Assessment of clopidogrel non-response by the PFA-100® system using the new test cartridge INNOVANCE® PFA P2Y

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Abstract: Until now, the PFA-100® system has been considered unsuitable for monitoring clopidogrel efficacy. The authors evaluated platelet function in PAOD (peripheral arterial occlusive disease) patients using a new PFA-100® test cartridge (product name: INNOVANCE® PFA P2Y*) specifically designed for this purpose. Twenty-two stable PAOD patients on antithrombotic therapy with clopidogrel alone (n = 22) and 18 patients undergoing a peripheral catheter intervention, preliminarily treated with 100 mg/d aspirin followed by co-administration of clopidogrel (loading dose 300 mg, maintenance dose 75/d), were enrolled in this study. Defining non-responsiveness to clopidogrel as an aggregation response within the reference range (90% central interval), four (18.2%) non-responders using LTA induced by 5 µM ADP and six (27.3%) non-responders using LTA induced by 2 µM ADP (LateAggr >72.1% and >42.9%, respectively) were identified. INNOVANCE® PFA P2Y* determined six (27.3%) non-responders (CT<87s). Agreement between the two aggregometry assays and INNOVANCE® PFA P2Y* on the definition of clopidogrel response and non-response exceeded 70%. Only three patients were uniformly identified as clopidogrel non-responders by all three assays. When clopidogrel was co-administered with aspirin, two (11.1%) non-responders to clopidogrel were detected with INNOVANCE® PFA P2Y*, whereas ADP-induced LTA found all patients to be responsive. INNOVANCE® PFA P2Y* appears to be suitable for monitoring the effect of clopidogrel on platelet function. Its sensitivity in detecting responsiveness or non-responsiveness to clopidogrel is comparable to ADP-induced LTA. Additional prospective studies are needed to clarify the clinical relevance of the test results and classification obtained with INNOVANCE® PFA P2Y*.

Response to Reviewers: FFM, 08/11/2009

Dear editor and reviewers,

Thank you for your comments on our manuscript. We performed the changes that you asked us to do. The changes are highlighted in blue. You will find a point-by-point response in the following.
We hope that we addressed your concerns satisfactorily and that our manuscript is now acceptable for publication.

Yours sincerely

Dr. Birgit Linnemann, M.D.

Ad reviewer 1:

Ad 1: The reasons why we used lower ADP concentration in LTA was added. In a previous work we have shown that late aggregation using 2 µM ADP in non-adjusted PRP provides a good discrimination between clopidogrel treated patients and healthy subjects. However, we were also able to show that in platelet count-adjusted PRP, higher concentrations were necessary to differentiate between patients on clopidogrel treatment and subjects without clopidogrel medication. See reference 22.

Ad 2: We agree that the figures 1-3 are quite similar. So we decided to remove fig. 1 and 3 and to keep fig. 2.

Ad 3: We discussed the variability of test results and CVs more in detail and added duplicate measurements of patients on dual antiplatelet therapy in figure 2 (formerly 4). Changes in the text are highlighted in blue.

ORIGINAL ARTICLE

Assessment of clopidogrel non-response by the PFA-100® system using the new test cartridge INNOVANCE® PFA P2Y*

*product under development – not available for sale

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Disclosures

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Abstract

Until now, the PFA-100® system has been considered unsuitable for monitoring clopidogrel efficacy. The authors evaluated platelet function in PAOD (peripheral arterial occlusive disease) patients using a new PFA-100® test cartridge (product name: INNOVANCE® PFA P2Y*) specifically designed for this purpose. Twenty-two stable PAOD patients on antithrombotic therapy with clopidogrel alone (n = 22) and 18 patients undergoing a peripheral catheter intervention, preliminarily treated with 100 mg/d aspirin followed by co-administration of clopidogrel (loading dose 300 mg, maintenance dose 75/d), were enrolled in this study. Defining non-responsiveness to clopidogrel as an aggregation response within the reference range (90% central interval), four (18.2%) non-responders using LTA induced by 5 µM ADP and six (27.3%) non-responders using LTA induced by 2 µM ADP (LateAggr >72.1% and >42.9%, respectively) were identified. INNOVANCE® PFA P2Y* determined six (27.3%) non-responders (CT<87s). Agreement between the two aggregometry assays and INNOVANCE® PFA P2Y* on the definition of clopidogrel response and non-response exceeded 70%. Only three patients were uniformly identified as clopidogrel non-responders by all three assays. When clopidogrel was co-administered with aspirin, two (11.1%) non-responders to clopidogrel were detected with INNOVANCE® PFA P2Y*, whereas ADP-induced LTA found all patients to be responsive. INNOVANCE® PFA P2Y* appears to be suitable for monitoring the effect of clopidogrel on platelet function. Its sensitivity in detecting responsiveness or non-responsiveness to clopidogrel is comparable to ADP-induced LTA. Additional prospective studies are needed to clarify the clinical relevance of the test results and classification obtained with INNOVANCE® PFA P2Y*.

Keywords: clopidogrel, antiplatelet therapy, non-response, PFA-100® System, INNOVANCE® PFA P2Y*

Introduction

Light transmittance aggregometry (LTA) was invented by Born in 1962 and is still regarded as the method of choice for the assessment of platelet function and its pharmacologic inhibition [1,2]. In LTA, the increase in light transmission through platelet-rich plasma following the addition of a platelet agonist is measured. Measurement occurs when platelets become activated by the agonist and form aggregates. However, LTA is time-consuming, not standardized, and its use is limited to specialized laboratories. A number of different agonists are used for LTA, including arachidonic acid (ARA), epinephrine, adenosine diphosphate (ADP), collagen, thrombin, thrombin receptor activating peptide (TRAP), phorbol myristate acetate (PMA), and ristocetin, and different optical aggregometers are available. However, the concentrations of agonists used for testing vary widely, and accepted guidelines on how laboratories should perform LTA are lacking [2,3].

There is a growing body of evidence that residual platelet function, despite antithrombotic therapy, identifies patients at risk of future cardiovascular events [4-11]. Therefore, interest in whole blood point-of-care methods has increased; these techniques offer the possibility of rapid and reliable identification of patients with antiplatelet “drug resistance” and tests may be routinely performed. Thus, the PFA-100® System, originally introduced as an aid to test for defects of primary hemostasis [12,13], has become one of the most widely-employed tests to assess aspirin efficacy. The PFA-100® System is a global test of high-shear-dependent platelet function. In disposable test cartridges, anticoagulated whole blood is aspirated from the sample reservoir through a capillary and an aperture in a platelet agonist coated membrane, thereby exposing platelets to high shear flow conditions and the platelet agonists of the membrane coating. Two test cartridges are commercially available with a membrane coated either with collagen and epinephrine or collagen and ADP. The closure time depends on different variables, such as von Willebrand factor levels, platelet count, or haematocrit and is usually affected by aspirin intake when using the Collagen/EPI Test Cartridge (CEPI) [12,13].

Theoretically, the Dade® PFA Collagen/ADP Test Cartridge (CADP) should be suitable for the assessment of P2Y12-receptor inhibition. However, activation of the platelets by collagen and the residual activation potential of ADP via the P2Y1-receptor seem to be sufficient to overcome the effect of P2Y12-receptor blockade on platelet function [14]. In earlier studies, the CADP cartridge was

indeed shown to be unsuitable for the detection of the efficacy of clopidogrel therapy [15-19]. A recent in vitro study by Pidcock et al. provided evidence that the P2Y1-receptor is an important contributor to platelet functionality as measured by CADP, despite an inhibition of the P2Y12-receptor [20]. The authors showed that a secondary addition of MRS 2179, a P2Y1-receptor antagonist, to a blood sample, already treated with the P2Y12-receptor antagonist cangrelor without effect, resulted in significant prolongation of the CADP CT. They concluded that this method might facilitate the assessment of clopidogrel treatment and related P2Y12-receptor antagonists using the CADP cartridge.

In the present study, we aimed to evaluate a new, but not yet commercially available PFA-100® test cartridge, product name INNOVANCE® PFA P2Y*, which was designed for the assessment of P2Y12 inhibitory effects of clopidogrel, either in monotherapy or in combination with aspirin.

Materials and methods

Study population

Twenty-two subjects (11 males, 11 females, aged 55 to 86 years) with peripheral arterial occlusive disease (PAOD), treated with 75 mg/d clopidogrel as the only antithrombotic drug were enrolled in the study (Group 1). PAOD was defined as an ankle brachial index ≤0.9 as measured with Doppler ultrasound [21]. Atherosclerotic disease was required to be stable (i.e., without clinical deterioration within the last 3 months) and patients had to have been treated with clopidogrel for at least 14 days. We additionally investigated 18 PAOD patients (11 males, 7 females, aged 43 to 84 years) undergoing a peripheral catheter intervention procedure due to intermittent claudication (Group 2). At baseline, these patients were treated with 100 mg/d aspirin. On the day of intervention, a loading dose of 300 mg clopidogrel was administered followed by a maintenance dose of 75 mg/d for at least 4 weeks, while the aspirin treatment was continued. Patients with additional medication known to influence platelet function (e.g., non-steroidal anti-inflammatory drugs (NSAIDs)) were excluded, as well as patients with known coagulation disorders, liver cirrhosis, alcohol abuse, end-stage renal failure, or malignant disease. A platelet count < 100/nl, hemoglobin < 9 g/dl and a haematocrit < 28% were additional exclusion criteria. The study was approved by the local Ethics Committee, and all patients provided written informed consent prior to participation. Using a standardized questionnaire, clinical

data detailing vascular and concomitant disease, cardiovascular risk factors, and co-medication were recorded. Compliance with the antithrombotic therapy was determined through patient interviews both at study entry and at follow-up. All patients confirmed that they had taken their medication as directed. The last dose of antithrombotic medication was administered one to 24 hours before blood sampling.

Blood sampling

Blood sampling was performed once in patients under chronic clopidogrel treatment. In patients undergoing peripheral catheter intervention, blood sampling was performed at baseline when treated with aspirin only and three weeks after intervention when patients were on stable combination therapy of aspirin and clopidogrel. Blood was drawn from an antecubital vein using a 21-gauge butterfly cannula system (Multifly®-Set, 21 G x 1½ TW, 0.8 x 19 mm, Sarstedt, Nümbrecht, Germany). EDTA and citrate (0.129 M (3.8%) and 0.106 M (3.2%) trisodium citrate)-supplemented blood was collected with plastic syringes (Monovette®, Sarstedt, Nümbrecht, Germany). Platelet count was measured on the Sysmex® KX-21 (Roche Diagnostics, Basel, Switzerland), an automatic multi-parameter blood cell counter. Platelet counts between 100 and 500/nl were required for subsequent platelet function testing. Platelet-rich plasma (PRP) was obtained by centrifuging 0.106 M (3.2%) citrated whole blood at room temperature at 140 x g for 5 min. Platelet-poor plasma (PPP, platelet count < 10/nl) was derived from citrated whole blood centrifuged more vigorously at 1,500 x g for 15 min. The time interval between blood sampling and testing was at least 1 h and did not exceed 3 h. All platelet function tests were performed in duplicate using blood or plasma from the same tube.

PFA-100® System

The PFA-100® System (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) is a system for assessment of high shear stress-dependent platelet function by a procedure simulating the complex process of primary haemostasis *in vitro*. The device aspirates 800 µl of whole blood, anticoagulated with 0.129 M (3.8%) trisodium citrate, at high shear rates (5,000-6,000 s^{-1}), first through a stainless steel capillary and then through an aperture in a membrane coated with agonists. The agonists at the membrane trigger platelet adhesion, activation, and aggregate formation at the aperture. The membrane of INNOVANCE® PFA P2Y* is coated with 20 µg ADP, 5 ng prostaglandin

E1 and 459 µg calcium chloride. The time required to occlude the central aperture (diameter: 100 µm) resulting in the cessation of blood flow is defined as the closure time (CT); the maximum value for CT is determined by the maximum measurement time of 300 s. If a closure of the aperture was not achieved within the time period of 300 s, the system reports a non-closure. For non-closures, a CT value of 300 s was used in the calculations. A CT within the reference range was considered to indicate non-responsiveness to clopidogrel. This study evaluated a new, as yet commercially unavailable, test cartridge designed to assess the efficacy of clopidogrel treatment. The preliminary cut-off for INNOVANCE® PFA P2Y* was defined as 87 s based on closure times of 134 ostensibly healthy volunteers (83 males, 51 females, aged 19 to 58 years) not taking any antithrombotic drug. The cut-off was statistically derived from the 95th percentile of the quadruple measurements in blood buffered with 3.8% sodium citrate.

Light transmittance aggregometry (LTA)

The LTA procedure was performed on the BCT* System (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). The BCT* System is a fully automatic machine used for routine and special coagulation testing. The BCT* System detects platelet aggregate formation in PRP by changes in light transmission (monochromatic light; wavelength: 620 nm) at 37°C. Platelet agonists (15 µl reagent) were automatically added to PRP (135 µl plasma) and stirred at a velocity of 600 rpm. In this study, adenosine 5-diphosphate (ADP; AppliChem, Darmstadt, Germany), at a final concentration of 2 and 5 µM, was used to induce platelet aggregation. The aggregation response following the addition of the agonist was monitored via the change in light transmission over time relative to PPP derived from the same blood sample. Measurements were performed in native PRP unadjusted for platelet count. As disaggregation is a frequent phenomenon in clopidogrel-treated patients, we recorded the late aggregation (LateAgg) response after 10 min. Light transmission through PRP relative to PPP was measured prior to the addition of the agonist and at the end of the measurement (10 min). LateAgg was calculated using the formula:

$$\text{LateAgg [\%]} = 100\% * \frac{\text{(Aggregation at start – Aggregation at end)}}{\text{(Aggregation at start – Platelet-Poor Plasma)}}$$

In a previous investigation, we demonstrated that LateAgg induced by 2 and 5 µM ADP in non-adjusted PRP offered an accurate index for distinguishing between patients on clopidogrel therapy and healthy volunteers not taking any antithrombotic drug [22]. We determined the within-day precision of LTA by calculating the coefficient of variation (CV) of the aggregation response in blood samples collected from 5 healthy volunteers on 5 consecutive days. The CV, using ADP 2 and 5 µM as agonist in non-adjusted PRP, was 2.72% and 1.40%, respectively. In accordance with the latest recommendations provided at the 53rd Annual Scientific and Standardization Committee Meeting during the International Society of Thrombosis and Haemostasis congress in Geneva in 2007, a reference range was derived from the 5th-95th percentiles of duplicates from a group of healthy volunteers (n = 20). Non-responsiveness to clopidogrel was defined as aggregation responses within the reference range despite clopidogrel medication (i.e., LateAgg ≥ 42.9% and ≥ 72.1% for ADP 2 and 5 µM, respectively).

Fibrinogen and von-Willebrand factor

Fibrinogen was assayed by the Clauss method on the BCT® System, using the Multifibren U reagent (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). Von Willebrand Factor (vWF) was also assayed on the BCT® System, using vWF:Ag medium for determination of vWF antigen and BC von Willebrand Factor Low setting for the measurement of vWF ristocetin cofactor (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 15.0, Chicago, IL, USA). Aside from descriptive statistics including frequencies, mean and standard deviation, median and range, we calculated the coefficient of variation using the formula CV = standard deviation/mean and performed the Mann-Whitney U-Test for comparison of metric variables. Cohen's kappa coefficient was calculated as a measure of agreement between LTA and PFA-100® System results. The criterion for statistical significance was a p-value less than 0.05. Results are also presented as box plots with the bar length indicating the interquartile range (25th–75th percentile).

Outliers were defined as values differing 1.5–3.0 bar lengths, whereas extreme values are those differing > 3.0 bar lengths from the upper or lower edge of the box. In the figures, outliers are represented as circles and extreme values as stars.

Results

PAOD patients receiving 75 mg clopidogrel daily, either in monotherapy (group 1; n = 22) or in combination with aspirin (group 2; n = 18), were enrolled in this study. Baseline characteristics of the patients are listed in Table 1.

Figure 1 illustrates the variability of responsiveness to the different antithrombotic regimens assessed with INNOVANCE® PFA P2Y* on the PFA-100® System as well as that determined by 2 and 5 µM ADP-induced LTA.

Non-responsiveness to clopidogrel monotherapy

The variability of responsiveness to clopidogrel monotherapy was high. While 11/22 patients (50.0%) on clopidogrel monotherapy (group 1) had a non-closure with INNOVANCE® PFA P2Y*, 6/22 (27.3%) achieved CT values within the reference interval provided, indicating a non-responsiveness to clopidogrel (Fig. 1). Considering an aggregation response within the reference range as an indication for non-responsiveness, we identified 6/22 (27.3%) or 4/22 (18.2%) patients as non-responders when assessing platelet function by conventional LTA induced by 2 or 5 µM ADP, respectively. Interestingly, only three patients were concordantly identified as non-responders by INNOVANCE® PFA P2Y* and by LTA with both ADP concentrations. Three patients were identified as non-responders only by INNOVANCE® PFA P2Y*, one patient only by LTA induced by 2 and 5 µM ADP and, finally, two patients only by 5 µM ADP-induced LTA. Thus, the agreement between the two aggregometry assays and INNOVANCE® PFA P2Y* slightly exceeded 70%. Consequently, Cohen's kappa coefficients, as a measure of agreement between ADP-induced aggregometry and INNOVANCE® PFA P2Y*, were only 0.32 and 0.24 for 2 and 5 µM ADP, respectively, indicating only a fair agreement.

Non-responsiveness to clopidogrel in addition to aspirin

In the subgroup of patients undergoing peripheral catheter intervention (group 2), all except two patients under pre-treatment with 100 mg/d aspirin showed CT values within the normal range (< 87 s). Under dual antiplatelet therapy (100 mg aspirin and 75 mg clopidogrel daily), 16/18 patients (88.9%) exhibited non-closure using INNOVANCE PFA P2Y*. The CT of two patients (11.1%) was minimally affected following the addition of clopidogrel to the antiplatelet therapy. These patients are considered non-responders to clopidogrel as defined by INNOVANCE® PFA P2Y*. In contrast, using LTA induced by 2 and 5 µM ADP, the results from tests of those two patients indicated substantial responsiveness to clopidogrel. In patients on aspirin monotherapy, ADP-induced LTA was clearly affected, resulting in a higher variability as well as a reduction of the aggregation response. In contrast, aspirin therapy had in all but two patients no influence on INNOVANCE PFA P2Y* closure times. The variability of the aggregation response assessed by LTA remained high following the addition of clopidogrel (Fig. 1). According to the above-mentioned cut-off values, non-responsiveness to clopidogrel as defined by ADP-induced LTA was not observed in patients on dual antiplatelet therapy.

Von Willebrand Factor and fibrinogen

Irrespective of the assay used, no statistically significant differences between defined non-responders and responders to clopidogrel were observed regarding vWF antigen, vWF ristocetin cofactor activity or fibrinogen levels of the blood (Table 2).

Coefficients of variation of duplicates

All values of INNOVANCE® PFA P2Y* and LTA were obtained in duplicate from the same collection tube. The results of duplicate testing in patients under clopidogrel monotherapy (group 1) are presented in Figure 2A. Comparison of the two test results measured with INNOVANCE® PFA P2Y* showed a large variation in some patient samples. In 11/22 cases (50%), both measurements were non-closures (i.e., CT > 300 s), while in 6/22 cases (27%), the difference between the two measurements exceeded 30%. Moreover, 2/22 patients (9%) displayed an ambiguous duplicate with respect to the preliminary cut-off value of 87 s, which would lead to a contrasting classification of

responsiveness if each result were used independently. Interestingly, based on the mean of these highly varying duplicates, all patients are classified as clopidogrel responders based on the preliminary cut-off. The large differences between the two measurements in some samples resulted in a coefficient of variation of 11.3% for INNOVANCE® PFA P2Y*, whereas the corresponding values for LTA performed with 2 and 5 µM ADP were 8.4% and 9.7%, respectively. However, in the subgroup on dual antiplatelet therapy (group 2), the results of INNOVANCE® PFA P2Y* were less variable (Fig 2B). Only two patients in this subgroup presented with duplicate measurements within the reference range, whereas all except for these two yielded a CT ≥ 300 ms. The CVs were 0.8% for INNOVANCE® PFA P2Y* and 10.3% and 6.4%, for LTA with 2 and 5 µM ADP, respectively.

Discussion

The results of the present study indicate that INNOVANCE® PFA P2Y* for the PFA-100® system is suitable for assessing clopidogrel therapy. The sensitivity of detecting clopidogrel non-responders was comparable to that of ADP-induced LTA.

Following absorption, the thienopyridine clopidogrel is metabolized by the hepatic cytochrome P450 system. Its active metabolite irreversibly binds to and blocks the P2Y12-receptor, one of the two ADP-receptors of platelets. Consequently, laboratory response to clopidogrel is generally determined by measuring the extent of inhibition of ADP-induced platelet aggregation. ADP-induced LTA is currently considered to be the standard platelet function test, but it cannot be practically applied to large numbers of clinical samples due to the labour-intensive sample preparation. A simple, reliable whole blood test system able to assess the antiplatelet effect of clopidogrel administration would be of great value in clinical practice. In addition, the contribution of the P2Y1-receptor to residual platelet function on therapy with P2Y12-receptor inhibitors varies widely. This variable contribution may account for the heterogeneity of the LTA results and indicates that ADP alone may not be appropriate to specifically measure the effect of clopidogrel and other P2Y12-receptor antagonists.

Using INNOVANCE® PFA P2Y*, 16/22 patients with clopidogrel monotherapy (73%) and 16/18 patients on dual therapy of clopidogrel and aspirin (89%) presented with a CT prolongation (> 87 s). According to the working definition in this study of non-responsiveness to clopidogrel therapy (i.e., values within the reference range), non-responsiveness was observed in 27% of the patients on monotherapy and in 11% of the patients on dual therapy. The corresponding results of LTA performed

with 2 or 5 µM ADP were 18% (4/22) and 27% (6/22), respectively, for clopidogrel monotherapy patients, whereas all patients on dual therapy showed full responsiveness to the therapy according to LTA. The most likely reason for this result might be the impact of aspirin on ADP-induced LTA, which is supported by the altered LTA result obtained in patients on aspirin monotherapy, when compared to control values.

Interestingly, the various assays differed in their classification of patients as clopidogrel non-responders. Only three patients were concordantly identified as non-responders to clopidogrel by INNOVANCE® PFA P2Y* and LTA induced by 2 or 5 µM ADP. To date, most studies utilize higher ADP concentrations (i.e., 5 and 20 µM), and many laboratories still perform platelet count adjustments. However, in a previous investigation, we demonstrated that late aggregation using 2 µM ADP in non-adjusted PRP provides a good discrimination between clopidogrel treated patients and healthy subjects [22]. A discrepancy between test methods has been observed before when response to aspirin was evaluated by different platelet function tests. Comparing arachidonic acid-induced LTA and the CEPI-CT on the PFA-100® System, Gum et al. observed that the agreement of tests was poor [23]. The difference between methods has been attributed to the fact that the two methods measure different aspects of platelet function. First, LTA is performed in platelet-rich plasma, whereas the PFA-100® system utilizes whole blood samples. Secondly, shear stress is low in the context of LTA, whereas the PFA-100® System is a flow-based system with high shear stress. Both factors are known to influence platelet function. It has, for example, been shown that due to the shear forces during measurement, closure time is strongly dependent on the amount of von Willebrand-factor (vWF) [13]. High plasma levels of vWF as well as high vWF-ristocetin cofactor activity have been related to shorter CT values for the two commercially available PFA-100® cartridges [24-26]. However, the analysis of vWF-ristocetin cofactor activity, vWF-antigen, and fibrinogen levels in the present investigation did not reveal a statistically significant difference between non-responders and responders to clopidogrel. Thus, the disagreement of the test results in our patients cannot be attributed to differences in these parameters.

In patients on clopidogrel monotherapy, the mean CV of duplicate measurements with INNOVANCE® PFA P2Y* was calculated to be 11.3% compared to 8.4% and 9.7% with LTA induced by 2 and 5 µM, respectively. Whereas about 50% of patients on clopidogrel consistently showed non-closure with INNOVANCE® PFA P2Y*, there was a substantial number of patients (6/22) with highly varying CT results of duplicate measurement who achieved a > 30% CT variation. In two patients from this

subgroup (9%), the variation of the individual results would result in different classifications with respect to responsiveness or non-responsiveness. An even higher variance of test results, i.e., a CV of 32%, has recently been reported by Madsen et al. [27], who performed duplicate measurements of CEPI-CT in patients treated with aspirin. In addition, the authors performed platelet function tests at baseline and after three months, and found that, using a cut-off value of 170 s, about 30% were classified as responsive at one visit, but non-responsive at the other visit. The reasons for this observation are not clear. A possible reason for the high variance observed in some patients in this study as well as in the study of Madsen et al. might be an intermediate response to the antiplatelet therapy with clopidogrel or aspirin for those patients, resulting in either almost normal (normal CT) or insufficient (non-closure) clot formation under high-shear flow conditions. However, due to the high intra-assay variation, it does not seem to be advisable to make a clinical decision about responsiveness to antiplatelet therapy based on a single test result.

In studies performed on normal subjects not receiving any medication known to affect platelet function, CVs of duplicates of 9 to 13% have been reported for the commercially available CEPI- and CADP-cartridges [28, 29]. Further investigations revealed a day-to-day variability of 9 to 12% and a diurnal variation as well, with about 30% longer CEPI-CT values in the afternoon than in the morning [30-32]. Only recently, the College of American Pathologists developed a survey to evaluate platelet function tests. In this survey, the precision of the closure time in normal subjects using the CEPI- and CADP-cartridges was also shown to be relatively low (CV 21.1% and 18.6%, respectively) [33]. Despite this, for normal subjects not on antithrombotic therapy, the results are interpreted correctly as normal in 92.4% and 94.0% of cases. Ingestion of aspirin increases the CV of the CEPI-cartridge in a dose-dependent manner [34], and testing patients with ischemic heart disease instead of healthy volunteers results in higher CVs as well [35]. In two studies, a change in responsiveness to aspirin over time has been described which may at least partly be explained by the variation in CT values [36, 37]. Based on these observations duplicate measurements and in case of inconsistent results repetitive platelet function testing should be performed to prevent misclassification. The high variability of test results after aspirin or clopidogrel intake should also be taken into account when interpreting clinical studies or planning future investigations.

However, in patients prescribed combined use of aspirin and clopidogrel, the results of INNOVANCE® PFA P2Y* seem to be more consistent: two patients presented with duplicate measurements within the reference range, whereas all except for these two yielded two non-closures. In patients with dual

antiplatelet therapy, this all-or-none pattern of duplicate measurements resulted in an extremely low mean CV for INNOVANCE® PFA P2Y* of only 0.8%. This value was lower than expected but can at least partially be explained by the fact that measurement on the PFA-100® system stops after 5 minutes resulting in a maximum CT of 300 s. In addition, the new test cartridge was almost independent of any aspirin-related effect, in contrast to ADP-induced LTA. As clopidogrel is usually administered to patients already on chronic aspirin therapy, e.g., patients with cardiovascular disease undergoing catheter interventional procedures, it may be advantageous to differentiate between the effects of aspirin and clopidogrel on platelet function.

Several studies have been published in recent years suggesting an association of a normal CEPI-CT in aspirin-treated patients with an unfavorable cardiovascular outcome; the results of these studies have been summarized in two recent meta-analyses [38,39]. In addition, on the basis of prospective studies, there is now evidence that high residual platelet reactivity determined by ADP-induced LTA predicts future ischemic events in patients on dual antiplatelet therapy [5-9]. However, the clinical relevance of INNOVANCE® PFA P2Y* results in assessing clopidogrel efficacy remains to be established. As the PFA-100® system is already widely used, the new test cartridge may be a useful tool for the assessment of clopidogrel effects. Most importantly, it remains to be elucidated which method of platelet function testing provides the best risk estimation for recurrent thrombotic events.

We conclude that INNOVANCE® PFA P2Y* is suitable for assessing the effect of clopidogrel on platelet function. Its sensitivity in detecting responsiveness or non-responsiveness to clopidogrel is comparable to ADP-induced LTA although the classification as responder or non-responder was discordant in some patients. The variability of CT values among patients treated with clopidogrel as the only antithrombotic drug is high. However, when used in patients on a combination therapy of aspirin and clopidogrel, INNOVANCE® PFA P2Y* seems to be a useful tool for differentiating between clopidogrel responders and non-responders. Additional prospective studies are needed to clarify the clinical relevance of the test results and classifications obtained with INNOVANCE® PFA P2Y*.

Disclosures

The study was supported by Siemens Healthcare Diagnostics Products GmbH, Germany, of which Dr. Andreas R. Rechner is an employee.

Disclaimer

*Product under development – not available for sale

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Table 1 Baseline characteristics in terms of cardiovascular risk factors and comorbidities.

	Clopidogrel monotherapy (group 1, n=22)	Dual antiplatelet therapy (group 2, n=18)
Sex	11 males, 11 females	11 males, 7 females
Age	69 years [range 55-86]	70 years [43-84]
Vascular risk factor		
- Smoking	5 (22.7%)	8 (44.4%)
- Arterial hypertension	14 (63.6%)	12 (66.7%)
- Diabetes mellitus	5 (22.7%)	9 (50.0%)
- Hyperlipidemia	16 (72.7%)	12 (66.7%)
- Obesity (BMI ≥ 30 kg/m²)	5 (22.7%)	4 (22.2%)
- Chronic Renal Failure	2 (9.1%)	3 (16.7%)
Peripheral Arterial Occlusive Disease (PAOD)	22 (100.0%)	18 (100.0%)
- Prior amputation	0 (0.0%)	1 (5.6%)
- Prior vascular surgery	3 (13.6%)	1 (5.6%)
- Prior foot ulcer/gangrene	0 (0.0%)	2 (11.1%)
- Prior catheter intervention (PTA/Stent)	12 (54.5%)	7 (38.9%)
Ischemic Heart Disease (IHD)	4 (18.2%)	8 (44.4%)
- Prior myocardial infarction	2 (9.1%)	3 (16.7%)
- Prior aorto-coronary bypass surgery	1 (4.5%)	3 (16.7%)
- Prior percutaneous coronary intervention (PCI)	2 (9.1%)	4 (22.2%)
Cerebrovascular Disease (CVD)	5 (22.7%)	3 (16.7%)
- Prior cerebral infarction	3 (13.6%)	2 (11.1%)
- Carotid artery stenosis/occlusion	4 (18.2%)	3 (16.7%)
- Prior carotid artery revascularisation (PTA/Stent or surgery)	1 (4.5%)	2 (11.1%)

Table 2 Comparison of von Willebrand factor antigen (vWF-Ag), vWF-ristocetin cofactor activity (vWF:RCo), and fibrinogen blood levels in clopidogrel non-responders and responders as defined by the different assays.

	Non-Response N/N	**vWF-Ag (%) NR vs. R**	U-Test (p value)	**vWF:RCo (%) NR vs. R**	U-Test (p value)	**Fibrinogen (mg/dl) NR vs. R**	U-Test (p value)
INNOVANCE® PFA P2Y*	6/22	164 vs. 158	0.693	141 vs. 144	0.641	327 vs. 348	0.494
LTA with ADP 2 µM	4/22	188 vs. 156	0.538	138 vs. 144	1.000	336 vs. 335	0.837
LTA with ADP 5 µM	6/22	166 vs. 152	0.641	167 vs. 141	0.407	339 vs. 335	0.914

vWF-Ag = vWF antigen; vwF:RCo = vWF ristocetin cofactor activity; NR = Non-responder, R = Responder.

Fig. 1 Comparison of test results of the different test systems (i.e., the INNOVANCE® PFA P2Y* test cartridge on the PFA-100® System and LTA induced by 2 and 5 µM) in patients on different antiplatelet therapy regimens (i.e., clopidogrel (group 1, n = 22)), aspirin and combined aspirin and clopidogrel therapy (group 2, n = 18)).

Fig. 2A und 2B Duplicate measurements (Test 1 and Test 2) and distribution of test results obtained with INNOVANCE® PFA P2Y* and LTA in patients on clopidogrel monotherapy (group 1, n = 22) and patients on dual antiplatelet therapy (group 2, n = 18). (* 11/22 and ** 16/18 with non-closure (CT ≥ 300 s)).

All figures have been created with SPSS version 15.0

Figure

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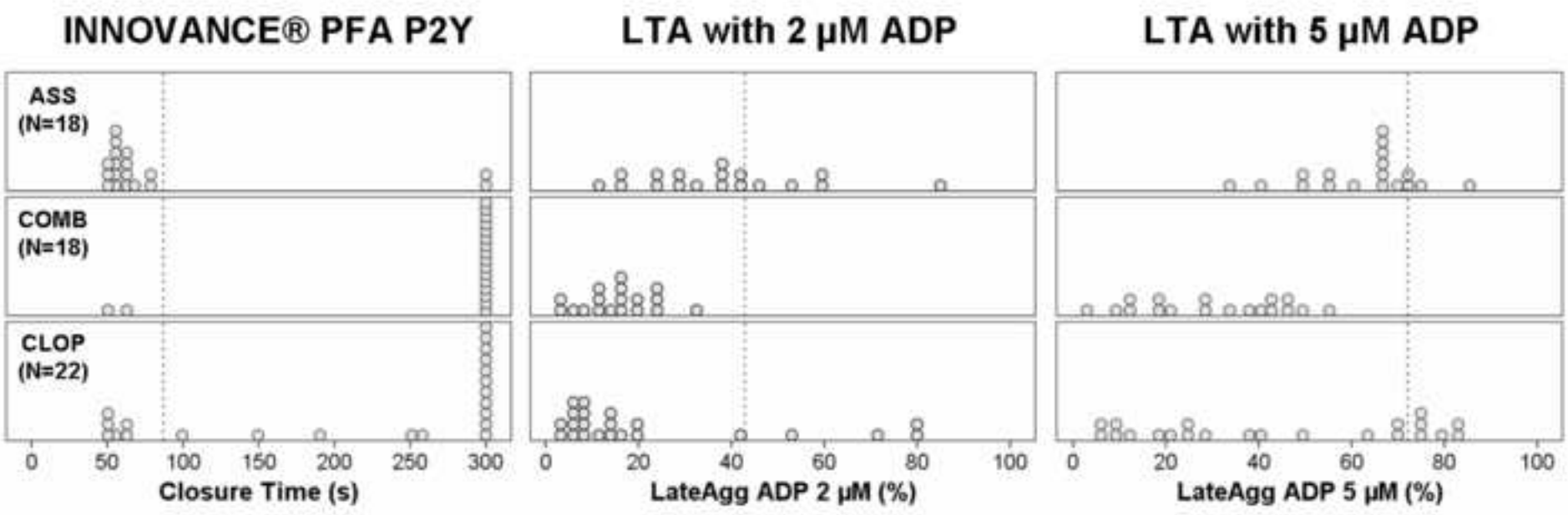

Figure

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