Changes in response to clopidogrel therapy in patients after percutaneous coronary interventions as assessed by different platelet function tests

Jacek Golański¹, Kamila Syska¹, Krzysztof Chiżynski², Hassan Kassassir¹, Cezary Watałe¹, Agata Sakowicz³, Wiktor Kuliczkowski⁴

¹ Department of Haemostasis and Haemostatic Disorders, Medical University of Lodz, Łódź, Poland
² Department of Intensive Cardiac Therapy, Medical University of Lodz, Łódź, Poland
³ Department of Medical Biotechnology, Medical University of Lodz, Łódź, Poland
⁴ Department of Cardiology, Wrocław Medical University, Wrocław, Poland

KEY WORDS
- method agreement
- platelet function tests
- responder status to clopidogrel

ABSTRACT

INTRODUCTION
Recently, the responder status to clopidogrel therapy has been observed to change over time.

OBJECTIVES
The aim of the study was to investigate changes in the responder status to clopidogrel therapy over time with the use of 4 platelet function tests (light transmission aggregometry [LTA], multiple electrode aggregometry [MEA], vasodilator-stimulated phosphoprotein (VASP) phosphorylation, and INNOVANCE® PFA P2Y assays [PFA]) in patients after percutaneous coronary intervention (PCI). We also compared the results of these tests to determine the most reliable method.

PATIENTS AND METHODS
The study included 35 patients after PCI, receiving acetylsalicylic acid (75 mg/d) and clopidogrel (75 mg/d). The control group included 50 healthy volunteers. Platelet function was measured at 3 different time points (4 ± 2 days after PCI, and then after 6 and 12 weeks).

RESULTS
The responder status to clopidogrel changed in 5 patients (14%) as shown by MEA; in 7 patients (20%), by LTA and PFA; and in 13 patients (37%), by VASP. The Cohen’s κ coefficient showed a moderate or poor agreement between the tests. The strongest agreement was between MEA and PFA (80%; κ = 0.46, P = 0.003), PFA and LTA (82%; κ = 0.41, P = 0.004), and MEA and LTA (80%; κ = 0.36, P = 0.008). The κ coefficient for all comparisons with VASP was less than 0.30.

CONCLUSIONS
Changes in the responder status over time are present for all platelet function tests, but a large discrepancy between the tests does not allow a careful assessment of this phenomenon. The tests showed only moderate agreement (in relation to one another and to time points), which significantly limits their interchangeable use in clinical practice.

INTRODUCTION
Although the use of routine laboratory testing for antiplatelet therapy monitoring is not recommended by any guidelines, there is still a considerable interest in this subject among researchers. Despite the fact that numerous platelet function tests (PFTs) have been developed, there is no consensus as to which one is the most appropriate for the monitoring of antiplatelet therapy. All methods have 2 main weaknesses. First, none of them has been fully standardized, which makes it difficult to compare results from independent laboratories. Second, as all methods have low positive predictive values for future ischemic events, they are associated with an increased risk of false positive results.

Nevertheless, PFTs revealed high on-treatment platelet reactivity (HPR) in approximately 20% of patients after elective percutaneous
coronary intervention (PCI). This phenomenon typically occurs on clopidogrel therapy and can be partially attributed to the presence of diabetes, genetic polymorphisms, overweight, platelet count, C-reactive protein (CRP) level, reduced renal function, active smoking, older age, or acute coronary syndrome.\textsuperscript{10–18} It is independently associated with a 2- to 5-fold higher risk of recurrent ischemic events compared with that in good responders to clopidogrel. However, this is only applicable to the whole groups of HPR patients, as all available PFTs have a low positive predictive value for individual risk estimation.

The limitation of PFTs in the prediction of future ischemic events can be attributed to the methodology of platelet reactivity measurement. Although most studies measure the response to clopidogrel only at a single time point, typically during hospitalization, some studies have suggested that susceptibility to antiplatelet therapy can vary between individuals and may differ over time. This is a relatively new phenomenon, but its occurrence can be predicted.\textsuperscript{19} Considering that the results of platelet reactivity obtained from different tests may be inconsistent and may depend on the duration of antiplatelet therapy, the use of single time-point testing to classify patients as poor or good responders seems to be inaccurate.\textsuperscript{9} Therefore, numerous authors refrain from recommending any particular assay to evaluate the effectiveness of antiplatelet treatment in routine clinical practice.\textsuperscript{1,2,20–22}

The aim of our study was to investigate changes in the responder status to clopidogrel therapy over time with the use of 4 PFTs (light transmission aggregometry [LTA], multiple electrode aggregometry [MEA], vasodilator-stimulated phosphoprotein [VASP] phosphorylation, and INNOVANCE\textsuperscript{®} PFA P2Y [PFA] assays) in a specially selected group of patients after PCI. The study also attempted to determine the most reliable PFA by comparing the results obtained using the 4 different methods.

**PATIENTS AND METHODS** The study included 50 patients (all males, aged 42–70 years) with coronary artery disease, who underwent elective PCI at the Department of Invasive Cardiology of the Medical University of Lodz, Lodz, Poland. Patients were receiving aspirin therapy (75 mg of acetylsalicylic acid daily). They were given clopidogrel at a dose dependent on the time of PCI intervention: from 600 mg during PCI to 75 mg/d after the procedure.

Clopidogrel was administered in the morning at around 7 AM. Blood was collected at around 9 AM, and the tests were performed at 10 to 11 AM. The exclusion criteria were as follows: platelet count of less than 100 × 10\textsuperscript{9}/l or exceeding 400 × 10\textsuperscript{9}/l, diabetes mellitus, CRP level exceeding 5 mg/l, CYP2C19*2 allele, glomerular filtration rate of less than 60 ml/min/1.73 m\textsuperscript{2}, age older than 75 years, and use of any anti-inflammatory drugs during the 2 weeks preceding the study. The use of eptifibatide or tirofiban was allowed during the PCI procedure because blood collection took place more than 4 days after PCI. The on-treatment platelet reactivity of each patient was evaluated at 3 different time points: 4 ±2 days after PCI (considered as baseline [point A]), and then after 6 (point B) and 12 weeks (point C).

The control group consisted of 50 healthy volunteers (21 men, 29 women; mean age, 40 ±12 years). Controls did not take any drugs that might have affected platelet reactivity, including non-steroidal anti-inflammatory drugs, for at least 7 days before the study.

The study was conducted in accordance with the Declaration of Helsinki, and approved by an institutional ethics committee. All participants confirmed the voluntary and conscious participation in the study.

**Blood sampling** Blood was collected by peripheral venipuncture into 2 sets of plastic tubes (S-Monovette, Sarstedt, Nümbrecht, Germany). The first set contained buffered sodium citrate, 3.2% (Becton Dickinson, Plymouth, United Kingdom) with a final citrate: blood ratio of 1:9 vol/vol, while the second contained 250 µg/ml hirudin (Refudan\textsuperscript{®}, Schering AG, Germany), yielding a final concentration of 25 µg/ml in the blood sample. Whole blood was kept at room temperature. Platelet-rich plasma (PRP) was prepared by centrifugation of the sodium citrate-anticoagulated whole blood at 250 g for 6 minutes, and kept in a capped plastic tube at 37°C. Platelet count was measured with the ABX MICROS CRP hematology analyzer (Horiba ABX, New Jersey, United States). Platelet-poor plasma (PPP) was isolated by centrifuging the blood remaining after PRP removal at 2000 g for 15 minutes.

**Genotype determination** DNA was obtained from 1-ml samples of sodium citrate-anticoagulated whole blood, which had been stored at −20°C. Genomic DNA was extracted from blood leukocytes using a Chemagic DNA Blood100 commercial kit (Chemagen, Germany). The polymerase chain reaction (PCR) amplification of CYP2C19*1, *2 alleles used 100-ng samples of genomic DNA template and 50 pmol/l of each primer as follows: forward primer 5′–ATT ACA ACC AGA GCT TGG C–3′ and reverse primer 5′–TAT CAC TTT CCA TAA AAG CAA GG–3′. The procedure was as follows: denaturation at 94°C for 60 seconds and annealing at 57°C for 60 seconds, with extension at 72°C for 45 seconds, with a 30-cycle amplification. The product of PCR was digested using the Smalenzyme (Fermentas, Finnzymes, Abgene). The product bands were separated on polyacrylamide gel (6%), treated with ethidium bromide, and visualized with ultraviolet light. Positive and negative controls were included in all PCR analyses, and samples were digested with restriction enzymes. The sequences of the primers after slight modification were as described by Adithan et al.\textsuperscript{23}
Platelet reactivity measurement Platelet reactivity was measured using 4 different adenosine diphosphate (ADP)-induced PFTs. To obtain reliable results, all experiments were repeated at least once, and the means of 2 records were used for the analyses. The measurements were carried out within 2 hours of blood sampling, except for the analysis of VASP phosphorylation by flow cytometry. To define HPR, the cut-off values for each method were determined for better discrimination of the differences between the methods used in the study.6,7,24

Light transmission aggregometry Optical platelet aggregation was measured within 2 hours of blood sampling in platelet-rich plasma using the 490-2D Chrono-Log Aggregometer and Aggrolink Software (Chrono-Log Corporation, Haverton, Pennsylvania, United States). Platelet count was adjusted to 200 × 10⁹/l with PPP. PPP was set as a reference for 100% of light transmission. Platelets in 300 µl of PRP were stimulated with ADP (Sigma-Aldrich Co., Munich, Germany) at 5 µmol/l (final concentration), and the extent of aggregation was defined as the maximum percent change in light transmission from baseline after the addition of an agonist.

Multiple electrode aggregometry Whole blood aggregation was measured using a 5-channel, semiautomated, dual measurement aggregometer (Multiplate® Analyzer, Roche Diagnostics GmbH, Mannheim, Germany).25 ADP (Roche Diagnostics GmbH; final concentration, 6.4 µmol/l) was added as a platelet agonist, and an increase in electrical impedance caused by the growing platelet attachment to the electrodes was recorded continuously for 6 minutes. The area under the aggregation curve was used to express the aggregation response over the measured time after 6 minutes of measurement.

Determination of PFA-100 closure time The closure time (CT expressed in seconds, maximum recorded value of 300 seconds) was measured with the PFA-100 System (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany).26 The new INNOVANCE PFA P2Y® cartridges (Siemens Healthcare Diagnostics Products GmbH) were used. The membrane in INNOVANCE PFA P2Y® cartridges was coated with 20 µg of ADP, 5 ng of prostaglandin E₁, and 125 µg of ionic calcium. A threshold CT value of 300 seconds was used for results greater than 300 seconds in data analysis.

Analysis of vasodilator-stimulated phosphoprotein phosphorylation by flow cytometry A standardized flow cytometric assay, PLT VASP/P2Y12 (BioCytex, Marseille, France) according to Alell et al27 with some adaptations, was used to determine the VASP phosphorylation level in platelets in whole blood. The analysis was performed at room temperature. Platelet reactivity was measured using a FACSCanto II flow cytometer (BD Biosciences, San Jose, California, United States). The analysis was performed using BD FACSDiva Software (BD Biosciences). Platelet reactivity was expressed as platelet reactivity index.27 Samples for the determination of VASP phosphorylation were prepared and measured within 24 hours from blood collection (VASP phosphorylation assay gives stable results for 48 hours after blood sampling).

Statistical analysis Normally distributed variables were presented as mean ± SD. Nonnormally distributed parameters were presented as medians and interquartile ranges (from the 25% quartile to the 75% quartile, as indicated by the Shapiro–Wilk test).

We calculated the intra-assay precision (coefficients of variation [CV]) among all 35 patients at 3 time points of the measurement, according to the guidelines from the Clinical and Laboratory Standards Institute.

We calculated the change in the responder status (CRS) as: CRS% = ([HPR(B) and non-HPR(C) + non-HPR(B) and HPR(C)/35] × 100, where CRS% stands for the CRS (%) of all patients (n = 35); HPR(B) and non-HPR(C), for the number of patients with HPR after 6 weeks and without HPR after 12 weeks, respectively; and non-HPR(B) and HPR(C), for the number of patients without HPR after 6 weeks and with HPR after 12 weeks, respectively.

For patient characteristics and platelet reactivity, the continuous variables were analyzed using the 1-way analysis of variance or nonparametric Kruskal–Wallis test and the all-pairwise comparisons Conover–Inman test. Ordinal data were analyzed with the Yates-corrected χ² or the Fisher exact test. The Cohen’s κ coefficient was calculated as a measure of agreement. A κ statistic value of less than 0.4 represented a poor-to-fair agreement, a value of 0.41 to 0.60 reflected a moderate agreement, a value of 0.61 to 0.80 was considered a good agreement, and a value of 0.81 to 1.0 was considered a very good agreement between methods.

We used an unpaired 2-sample t test for minimum sample size estimation. For leading variables, we assumed the values of the standardized effects equal 1.0 (for variables, the differences between means originating from 2 compared methods equaled 20% and pooled measures of dispersion [SD] equaled 20%). The calculated sample size for the power and significance equal to at least 0.8 and 0.05 was 32 persons. During the study, the above parameters occurred to be higher than 20%; therefore, the sample size was increased to 40 persons. Analyses were performed with the use of StatsDirect statistical software, version 2.7.8 (StatsDirect Ltd, Altrincham, United Kingdom).

RESULTS Of the 50 screened patients, 7 withdrew their informed consent for personal reasons and another 8 patients were found to be
The HPR values of each patient were evaluated 4 ± 2 days after PCI (point A) and after 6 (point B) and 12 weeks (point C) of continuous clopidogrel treatment (75 mg/d). Because the mean platelet reactivity (for all methods except PFA) and the HPR status remained stable between points A and B, in TABLE 4 we only showed CRSs at 6 and 12 weeks. For the MEA, the CRS was present in 5 patients (14.3%); for LTA and PFA, in 7 patients (20%); and for VASP phosphorylation, in 13 patients (37%). TABLE 4 shows the percentage of CRS compared to the acceptable hypothetical 5% of CRS proposed by Hochholzer et al., and to 0% of CRS with our modification.

The changes in platelet reactivity determined by various methods in all patients participating in the study are presented in FIGURE 1. The relationship between the outcomes recorded for various tests was estimated using the Cohen’s k test. The agreement between measurements at different time points was moderate to poor. Between 6 and 12 weeks of continuous clopidogrel treatment, the strongest agreement was revealed for the MEA and PFA (κ = 0.58; P = 0.0002).

Platelet reactivity measurements after 12 weeks of clopidogrel treatment were used to assess the compatibility between the methods. The Cohen’s κ test showed that the level of agreement between various tests was moderate or poor. The strongest agreement occurred between the MEA and PFA (80%; κ = 0.46, P = 0.003), PFA and LTA (82%; κ = 0.41, P = 0.004), and MEA and LTA (80%; κ = 0.36, P = 0.008). Cohen’s κ coefficients for all other comparisons with VASP phosphorylation were less than 0.30.

According to the additional analysis, the platelet and leukocyte counts, together with the CRP level, could influence the development of HPR. The listed variables were significantly reduced in the non-HPR group. Data are presented in TABLE 5. The percentage of smokers did not differ between the HPR and non-HPR groups.

### DISCUSSION
This was a novel study involving the assessment of 4 ADP-induced platelet reactivity tests (LTA, MEA, VASP phosphorylation, and INNOVANCE PFA P2Y assays) over 12 weeks in patients receiving stable dual therapy of aspirin (75 mg/d) and clopidogrel (75 mg/d). All examined methods except for the PFA assay have been reported to have predictive value for future adverse events in cardiac patients on dual anti-platelet therapy.22,29,30

The measurements of platelet reactivity varied over time in a significant proportion of patients. Interestingly, no significant changes were observed in total platelet reactivity and HPR frequency. In contrast, when evaluating each patient individually, CRS was present in a considerable percentage of patients when tested at 2 different time points. The lowest CRS values were observed for MEA (14%) and the highest for VASP phosphorylation (37%), which is to some extent

### TABLE 1 Baseline characteristics of the study cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study cohort, n = 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>age, y</td>
<td>60.0 ± 7.8</td>
</tr>
<tr>
<td>male sex</td>
<td>35 (100.0)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.4 ± 4.5</td>
</tr>
<tr>
<td>diabetes mellitus</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>hypertension</td>
<td>27 (77.1)</td>
</tr>
<tr>
<td>hyperlipidemia</td>
<td>23 (65.7)</td>
</tr>
<tr>
<td>hypercholesterolemia</td>
<td>19 (54.3)</td>
</tr>
<tr>
<td>current smoking</td>
<td>12 (34.3)</td>
</tr>
<tr>
<td>smoking history</td>
<td>21 (60.0)</td>
</tr>
<tr>
<td>previous myocardial infarction</td>
<td>34 (97.1)</td>
</tr>
<tr>
<td>previous PCI</td>
<td>35 (100.0)</td>
</tr>
<tr>
<td>previous bypass surgery</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>platelet count, ×10⁹/l</td>
<td>224.5 ± 54.4</td>
</tr>
<tr>
<td>WBC count, ×10⁹/l</td>
<td>8.3 ± 1.9</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>2.0 ± 2.8</td>
</tr>
<tr>
<td>aspirin, 75 mg/d</td>
<td>35 (100.0)</td>
</tr>
<tr>
<td>ACEIs</td>
<td>33 (94.3)</td>
</tr>
<tr>
<td>statins</td>
<td>35 (100.0)</td>
</tr>
<tr>
<td>calcium channel blockers</td>
<td>5 (14.3)</td>
</tr>
<tr>
<td>proton pump inhibitors (pantoprazole)</td>
<td>33 (94.3)</td>
</tr>
<tr>
<td>β-blockers</td>
<td>18 (51.4)</td>
</tr>
<tr>
<td>carriers of the CYP2C19*2 allele</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

Categorical variables are expressed as absolute numbers (percentages) and continuous variables, as mean ± SD.

### TABLE 2 Cut-off values for adenosine diphosphate-induced platelet function tests

<table>
<thead>
<tr>
<th>Platelet reactivity test</th>
<th>LTA</th>
<th>MEA</th>
<th>PFA</th>
<th>VASP</th>
</tr>
</thead>
<tbody>
<tr>
<td>cut-off value</td>
<td>max. agg. &gt; 44%</td>
<td>AUC &gt; 47 U</td>
<td>CT &lt; 96 s</td>
<td>PRI &gt; 52%</td>
</tr>
</tbody>
</table>

The estimates for the group of healthy volunteers (n = 50). Data are presented in TABLE 5. The percentage of smokers did not differ between the HPR and non-HPR groups.

### TABLE 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study cohort, n = 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19*2 carriers</td>
<td></td>
</tr>
</tbody>
</table>

After exclusion of these 2 groups, the study population consisted of 35 patients. The detailed baseline demographic characteristics of these patients (all CYP2C19*2 non-carriers) are presented in TABLE 1. The cut-off values for HPR, determined as the 5th or 95th percentiles, were defined on the basis of reactivity values recorded for platelets originating from healthy volunteers not taking antiplatelet drugs (TABLE 2).

All assays were run in duplicate. Intra-assay CVs for each of the methods employed, calculated for all studied patients are given in TABLE 3. The Kruskal–Wallis test revealed that the CV of duplicate measurements for VASP was always significantly lower than that for the LTA, MEA, and PFA assays.

The changes in platelet reactivity determined by various methods in all patients participating in the study are presented in FIGURE 1. The relationship between the outcomes recorded for various tests was estimated using the Cohen’s k test. The agreement between measurements at different time points was moderate to poor. Between 6 and 12 weeks of continuous clopidogrel treatment, the strongest agreement was revealed for the MEA and PFA (κ = 0.58; P = 0.0002).

Platelet reactivity measurements after 12 weeks of clopidogrel treatment were used to assess the compatibility between the methods. The Cohen’s κ test showed that the level of agreement between various tests was moderate or poor. The strongest agreement occurred between the MEA and PFA (80%; κ = 0.46, P = 0.003), PFA and LTA (82%; κ = 0.41, P = 0.004), and MEA and LTA (80%; κ = 0.36, P = 0.008). Cohen’s κ coefficients for all other comparisons with VASP phosphorylation were less than 0.30.

According to the additional analysis, the platelet and leukocyte counts, together with the CRP level, could influence the development of HPR. The listed variables were significantly reduced in the non-HPR group. Data are presented in TABLE 5. The percentage of smokers did not differ between the HPR and non-HPR groups.

### DISCUSSION
This was a novel study involving the assessment of 4 ADP-induced platelet reactivity tests (LTA, MEA, VASP phosphorylation, and INNOVANCE PFA P2Y assays) over 12 weeks in patients receiving stable dual therapy of aspirin (75 mg/d) and clopidogrel (75 mg/d). All examined methods except for the PFA assay have been reported to have predictive value for future adverse events in cardiac patients on dual anti-platelet therapy.22,29,30

The measurements of platelet reactivity varied over time in a significant proportion of patients. Interestingly, no significant changes were observed in total platelet reactivity and HPR frequency. In contrast, when evaluating each patient individually, CRS was present in a considerable percentage of patients when tested at 2 different time points. The lowest CRS values were observed for MEA (14%) and the highest for VASP phosphorylation (37%), which is to some extent
Our findings are rather consistent with those of previous studies. Nuhrenberg et al. showed that the LTA-measured CRS is present in 20% comparable to the HPR “diagnostic” specificity of MEA (83%) vs VASP (38%), reported by Winter et al. Our findings are rather consistent with those of previous studies. Nuhrenberg et al. showed that the LTA-measured CRS is present in 20%

**TABLE 3** The intra-assay variability of platelet reactivity measurements

<table>
<thead>
<tr>
<th>Platelet function test</th>
<th>1st visit</th>
<th>2nd visit</th>
<th>3rd visit</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP-induced LTA</td>
<td>21.0</td>
<td>17.8</td>
<td>13.8</td>
<td>17.5 ± 3.6</td>
</tr>
<tr>
<td>ADP-induced MEA</td>
<td>21.1</td>
<td>20.0</td>
<td>16.5</td>
<td>19.2 ± 2.4</td>
</tr>
<tr>
<td>VASP phosphorylation</td>
<td>9.3±</td>
<td>10.5±</td>
<td>9.2±</td>
<td>9.7 ± 0.7</td>
</tr>
<tr>
<td>PFA</td>
<td>23.7</td>
<td>17.8</td>
<td>15.9</td>
<td>19.1 ± 4.1</td>
</tr>
</tbody>
</table>

Data are shown as intra-assay coefficients of variation.

* Data were analyzed with the Kruskal–Wallis test and the post-hoc all-pairwise comparisons Conover–Inman test; *P* < 0.05 vs any other test.

**Abbreviations:** ADP, adenosine diphosphate; others, see **TABLE 2**

**TABLE 4** Platelet reactivity, high on-treatment platelet reactivity, as well as frequency and change in the responder status in patients on stable clopidogrel treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Data presentation</th>
<th>LTA, %</th>
<th>MEA, U</th>
<th>PFA, s</th>
<th>VASP, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 weeks of treatment</td>
<td>PR</td>
<td>30.5 (20.3–37.8)</td>
<td>20.2 (12.4–37.6)</td>
<td>159.7 (73.3–268.0)</td>
<td>58.3 (41.7–68.5)</td>
</tr>
<tr>
<td></td>
<td>HPR, % (n)</td>
<td>8.6 (3)</td>
<td>17.1 (6)</td>
<td>34.3 (12)</td>
<td>54.2 (20)</td>
</tr>
<tr>
<td>12 weeks of treatment</td>
<td>PR</td>
<td>31.5 (20.8–38.0)</td>
<td>23.9 (9.9–43.7)</td>
<td>220.6 (128.5–300.0)</td>
<td>55.5 (40.2–69.4)</td>
</tr>
<tr>
<td></td>
<td>HPR, % (n)</td>
<td>11.4 (4)</td>
<td>25.7 (9)</td>
<td>20.0 (7)</td>
<td>54.2 (20)</td>
</tr>
<tr>
<td>real CRS, % (n)</td>
<td></td>
<td>20.0 (7)</td>
<td>14.3 (5)</td>
<td>20.0 (7)</td>
<td>37.1 (13)</td>
</tr>
<tr>
<td>significant difference related to hypothetical CRS, 5%</td>
<td><em>P</em> = 0.04</td>
<td><em>P</em> = 0.13</td>
<td><em>P</em> = 0.04</td>
<td><em>P</em> = 0.0007</td>
<td></td>
</tr>
<tr>
<td>significant difference related to hypothetical CRS, 0%</td>
<td><em>P</em> = 0.003</td>
<td><em>P</em> = 0.01</td>
<td><em>P</em> = 0.003</td>
<td><em>P</em> &lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Platelet reactivity is presented as median (interquartile range).

Data from 2 points of measurement were analyzed using the Kruskal–Wallis test and the all-pairwise comparisons Conover–Inman test.

* Data were analyzed with the Kruskal–Wallis test and the post-hoc all-pairwise comparisons Conover–Inman test.

**Abbreviations:** CRS, change in the responder status; HPR, high on-treatment platelet reactivity; PR, platelet reactivity; others, see **TABLE 2**

**TABLE 5** Confounding variables affecting platelet reactivity recorded with multiple electrode aggregometry

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>6 weeks of treatment</th>
<th>12 weeks of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>platelet count, ×10⁹/l</td>
<td>HPR</td>
<td>244.8 ± 52.1</td>
<td>252.8 ± 61.6</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.035</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>non-HPR</td>
<td>215.3 ± 48.2</td>
<td>212.5 ± 49.0</td>
</tr>
<tr>
<td>WBC count, ×10⁹/l</td>
<td>HPR</td>
<td>8.9 ± 1.7</td>
<td>7.6 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.043</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>non-HPR</td>
<td>7.8 ± 2.1</td>
<td>6.9 ± 1.7</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>HPR</td>
<td>3.3 ± 3.3</td>
<td>3.8 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>non-HPR</td>
<td>1.2 ± 1.0</td>
<td>2.0 ± 2.5</td>
</tr>
</tbody>
</table>

Categorical variables are expressed as absolute number (percentages) and continuous variables as mean ± SD.

Data recorded at different time points analyzed with the Kruskal–Wallis test and the post-hoc all-pairwise comparisons Conover–Inman test.

**Abbreviations:** see **TABLES 1 and 3**
agreements have been observed between MEA and LTA ($\kappa = 0.53$).

Our previous findings indicate that MEA and VASP phosphorylation are not interchangeable because they measure different aspects of the P2Y12 receptor blockade.

With respect to the above relationship, our findings are similar to those of Tsantes et al, indicating that the agreement between VASP and other PFTs is poor ($\kappa <0.3$).

Our study showed a possible link between HPR and CRP levels. This is in line with the findings of Mostowik et al, who demonstrated the relationship between prolonged post-PCI inflammatory reaction, reflected by elevated CRP levels, and platelets’ response to clopidogrel. The same link was demonstrated between platelet count and HPR phenomenon in another study.

On the contrary, we were not able to confirm the finding of Reed et al, who demonstrated that smokers treated with clopidogrel exhibited reduced platelet reactivity and were less likely to have HPR than nonsmokers. Most reasonably, it could be explained by a relatively small size of our group.

Changes in platelet reactivity over time, also in patients on antiplatelet drugs, may well be attributed to platelet physiology, clopidogrel bioavailability, or the already known HPR causes that can emerge or disappear during a long-term follow-up. The key question is whether the frequency of patients from 1 to 6 months after PCI, while Codner et al observed changes in HPR on clopidogrel treatment established by MEA in 26.3% of patients during a 6-month follow-up. Slightly different percentages were observed in a study by Jaitner et al, where CRS was present in 6.5% of patients, as assessed by MEA, and in 32% of patients, as assessed by LTA, although this study used a shorter follow-up of 15 days following PCI. Using another PFT method (VerifyNow), Hochholzer et al reported CRS at 2 different time points: 15.7% in patients taking clopidogrel at a dose of 75 mg/d and 11.4% in patients taking clopidogrel at a dose of 150 mg/d.

All previous studies, as well as our present study, indicate a rather high percentage of CRS (or interassay CV) in patients on clopidogrel therapy, no matter which PFT was used to monitor platelet reactivity. The impact of this phenomenon on clinical outcome needs further investigation.

In our study, the concordance between the results of measurements at different time points and between different PFTs was moderate to poor. Based on the Cohen’s $\kappa$ test ($\kappa = 0.46$), we showed the strongest agreement between MEA and PFA. Tsantes et al reported a moderate-to-poor agreement between various platelet function methods, with a lower concordance between MEA and PFA compared to that in our study ($\kappa = 0.37$). Higher agreements have been observed between MEA and LTA ($\kappa = 0.53$). Our previous findings indicate that MEA and VASP phosphorylation are not interchangeable because they measure different aspects of the P2Y12 receptor blockade.

FIGURE 1 Platelet function data obtained with light transmission aggregometry (A) after stimulation with 5 $\mu$mol/l of adenosine diphosphate (ADP); multiple electrode aggregometry (B) after stimulation with 6.4 $\mu$mol/l ADP after 6 minutes of measurement; platelet function analyzer (INNOVANCE® PFA P2Y test cartridge) (C); and vasodilator-stimulated phosphoprotein phosphorylation assay (D) in patients ($n = 35$) undergoing percutaneous coronary intervention on clopidogrel treatment at different time points. The cut-off values are shown as a dashed line. Cohen’s $\kappa$ coefficient was computed as a measure of agreement between the results of platelet reactivity tests from different time points. Abbreviations: see TABLE 2
or duration of a change, or even its existence, has an impact on future thrombotic or bleeding events, and also which PFT can reliably predict these events. Changes in platelet reactivity or responses to therapy may account for the known low positive predicting value of PFTs when performed only at a single time point.

The dependent variability (intra-assay and interassay) in response to clopidogrel observed in our present and earlier studies further blurs the future of tailored antiplatelet therapy. Further studies are required to better determine the impact of antiplatelet therapy, if any, on clinical endpoints, as well as the changes over time when recorded with the use of various PFTs. For the time being, it is recommended to avoid routine use of PFTs in clinical practice.

Study limitations The study group was small, as it was intentionally selected to exclude possible factors that could influence the occurrence of HPR (compounding variables), such as diabetes, genetic polymorphisms, overweight, platelet count inflammation state (CRP level), reduced renal function, old age, and use of anti-inflammatory drug (other than aspirin). Even so, the observed CRS was relatively high and comparable to that reported by other studies, which confirms the presence of the phenomenon and argues against its random nature. In addition, the study did not measure the level of the clopidogrel active metabolite. This might have confirmed whether the differences observed in PFT results were due to changes in clopidogrel bioavailability or changes in platelet activation per se. Our study included men only, which should be taken into account in future analysis.

Generally, it has to be stressed that HPR is a multifactorial phenomenon, and we do not have one platelet reactivity test that could describe and measure all aspects of platelet behavior at once. The possible influence of platelet and leukocyte count as well as of the CRP level on HPR, detected also in our study, shows that each PFT result should be looked at in view of clinical variables and patient status and not as the 0/1 value.

In conclusion, changes over time do occur in the course of clopidogrel therapy, but the broad differences between different PFTs do not allow for the exact estimation of its incidence. All analyzed PFTs (LTA, MEA, VASP phosphorylation, and INNOVANCE PFA P2Y assays) show only moderate concordance and should not be used interchangeably or employed in routine clinical practice.

Contribution statement JG and WK contributed to the concept and design of the study as well as the analysis and interpretation of the data. JG and KS interpreted the data and conducted statistical analyses. KCh and WK were responsible for clinical evaluation of the patients. HK and AS conducted the analysis. CW was responsible for critical writing, revising the manuscript for intellectual content, and the final approval of the manuscript. All authors edited and approved the final version of the manuscript.

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ORIGINAL ARTICLE Change in response to clopidogrel in patients after PCI 659


ARTYKUŁ ORYGINALNY

Zmienność odpowiedzi na klopidogrel u pacjentów po przezskórnej interwencji wieńcowej oceniana za pomocą różnych metod badania funkcji płytek krwi

Jacek Golański¹, Kamila Syska¹, Krzysztof Chiżynski², Hassan Kassasir¹, Cezary Watała¹, Agata Sakowicz³, Wiktor Kuliczkowski⁴
¹ Zakład Zaburzeń Krzepnięcia Krwi, Uniwersytet Medyczny w Łodzi, Łódź
² Klinika Intensywnej Terapii Karłowicznej, Uniwersytet Medyczny w Łodzi, Łódź
³ Zakład Biotechnologii Medycznej, Uniwersytet Medyczny w Łodzi, Łódź
⁴ Klinika Kardiologii, Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu, Wrocław

STRESZCZENIE

WPROWADZENIE Ostatnio zaobserwowano, że status odpowiedzi na leczenie klopidogrelem zmienia się w zależności od czasu.

CELE Celem pracy było zbadanie zmian w statusie odpowiedzi na leczenie klopidogrelem w zależności od czasu za pomocą czterech metod oceny funkcji płytek krwi (agregacja optyczna [LTA], agregacja impedancyjna [MEA], fosforylacja białka VASP [vasodilator-stimulated phosphoprotein – VASP] i metoda kartridżowa INNOVANCE® PFA P2Y [PFA]) w grupie pacjentów po zabiegu przezskórnej interwencji wieńcowej (percutaneous coronary intervention – PCI). Dodatkowo porównywano wyniki tych testów w celu wskazania najbardziej wiarygodnej metody.

PACJENCI I METODY Do badania włączono 35 pacjentów po PCI przyjmujących po 75 mg/d kwasu acetylosalicylowego i klopidogrelu. Grupę kontrolną stanowiło 50 zdrowych ochotników. Reaktywność płytek krwi oceniano w trzech różnych punktach czasowych (4 ±2 dni po PCI oraz 6 i 12 tygodni po zabiegu).

WYNIKI Zmianę w statusie odpowiedzi na klopidogrel zarejestrowano u 5 pacjentów (14%) w przypadku testu MEA, u 7 pacjentów (20%) w przypadku LTA i PFA oraz u 13 pacjentów (37%) w przypadku VASP. Wyniki porównań wyrażone współczynnikiem κ Cohena wykazały umiarkowaną lub słabą zgodność między testami. Największą zgodność wykazano między MEA i PFA – 80% (κ = 0,46; p = 0,003), PFA i LTA – 82% (κ = 0,41; p = 0,004) oraz MEA i LTA – 80% (κ = 0,36; p = 0,008). Dla wszystkich porównań z VASP współczynnik κ wynosił <0,30.

WNIOSKI Zależne od czasu zmiany w odpowiedzi na klopidogrel są rejestrowane dla wszystkich metod, ale duża niezgodność między testami uniemożliwia dokładną ocenę tego zjawiska. Porównywane metody wykazywały jedynie umiarkowaną zgodność (między sobą i między punktami czasowymi), co istotnie utrudnia ich zamienne stosowanie w praktyce klinicznej.

SŁOWA KLUCZOWE
metody badania funkcji płytek krwi, status odpowiedzi na terapię klopidogrelem, zgodność metod

ARTYKUŁ ORYGINALNY
Zmienność odpowiedzi na klopidogrel u pacjentów po PCI 661