Thrombosis Research 128 (2011) 352–357

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

The influencing factors for clopidogrel-mediated platelet inhibition are assay-dependent

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ARTICLE INFO

Article history:
Received 28 January 2011
Received in revised form 13 April 2011
Accepted 5 May 2011
Available online 28 May 2011

Keywords:
Influencing factors
clopidogrel
platelet reactivity

ABSTRACT

Background: Influencing factors for clopidogrel-mediated platelet inhibition have only been evaluated by one or two different test systems in the same population so far. Since previous studies revealed poor correlations between the various platelet function tests, the identification of influencing variables for clopidogrel response may vary from one test system to the next. We therefore investigated whether the influencing factors for clopidogrel-mediated platelet inhibition depend on the used assay.

Patients and methods: Adenosine diphosphate (ADP)-inducible platelet reactivity was assessed by light transmission aggregometry (LTA), the VerifyNow P2Y12 assay, the vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay, multiple electrode aggregometry (MEA), and the Impact-R in 288 patients after angioplasty and stenting for cardiovascular disease. By univariate and multivariate regression analyses, we evaluated the impact of age ≥ 75, gender, body mass index (BMI), diabetes, active smoking, hypertension, hyperlipidemia, C-reactive protein, platelet count, creatinine, use of calcium-channel blockers (CCBs), statins, proton pump inhibitors, beta blockers, angiotensin converting enzyme inhibitors, and angiotensin receptor blockers on clopidogrel-mediated platelet inhibition in each test system.

Results: None of the independent influencing variables was consistent through all test systems. Only by LTA and the VerifyNow P2Y12 assay, age ≥ 75 and the use of CCBs were independently associated with higher on-treatment platelet reactivity. Only by the VASP assay and MEA, on-treatment platelet reactivity increased linearly with BMI. Further, only by MEA, residual ADP-inducible platelet reactivity increased linearly with platelet count, whereas an increase in platelet count was independently associated with a decrease in ADP-inducible platelet activation by the Impact-R.

Conclusion: The influencing factors for platelet reactivity during clopidogrel therapy are assay-dependent.

Introduction

The measurement of platelet reactivity during antiplatelet therapy with clopidogrel became a major issue in the last few years [1,2]. A variety of platelet function tests have been developed to assess on-treatment residual adenosine diphosphate (ADP)-inducible platelet reactivity. It was shown, that the response to clopidogrel varies considerably from one individual to the next [3,4]. In several prospective studies it was shown that patients with high on-treatment residual platelet reactivity to ADP (HRPR) in one or several platelet function tests had a significantly increased risk for adverse outcomes after percutaneous intervention with stent implantation [5–17]. In further studies, numerous potential influencing variables for the antiplatelet effect of clopidogrel were identified. Besides genetic factors, age, body mass index (BMI), diabetes, treatment with proton pump inhibitors (PPIs), calcium-channel blockers (CCBs), and statins were associated with elevated ADP-inducible platelet reactivity during clopidogrel therapy [18–28]. Most of these variables possibly influence the activity of the cytochrome P450 isoenzymes, which are responsible for the conversion of clopidogrel to its active metabolite and thereby affect the platelet response to clopidogrel treatment.

So far, predictors of a decreased antiplatelet effect of clopidogrel have been evaluated by only one or two different test systems in the same patient population. Further, previous studies revealed poor correlations between the various test systems, and therefore poor

Abbreviations: ADP, adenosine diphosphate; BMI, body mass index; PPIs, proton pump inhibitors; CCBs, calcium-channel blockers; LTA, light transmission aggregometry; VASP, vasodilator-stimulated phosphoprotein; PRI, platelet reactivity index; PRU, P2Y12 Reaction Units; MEA, multiple electrode aggregometry; AU, aggregation units; SC, surface coverage.

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doi:10.1016/j.thromres.2011.05.008

agreement concerning the classification of patients as responders or non-responders to antiplatelet therapy [17,29–31]. Consequently, influencing factors for on-treatment residual ADP-inducible platelet reactivity may be seen with one test system, but not with another one. Based on the rather poor correlation between the diverse tests for the estimation of residual platelet reactivity in patients on clopidogrel treatment, we hypothesised that each of these test systems is sensitive to a different aspect of the fine regulated pathways of platelet activation. We therefore investigated whether the influencing factors are assay-dependent.

Materials and methods

Patients

This was an observational study. The study protocol was approved by the Ethics Committee of the Medical University of Vienna in accordance with the Declaration of Helsinki and written informed consent was obtained from all study participants.

The study population comprised 288 patients on dual antiplatelet therapy after percutaneous intervention with endovascular stent implantation. A part of the patients´ cohort has been included in previous studies [22,27,30]. All patients received daily acetylsalicylic acid therapy (100 mg/day). Except 62 patients (21.5%), who were already on continuous clopidogrel therapy, all patients received a loading dose of 300 (n = 148, 51.4%) or 600 mg (n = 78, 27.1%) clopidogrel prior to intervention followed by a daily dose of 75 mg clopidogrel. Exclusion criteria were a known aspirin or clopidogrel intolerance (allergic reactions, gastrointestinal bleeding), therapy with vitamin K antagonists (warfarin, phenprocoumon, acenocoumarol), treatment with ticlopidine, dipyridamol or nonsteroidal antiinflammatory drugs, a family or personal history of bleeding disorders, malignant paraproteinemias, myeloproliferative disorders or heparin-induced thrombocytopenia, severe hepatic failure, known qualitative defects in platelet function, a major surgical procedure within one week before enrollment, a platelet count <100.000 or >450.000/μl and a hematocrit <30%.

Blood sampling

Blood was drawn by aseptic venipuncture from an antecubital vein using a 21-gauge butterfly needle (0.8 × 19 mm; Greiner Bio-One, Kremsmünster, Austria) 24 hours after the percutaneous intervention [32]. To avoid procedural deviations, all blood samples were taken by the same physician applying a light tourniquet, which was immediately released and the samples were mixed adequately by gently inverting the tubes. After the initial 3 ml of blood had been discarded to reduce procedurally induced platelet activation, blood was drawn into a 3.8% sodium citrate Vacuette tube (Greiner Bio-One; 9 parts of whole blood, 1 part of sodium citrate 0.129 M/L) for evaluations by light transmission aggregometry (LTA) and the vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay, into a 3.2% sodium citrate Vacuette tube (Greiner Bio-One; 9 parts of whole blood, 1 part of sodium citrate 0.109 M/L) for the VerifyNow P2Y12 assay and into a Vacuette tube containing lithium heparin (18 IU/ ml) for the determinations by multiple electrode platelet aggregometry (MEA) and the Impact-R. The time interval between blood sampling and testing was at least 1 hour and did not exceed 3 hours. To avoid investigator-related variations of the results, each of the different tests was performed by just one corresponding operator, who was blinded to the results from the other operators.

Light transmission aggregometry

LTA was performed as previously described [30]. In brief, citrate-anticoagulated whole blood was centrifuged at 150 ×*g* for 10 minutes at room temperature to obtain platelet rich plasma (PRP). Platelet poor plasma (PPP) was obtained from the remaining specimen by re-centrifugation at 2000 ×*g* for 10 minutes. Platelet counts were not adjusted as the median platelet count was 208 G/L (range 106–431 G/L) [33,34]. The baseline optical density was set with PPP. Aggregation was performed using ADP (10 μM; Rolf Greiner Bio-Chemica, Flacht, Germany). Optical density changes were recorded photoelectrically for 10 minutes as platelets began to aggregate.

VerifyNow P2Y12 assay

The VerifyNow P2Y12 assay (Accumetrics, San Diego, CA, USA) was performed as previously described [30]. Briefly, citrate-anticoagulated whole blood was automatically dispensed from the blood collection tube into the assay device by the instrument. ADP was incorporated into the assay channel to induce platelet activation and light transmittance increased as activated platelets bound and aggregated fibrinogen-coated beads. The instrument measured this change in optical signal and reported results in P2Y12 reaction units (PRU). With this assay, a higher PRU reflects greater ADP-mediated platelet reactivity.

Vasodilator-stimulated phosphoprotein phosphorylation assay

The platelet reactivity index (PRI) was determined according to a standardized flow cytometric assay (Platelet VASP, Diagnostica Stago, Biocytex, Marseille, France) as previously described [30]. Samples of citrate-anticoagulated whole blood were incubated in *vitro* with prostaglandin E1 (PGE1), with or without ADP before fixation. After 10 minutes, platelets were permeabilized, labeled with a primary monoclonal antibody against serine 239-phosphorylated VASP (clone 16 C2) or its isotype, followed by a secondary fluorescein isothiocyanate-conjugated polyclonal goat anti-mouse antibody. All procedures were performed at room temperature and the samples were analyzed on a Becton Dickinson FACSCalibur system (BD Biosciences, Vienna, Austria). The platelet population was identified by its forward and side scatter distribution, and 10.000 platelet events were gated. The extent of VASP phosphorylation was measured by geometric mean fluorescence intensity (MFI) values in the presence of PGE1 without (T1) or with ADP (T2). After subtraction of the negative isotypic control values from the corresponding fluorescence values, PRI (%) was calculated according to the following formula:

$$\mathrm{PRI\%} = [\mathrm{T1(PGE1)}-\mathrm{T2(PGE1 + ADP)} / \mathrm{T1(PGE1)}] \times 100$$

The ratio was expressed as mean percentage platelet reactivity, inversely correlated with the clopidogrel-mediated platelet inhibition.

Multiple electrode platelet aggregometry

Whole blood impedance aggregometry was performed as previously described [30]. After dilution (1:2 with 0.9% NaCl solution) of heparin-anticoagulated whole blood and stirring in the test cuvettes for 3 minutes at 37 °C, ADP (6.4 μM; Dynabyte, Munich, Germany), respectively, were added, and aggregation was continuously recorded for six minutes. The adhesion of activated platelets to the electrodes led to an increase of impedance, which was detected for each sensor unit separately and transformed to aggregation units (AU) that were plotted against time.

Cone and Plate(let) analyzer Impact-R

The Impact-R (Matis Medical Inc, Beersel, Belgium) was performed as previously described [30]. In brief, 130 μL of lithium heparin-anticoagulated whole blood were placed on the polystyrene plate. Shear stress was immediately applied (2050 s^{-1}) using a acrylnitril-

butadien-styrene cone. Plates were then washed with tap water and stained with May-Grünwald solution following the manufacturer's instructions. Samples were analyzed with an inverted light microscope connected to an image analyzer (Galai, Migdal Haemek, Israel). Like in previous reports, platelet adhesion determined by examination of the percentage of total area covered with platelets (surface coverage, SC %) was used for statistical analyses [30,35,36]. Seven images were collected from each run and medians of these were calculated by the analyzing system.

In the ADP response test, the whole blood sample was pre-incubated with 1.36 μM ADP (DiaMed, Cressier, Switzerland) for 1 minute under gentle mixing (10 rpm) prior to the Impact-R test. This led to platelet activation and microaggregates formation in the tube resulting in reduced platelet adhesion to the well as reflected by reduced SC.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 15.0, SPSS, Chicago, Illinois, USA). Median and range of continuous variables are shown. Spearman rank correlation was used to test for correlations between the different platelet function tests. Univariate and multivariate linear regression analyses were used to identify influencing variables for ADP-inducible platelet reactivity in each test system. By multivariate regression analysis, we adjusted for age ≥ 75, gender, BMI, diabetes, active smoking, hypertension, hyperlipidemia, C-reactive protein, platelet count, serum creatinine, use of CCBs, statins, PPIs, beta blockers, angiotensin converting enzyme inhibitors (ACE), and angiotensin receptor blockers (ARBs). Two-sided P-values<0.05 were considered statistically significant.

Table 1
Clinical, laboratory and procedural characteristics of the study population.

Characteristics	n = 288
Age, years	66 (36–93)
Male sex	188 (65.3)
BMI, kg/m^2	27 (18.5-45.7)
Medical history	
Previous MI	120 (41.7)
Previous TIA/stroke	30 (10.4)
Hypertension	261 (90.6)
Hypercholesterolemia	269 (93.4)
Diabetes mellitus	95 (33)
Active smoking	116 (40.3)
Laboratory data	
Platelet count, G/L	208 (106–431)
Serum creatinine, mg/dl	1.02 (0.52-2.28)
C-reactive protein, mg/dl	0.94 (0.02-15.14)
Procedure	
Stent implantation	288 (100)
- peripheral	152 (52.8)
- coronary	102 (35.4)
- carotid	34 (11.8)
Number of stents/patient	1 (1–7)
Medication pre-intervention	
Clopidogrel	288 (100)
Aspirin	288 (100)
Statins	276 (95.8)
ACE inhibitors	182 (63.2)
Angiotensin receptor blockers	75 (26)
Beta blockers	205 (71.2)
Proton pump inhibitors	155 (53.8)
Calcium-channel blockers	88 (30.6)

Continuous data are shown as median (range). Dichotomous data are shown as n (%). BMI, body mass index; MI, myocardial infarction; TIA, transient ischaemic attack; ACE inhibitors, angiotensin converting enzyme inhibitors.

Results

Clinical, laboratory and procedural characteristics of the study population are shown in Table 1. Medians (range) of maximal aggregation % by LTA, PRU by the VerifyNow P2Y12 assay, PRI by the VASP assay, AU by MEA, and SC % by the Impact-R in patients were 46% (0–87.4%), 203 PRU (10–385 PRU), 49.5% (0-100%), 39 AU (6–110 AU), and 3.7% (0.6-11%), respectively. The results from all assays correlated significantly with each other but the correlations were at best moderate (Table 2).

Results of univariate and multivariate analyses of significant influencing factors for on-treatment residual ADP-inducible platelet reactivity by each test system are given in Table 3. In univariate analyses, age≥75 was associated with significantly higher ADP-inducible platelet reactivity by LTA, the VerifyNow P2Y12 assay, and the Impact-R. Further, LTA, the VerifyNow P2Y12 assay, the VASP assay, and the Impact-R identified the use of CCBs as predictor of decreased platelet inhibition by clopidogrel. The BMI was an influencing factor for on-treatment platelet reactivity by LTA, the VASP assay, MEA, and the Impact-R. Platelet counts were significantly influencing platelet reactivity by the VerifyNow P2Y12 assay, MEA, and the Impact-R. Smoking was identified as an influencing variable for ADP-inducible platelet reactivity by the VerifyNow P2Y12 assay and the Impact-R, whereas diabetes was significantly affecting the results from LTA and the VASP assay. Renal function was classified as influencing variable for platelet reactivity by the VerifyNow P2Y12 assay, MEA, and the Impact-R. The use of ACE and ARBs were only associated with platelet reactivity by the VASP assay, and the use of PPIs was only associated with surface coverage by the Impact-R.

In multivariate analyses, age≥75 and the use of CCBs were independently associated with higher on-treatment residual ADP-inducible platelet reactivity only by LTA and the VerifyNow P2Y12 assay (age≥75: p = 0.01 for LTA and p<0.001 for the VerifyNow P2Y12 assay; CCBs: p = 0.03 for LTA and p = 0.04 for the VerifyNow P2Y12 assay). By LTA there was also a trend towards higher aggregation values with increasing BMI (p = 0.09) in multivariate regression analysis.

Only by the VASP assay and MEA, an increasing BMI was independently associated with an increase of on-treatment residual ADP-inducible platelet reactivity (p<0.001 for the VASP assay and p = 0.004 by MEA). Further, by the VASP assay, there were trends towards higher reactivity values in patients with concomitant CCB and PPI therapy (both p = 0.09), whereas by MEA platelet reactivity increased linearly with platelet count (p<0.001).

By the Impact-R, there were trends towards higher aggregation values in patients aged≥75 years, and in patients taking CCBs and ACE inhibitors in multivariate regression analysis (all p = 0.08). Further, an increase in platelet count was independently associated with a decrease in ADP-inducible platelet activation by the Impact-R (p = 0.003).

Discussion

This is the first study investigating the impact of demographic, medication and physiologic factors on clopidogrel-mediated platelet inhibition by five different test systems in the same patient population. Our results indicate that the various influencing factors affecting *in vitro* assessment of clopidogrel-mediated platelet inhibition depend on the

Table 2
Correlations between the results of the different platelet function tests.

	VerifyNow P2Y12	VASP assay	MEA	Impact-R
LTA	0.65	0.37	0.45	-0.32
VerifyNow P2Y12		0.44	0.32	-0.52
VASP assay			0.33	-0.26
MEA				-0.2

LTA, light transmission aggregometry; VASP, vasodilator-stimulated phosphoprotein; MEA, multiple electrode aggregometry.

Table 3
Regression coefficients (B), 95% confidence intervals (CI), and p-values of univariate and multivariate analyses, and correlation coefficients (r) of univariate analyses of significant influencing variables for on-treatment residual ADP-inducible platelet reactivity by each test system.

	Univariate analysis				Multivariate analysis		
Variable	r	B	95% CI	p	B	95% CI	P
LTA							
Age ≥ 75	0.18	7.6	2.6 – 12.6	0.003	7.5	1.8 – 13.2	0.01
BMI	0.13	0.6	0.07 – 1.2	0.027	0.5	-0.07 – 1.1	0.09
DM	0.12	4.8	0.2 – 9.5	0.04	2.8	-2 – 7.6	0.26
CCBs	0.19	7.7	3 – 12.4	0.001	5.8	0.7 – 10.9	0.026
VerifyNow P2Y12 assay							
Age ≥ 75	0.41	83.4	61.4 – 105.3	<0.001	70.9	45.8 – 96	<0.001
Smoking	-0.19	-35.3	-56.2 – -14.4	0.001	-9.4	-31 – 12.2	0.39
Platelet count	-0.15	-0.2	-0.4 – -0.05	0.014	-0.1	-0.3 – 0.07	0.25
Creatinine	0.23	70.5	35.6 – 105.4	<0.001	24.1	-13.2 – 61.4	0.2
CCBs	0.2	38.4	16.2 – 60.6	0.001	23.6	1.1 – 46	0.04
VASP assay							
BMI	0.26	1.5	0.9 – 2.2	<0.001	1.3	0.7 – 2	<0.001
DM	0.13	6.3	0.6 – 12	0.03	4.5	-1.3 – 10.3	0.13
ACE	0.17	8.3	2.7 – 13.8	0.004	2.8	-5.7 – 11.2	0.52
ARBs	-0.19	-9.8	-15.9 – -3.8	0.001	-6.8	-16.2 – 2.5	0.15
CCBs	0.12	6.2	0.4 – 12	0.037	5.3	-0.8 – 11.4	0.09
MEA							
BMI	0.14	0.7	0.1 – 1.3	0.018	0.9	0.3 – 1.4	0.004
Platelet count	0.43	0.15	0.1 – 0.2	<0.001	0.15	0.1 – 0.2	<0.001
Creatinine	-0.14	-9.7	-18 – -1.4	0.022	-2.5	-11.2 – 6.3	0.58
Impact-R							
Age ≥ 75	-0.2	-1.1	-1.8 – -0.5	0.001	-0.7	-1.4 – 0.09	0.083
BMI	-0.12	-0.08	-0.15 – -0.02	0.044	-0.06	-0.1 – 0.02	0.13
Smoking	0.15	0.8	0.2 – 1.4	0.013	0.4	-0.3 – 1	0.25
Platelet count	0.24	0.01	0.006 – 0.016	<0.001	0.008	0.003 – 0.01	0.003
Creatinine	-0.12	-1.1	-2 – -0.4	0.042	0.06	-1.1 – 1.2	0.92
PPIs	-0.13	-0.7	-1.2 – -0.08	0.025	-0.4	-1 – 0.1	0.13

LTA, light transmission aggregometry; BMI, body mass index; CCBs, calcium-channel blockers; VASP, vasodilator-stimulated phosphoprotein; DM, diabetes mellitus; ACE, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; MEA, multiple electrode aggregometry; PPIs, proton pump inhibitors.

used test system. None of the independent influencing variables was consistent throughout all test systems. The best concordance was seen between LTA and the VerifyNow P2Y12 assay, which both classified age ≥ 75 and the use of CCBs as independent predictors of higher on-treatment residual ADP-inducible platelet reactivity.

The discrepancies concerning the identification of influencing factors for on-treatment platelet reactivity may be caused by the fundamentally different approaches of the various test systems: LTA and the VerifyNow P2Y12 assay measure the increase of light transmittance through the sample after the addition of ADP and use it as surrogate marker for the extent of platelet aggregation. In line with previous studies, these tests correlated best and identified the same variables as independent influencing factors for clopidogrel-mediated platelet inhibition [29–31]. In contrast, the VASP assay is a flow-cytometric method, which specifically captures a second- messenger reaction of the P2Y12 receptor [37–39]. MEA uses the principle of impedance aggregometry, whereas the Impact-R is based on pre-stimulation of platelets by ADP and the subsequent detection of non-activated platelets by high shear stress stimulation [35,40]. Since the Impact-R is laborious and time-consuming, it has not become a routine method for platelet function analysis and consequently, only few data are available on this assay, so far. Recently, Breet et al. reported that the Impact-R was not able to discriminate between patients without and with adverse ischemic events at 1-year follow-up after elective coronary stenting [17].

We used unfractionated heparin as anticoagulant for MEA. This approach is in line with a recent report by Kalb et al., who found that unfractionated heparin was suitable for whole blood impedance aggregometry, whereas aggregation in response to ADP was significantly impaired in citrated blood [41].

By showing that age ≥ 75 and the use of CCBs are independently associated with increased on-treatment platelet reactivity by LTA and the VerifyNow P2Y12 assay, our data are in line with previous findings by others and us [22,27,42,43]. By the VASP assay and the Impact-R, a significant influence of concomitant CCB medication was only seen by univariate, but not by multivariate analysis. Like in a recent study by Sarafoff et al., we did not observe an attenuation of clopidogrel-mediated platelet inhibition by CCB in MEA [44]. Thus, CCB seem to have some influence on ADP-mediated platelet activation, but the effect may be outweighed if other factors are also taken in consideration. Age ≥ 75 years had a significant detrimental effect if evaluated by LTA and the VerifyNow P2Y12 assay but no influence on the VASP assay, indicating that age ≥ 75 years is not affecting the receptor's susceptibility to ADP directly. Likewise, the response to shear stress by the Impact-R test is not strongly affected in elder patients. Besides age ≥ 75 years and the use of CCBs, the BMI was the only independent predictor of decreased clopidogrel-mediated platelet inhibition in more than one assay. In line with previous studies, ADP-inducible platelet reactivity by the VASP assay and by MEA increased with the BMI [23,24]. Further, we observed a trend towards higher aggregation values by LTA with increasing BMI. We therefore can conclude that age ≥ 75 years, the concomitant medication with CCBs, and the BMI indeed increase *in vitro* residual platelet reactivity, but not all test systems are sensitive to these factors to the same extent.

In contrast to previous findings, active smoking was not independently associated with an enhanced antiplatelet effect of clopidogrel. This may be due to the number of cigarettes smoked per day. Previous studies associated smoking ≥ 10 cigarettes/day with increased clopidogrel-mediated platelet inhibition by LTA and the VerifyNow P2Y12 assay [45–47]. However, in this study, we did not assess the exact number of cigarettes/day and therefore we cannot discriminate between patients with ≥ 10 and < 10 cigarettes/day. Further, in several larger studies smoking was associated with increased platelet reactivity by MEA [7,16].

Interestingly, MEA and the Impact-R yielded opposite results concerning the influence of platelet counts, while increasing platelet counts were not revealed as affecting the other test results. While residual platelet reactivity by MEA increased with platelet count, an increase in

platelet count was independently associated with a decrease of ADP-inducible platelet activation by the Impact-R. This finding may be due to the underlying principles of both systems. MEA directly measures adhesion of activated platelets to the electrodes after the addition of ADP. The more platelets, the more can adhere to the electrodes, the stronger the inhibition of the current. In contrast, in the Impact-R ADP response test, platelets are pre-incubated with ADP. Thereby, platelets which are not blocked by clopidogrel are activated and form micro-aggregates. These microaggregates do not adhere to the well and are washed away with tap water resulting in reduced SC. The amount of the platelets that adhere to the surfaces of the well is indicative of the platelets inhibited by clopidogrel. However, if the platelet count is higher also more inhibited platelets are present in the sample and this leads to a higher surface coverage. Thereby, the paradox influence of platelet count on results by the Impact-R may be due to its principle of "inverse aggregometry", i.e. detecting the inhibited but not the non-inhibited platelets. If the platelet count increases, the non-inhibited platelets rise too, but these are not detected by the Impact-R. To sum up, the major difference between MEA and the Impact-R is that MEA detects activated platelets, which adhere to its electrodes, while the Impact-R detects non-activated platelets.

In line with recent studies, the use of statins was not associated with an increase in on-treatment platelet reactivity [48,49]. Further, patients with PPIs exhibited similar reactivity values like patients without PPI therapy. This may be due to the small number of patients receiving omeprazole, which was shown to decrease clopidogrel-mediated platelet inhibition after coronary stenting [26,50].

In conclusion, influencing factors for platelet reactivity during clopidogrel therapy are assay-dependent. Results from LTA, the VerifyNow P2Y12 assay, MEA and the VASP assay were previously associated with adverse ischemic events after coronary stenting [8]. Consequently, influencing factors for clopidogrel-mediated platelet inhibition identified by these test systems need to be investigated regarding the clinical outcome of clopidogrel-treated patients after angioplasty and stenting for cardiovascular disease.

Limitations

The limitations of our study comprise the lack of clinical outcome data and cytochrome P450 2 C19 genotyping.

Conflict of Interest

None.

Acknowledgements

We would like to thank Beate Eichelberger and Daniela Koren for expert technical support.

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