0.22, 0.93)	<0.001
LTA-ADP, late (%)	0.44	0.001	0.18 (0.02, 0.35)	0.011	–	–
PRI (%)						
Cut-off: 50%	0.76	<0.001	0.40 (0.23, 0.57)	<0.001	0.57 (0.35, 0.79)	<0.001
Cut-off: 66%					0.69 (0.42, 0.95)	<0.001
<i>Blood parameters</i>						
WBC count (K/ μ L)	0.67	<0.001	0.21 (0.05, 0.37)	0.002	–	–
Hemoglobin (g/dL)	0.68	<0.001	0.29 (0.12, 0.46)	<0.001	–	–
Hematocrit (%)	0.73	<0.001	0.25 (0.08, 0.42)	0.001	–	–
Platelet count (K/ μ L)	0.77	<0.001	0.41 (0.24, 0.58)	<0.001	–	–
PRP (K/ μ L)	0.80	<0.001	0.32 (0.15, 0.49)	<0.001	–	–

 κ = kappa statistic; CI = confidence interval. Other abbreviations are as in Table 2.A rho or kappa (κ) statistic <0.2 translates into no correlation or agreement, 0.2–0.4 into poor correlation or agreement, 0.4–0.6 into moderate correlation or agreement, and 0.6–0.8 strong correlation or agreement which was indicated with shading from lighter to darker, respectively. Full agreement was pointed as bold.¹ κ statistics were calculated from the categorized data as quartiles.

² κ statistics were calculated from the categorized data as binary based on cut-off points of platelet function assays.

³ rho is the Spearman correlation.

⁴ p-value is the p-value of testing correlation.

Table 5

Correlation and agreement between platelet function assays.

A. Aspirin-response parameters.

	LTA-AA, maximum (%)	TxB ₂ (ng/mL)	11dhTxB ₂ (pg/mg of creatinine)
ARU			
rho, p-value	0.14, 0.279	0.24, 0.066	0.02, 0.871
κ quartile (95% CI), p-value	0.15 (−0.02, 0.31), 0.05	0.06 (−0.1, 0.21), 0.46	0.08 (−0.08, 0.24), 0.29
κ binary (95% CI), p-value	−0.03 (−0.61, 0.55), 0.67	0.19 (−0.13, 0.5), 0.11	−0.01 (−0.47, 0.45), 0.92
LTA-AA, maximum (%)			
rho, p-value		0.06, 0.667	0.26, 0.045
κ quartile (95% CI), p-value		0.06 (−0.1, 0.21), 0.45	0.14 (−0.03, 0.31), 0.07
κ binary (95% CI), p-value		−0.03 (−0.4, 0.33), 0.48	−0.03 (−0.69, 0.63), 0.71
TxB ₂ (ng/mL)			
rho, p-value			0.10, 0.462
κ quartile (95% CI), p-value			0.06 (−0.1, 0.21), 0.45
κ binary (95% CI), p-value			0.07 (−0.27, 0.4), 0.54

B. Clopidogrel-response parameters.

	LTA-ADP, maximum (%)	PRI (%)
PRU		
rho, p-value	0.60, <0.001	0.70, <0.001
κ quartile (95% CI), p-value	0.26 (0.08, 0.44), <0.001	0.36 (0.19, 0.54), <0.001
240-κ binary (95% CI), p-value	0.33 (−0.07, 0.73), <0.001	0.25 (−0.07, 0.56), 0.06 ¹
		0.24 (−0.13, 0.6), 0.08 ²
208-κ binary (95% CI), p-value	0.16 (−0.17, 0.49), 0.08	0.42 (0.16, 0.68), <0.001 ¹
		0.41 (0.13, 0.69), <0.001 ²
LTA-ADP, maximum (%)		
rho, p-value		0.49, <0.001
κ quartile (95% CI), p-value		0.19 (0.02, 0.36), 0.01
κ binary (95% CI), p-value		0.18 (−0.16, 0.52), 0.05 ¹
		0.18 (−0.25, 0.62), 0.1 ²

Abbreviations are as in Table 2.

A rho or kappa (κ) statistic <0.2 translates into no correlation or agreement, 0.2–0.4 into poor correlation or agreement, 0.4–0.6 into moderate correlation or agreement, and 0.6–0.8 strong correlation or agreement which was indicated with shading from lighter to darker, respectively.

¹ and ² PRI cut-off points are 50% and 66%, respectively.