MULTIPLE ELECTRODES AGGREGOMETRY - A NEW METHOD TO ASSESS THE PLATELET REACTIVITY IN NON-ST ELEVATION ACUTE CORONARY SYNDROME PATIENTS

Daniela Maximov¹, Adina Ionac², Alina Lupu¹, Cristian Mornos², Stefan I. Dragulescu²

INTRODUCTION

Dual antiplatelet therapy with clopidogrel and aspirin is the actual “gold standard” of treatment to prevent stent thrombosis in patients undergoing percutaneous coronary intervention (PCI) and to reduce major adverse cardiovascular events (MACE) in patients with non-ST segment elevation acute coronary syndrome (NSTA ACS).

Despite this data, a lot of studies have reported individual variability platelet response to aspirin...
and clopidogrel in atherothrombotic disease, and identified patients who did not achieve an appropriate platelet inhibition or with high on-treatment residual platelet reactivity as “low- responders” or “non-responders”.4-10

The term of “aspirin resistance” should be limited to situations in which failure of the drug to reach its pharmacological target has been documented with specific laboratory tests. The concept of “clinical resistance” to aspirin should not be used to detect situations in which aspirin is unable to prevent atherothrombotic events. Although global tests assessing platelet activation in vitro may identify patients with high residual platelet reactivity, they do not necessarily detect patients who are resistant to aspirin.11,12

The term of “clopidogrel resistance” has increasingly emerged in the literature and its clinical relevance, as a risk factor for cardiovascular ischemic events, has been explored in a few studies.13,14 In the majority of these studies, response to clopidogrel was defined as the difference between baseline and posttreatment maximal intensity of platelet aggregation.4-10 This approach can be criticized because of not taking into account the absolute level of pre- and posttreatment platelet activity. Other issues are represented by the facts that the determination is not suitable for routine clinical practice as the majority of ACS patients need urgently percutaneous coronary stenting and many others are already on chronic clopidogrel or aspirin therapy at the admission to the hospital. On the other hand, recent studies support that post-platelet reactivity is a better estimate of thrombotic risk rather than clopidogrel responsiveness.5,15

Accordingly, we analyzed the inter-individual variability in platelet response to clopidogrel and aspirin based on a single whole blood sample using multiple electrodes aggregation testing just before the PCI procedure in 100 patients admitted for NSTE ACS. We considered the maximal intensity of platelet aggregation using multiple electrode aggregometry (MEA) as a parameter for the antiplatelet responder status. We hypothesized that high level of posttreatment platelet reactivity predicts recurrent ischemic cardiovascular events at 30 days follow-up.

MATERIAL AND METHODS

Study patients

The study lot consisted of 100 consecutive patients admitted to the Institute of Cardiovascular Diseases Timisoara in 2010. Percutaneous coronary interventions (PCI) with stent implantation were performed in the CathLab of the Institute of Cardiovascular Diseases Timisoara in all study patients.

They were eligible for this prospective study if they had presented clinical symptoms defined as acute myocardial ischemia within 12 hours before admission and at least one of the following criteria: transient (<20 min) ST-segment elevation >0.1 mV, a new finding of ST-segment depression >0.05 mV, T-wave inversion in at least two contiguous leads, increased level of cardiac ischemic biomarkers or coronary disease documented by a previous coronary angiography, coronary revascularization or myocardial infarction. The exclusion criteria were: ST elevation ACS, NYHA class IV, PCI or coronary artery by-pass grafting (CABG) in the last 3 months, use of anti GP IIb/IIIa therapy before the PCI procedure, history of bleeding diathesis, contraindication to antiplatelet therapy, platelet count <100x10^9/L, creatinine clearance <30 mL/min.

Patients on chronic clopidogrel therapy with a daily dose of 75 mg > 5 days did not receive a loading dose of clopidogrel. Other patients received a loading dose of 300 mg clopidogrel at least 12 hours before the PCI. All patients received aspirin doses (75-300 mg) daily administered at least 12 hours before stenting procedure.

The study protocol was approved by the Institutional Ethics Committee of the Institute of Cardiovascular Diseases Timisoara, and patients gave informed consent for participation. PCI was performed within 48-72 hours after hospital admission.

Blood samples

Blood samples for testing the platelet activity by multiple electrodes aggregometry method were drawn after admission in the CathLab, before PCI, at least 12 h after the loading dose of clopidogrel and aspirin administration and before administration of anti GP IIb/IIIa therapy if needed. The blood was collected in special vacutainer tubes provided by the Multiplate® analyzer manufacturer, usually containing hirudin, filled to capacity, and then inverted three to five times for gentle mixing before the laboratory analysis testing.

Laboratory method

The aggregation testing was performed in Timisoara Institute of Cardiovascular Diseases Laboratory. We used the multiple electrodes aggregometry method performed with the Multiplate® analyzer, produced by Dynabyte Medical, Munich. Multiplate® reagents are also provided by the manufacturer, as reconstitute
solutions called ASPItest and ADPtest. In all patients we determined:

- Inhibition of arachidonic-acid-induced aggregation (ASPItest), which is in accordance with an adequate aspirin effect;

- Inhibition of ADP-induced aggregation (ADPtest), which is in accordance with an adequate clopidogrel effect.

The method is a fast and easy one, comprising a few specific steps, and allows obtaining the printed result in a short time (10 minutes). The results are represented by a graphic curve of aggregation (AU)/time (minutes). The parameters of the results are: the velocity (AU/min), the aggregation (AU), and the most important is the area under the curve (AUC) expressed in AU*min or U (10 AU*min = 1U).

**Clinical endpoint**

The clinical endpoint included all following major cardiovascular events (MACE): cardiovascular (CV) death, acute or subacute stent thrombosis, recurrent ACS and ischemic stroke. Follow-up events were assessed by regular clinical 1-month follow-up after PCI: 15 ACS was defined by the presence of symptoms compatible with recurrent ischemia needing new hospitalization and angiocoronarography, ischemic stroke was defined as a new focal neurological deficit without bleeding on computer tomodesitometry (CT) and confirmed by a neurologist.

Statistical analysis was performed with the SAS Software (v 8.01; SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as mean ± SD. Categorical variables are expressed as frequencies and percentages. The Wilcoxon rank-sum test was used to compare continuous variables in individuals with and without CV events. We used the Fischer’s exact test when frequencies were below five and the $\chi^2$- test to compare the categorical variables. $P$ for trend between quartiles of AUC values of AA- or ADP-induced aggregation and other variables was studied using a general linear model with AA- or ADP-induced aggregation as dependent variable. Comparison between individuals with maximal intensity of AA- or ADP-induced aggregation in the top vs. the three bottom quartiles were performed using logistic regression after adjustment for conventional CV risk factors, treatment and inflammatory parameters. ADP and AA-induced maximal intensity of platelet aggregation expressed as AUC value /patient were analyzed as potential predictors of the clinical endpoint both univariably and after adjustment for other baseline confounding variables. The value of $P<0.05$ was considered significant.

**RESULTS**

**Patient characteristics**

One hundred patients were included in our prospective study. Twenty one patients (20%) were on chronic clopidogrel therapy. Demographic and biological baseline data of the studied population are summarized in Table 1. The mean age was 63.2 ± 12 years, and 77% of the patients were males. Multiple CV risk factors were frequent (27% of patients were with diabetes mellitus, 57% were hypertensive, 64% presented dyslipidemia). For the patients who did not receive clopidogrel prior to the current admission, the mean time between the clopidogrel loading dose and blood sampling was 16 ± 2.5 hours. All patients received a daily 75 mg clopidogrel dose and a daily aspirin dose of 100 mg during the 1 month follow-up period. The mean time between angina symptoms and PCI was similar in patients with or without recurrent ischemic CV events (mean ± SD = 20 ± 4.63 vs. 20.24 ± 3.97, $P = 0.78$).

**Platelet response to clopidogrel**

We analyzed platelet response to clopidogrel using the multiple electrodes aggregometry performed with Multiplate® analyzer. In all patients we determined inhibition of ADP induced aggregation (ADPtest). The result of the test is represented by a curve of aggregation (AU)/time (minutes). The most important parameter is the area under the curve (AUC) expressed in AU*min or U (10 AU*min = 1U), which is in accordance with an adequate clopidogrel effect. Other important parameters assessed in the study population were the velocity (AU/min) and the aggregation (AU).

We observed that the distribution of the AUC values representing the ADP-induced platelet response intensity was consistent with a normal, bell-shaped distribution.

We classified the entire study group into four quartiles according to their ADP-induced platelet aggregation response, assessed by the AUC values, respectively. We considered the first quartile (Q1) of patients “clopidogrel high-responders” (AUC < 200 AU*min), the second quartile of patients (Q2) “clopidogrel responders” (200 AU*min < AUC < 500 AU*min), the third quartile (Q3) “clopidogrel intermediate responders” (500 AU*min < AUC < 700 AU*min) and the patients from the fourth quartile (Q4) were “clopidogrel low responders” (AUC > 700 AU*min).

The mean ADP-induced intensity of platelet aggregation response (represented by mean AUC value) in the described quartiles was: 155.5 ± 22.6
Table 1. Baseline characteristics of the patients with and without cardiovascular events.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group without CV events (n = 90)</th>
<th>Group with CV events (n=10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>61 ± 8</td>
<td>64 ± 4</td>
<td>0.84</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>70 (77)</td>
<td>8 (80)</td>
<td>0.62</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>50 (55)</td>
<td>9 (90)</td>
<td>0.48</td>
</tr>
<tr>
<td>History of ACS, n (%)</td>
<td>47 (52)</td>
<td>6 (60)</td>
<td>0.49</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>25 (27)</td>
<td>3 (30)</td>
<td>0.22</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 0.5</td>
<td>26 ± 1</td>
<td>0.42</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>52 (57)</td>
<td>6 (60)</td>
<td>0.53</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>58 (64)</td>
<td>7 (70)</td>
<td>0.79</td>
</tr>
<tr>
<td>Troponin positive patients, n (%)</td>
<td>25 (27)</td>
<td>7 (70)</td>
<td>0.24</td>
</tr>
<tr>
<td>ST-segment shift, n (%)</td>
<td>26 (28)</td>
<td>3 (33)</td>
<td>0.79</td>
</tr>
<tr>
<td>Number of stents, n (%)</td>
<td>19 (21)</td>
<td>5 (50)</td>
<td>0.071</td>
</tr>
</tbody>
</table>

**Medication**

| Statins, n (%)       | 54 (60)                          | 7 (70)                     | 0.58    |
| Beta-blockers, n (%) | 65 (72)                          | 8 (80)                     | 0.85    |
| IIECA, n (%)         | 48 (53)                          | 4 (40)                     | 0.63    |

**Biological parameters**

| hs-CRP (mg/L)        | 1.1 ± 0.5                        | 2.8 ± 0.9                  | 0.58    |
| Platelet count (10³/L) | 224 ± 15                         | 247 ± 29                   | 0.84    |
| Creatinine (mg/dL)   | 0.7 ± 0.1                        | 0.8 ± 0.02                 | 0.96    |
| AUC (AU*min) (MEA)   | 444.5 ± 361.3                    | 812 ± 172.5                | < 0.001 |

AU*min (Q1), 386.7 ± 86.13 AU*min (Q2), 599.3 ± 61.4 AU*min (Q3) and 819 ± 49.1 AU*min (Q4).

The range of ADP-induced intensity of platelet aggregation (AUC) in the fourth quartile (low-responders) was 701-940 AU*min, determining a cutoff value of 700 AU*min; this value is also present in a few number of studies available so far regarding this new method - multiple electrodes aggregometry. There were no significant differences between the quartiles one and four, excepting a significant increase of intensity of AA-induced platelet aggregation (AU)/time (minutes). The most important parameter is the area under the curve (AUC) expressed in AU*min or U (10 AU*min = 1U), which is in accordance with the velocity (AU/min) and the aggregation (AU).

We observed that the distribution of the AUC values representing the ADP-induced platelet aggregation using the Multiple electrodes aggregometry (AA) was 701-940 AU*min, determining a cut-off value of 700 AU*min; this study demonstrates that, among a high risk category of CV patients, treated by PCI with stenting, the single measurement of ADP-induced platelet aggregation using multiple electrodes aggregometry can be used for the identification of patients with low platelet responsiveness to clopidogrel.

**DISCUSSION**

So far, a large number of studies were focused on individual variability of platelet response to clopidogrel and the term of “clopidogrel resistance” is usual. Despite this facts, its definition is still controversial and mainly based on the percentage of changes in ADP-induced maximal intensity of platelet aggregation before and after initiation of clopidogrel treatment (clopidogrel responsiveness). In addition, the cut-off value to identify the low responders varied in large ranges (from <10% to 40%).

Our study demonstrates that, among a high risk category of CV patients, admitted for NSTE ACS treated by PCI with stenting, the single measurement of ADP-induced platelet aggregation using multiple electrodes aggregometry can be used for the identification of patients with low platelet responsiveness to clopidogrel.
electrodes aggregometry (MEA) was associated with the subsequent occurrence of major adverse CV events (MACE).

Light transmittance aggregometry (turbidimetric method, LTA) has been the most widely used technique to monitor the effect of antplatelet drugs, including aspirin, clopidogrel, other P2Y12 inhibitors, and platelet glycoprotein (GP) IIb/IIIa inhibitors.\textsuperscript{16,17}

In the studies using LTA, the historical “gold standard” test, based on the stimulation of platelet-platelet aggregation in platelet-rich plasma after stimulation with various agonists, we identified disadvantages related to the laboratory employees’ workload. They include the need for immediate processing, variable reproducibility, large volume samples required, lengthy processing time, and expenses of the aggregometer and trained operators. LTA has also been the most widely investigated method to predict clinical outcomes.\textsuperscript{18}

Impedance aggregometry is conceptually similar to LTA, but it uses whole blood instead of platelet-rich plasma and platelet aggregation is measured by impedance, not by light transmittance.\textsuperscript{19} Platelet function analysis using multiple electrode aggregometry (Multiplate\textsuperscript{®})-Dynabyte, Munich, Germany is a recent method which allows an easy and rapid assessment of platelet function, with the possibility to decide on treatment regimens when the patient is still in the CathLab (results in 10 minutes). In present, Multiplate is used in many expert centres and pharmaceutical companies throughout Europe. This method is also suitable for daily clinical practice for many reasons; time consuming stages related to rich platelet plasma preparation or light transmittance aggregometer manipulation were eliminated. This new method of aggregometry allows rapid and reliable results, and could be used also in the Coronary Unit or CathLab.

In our study, a baseline aggregation assessment could not be obtained because of certain factors: previous chronic clopidogrel therapy, patients’ admission through the Emergency Department. But recent studies demonstrated that pretreatment platelet activity did not predict the clopidogrel responsiveness.\textsuperscript{13,14} These trials have also shown the correlation between a low response to clopidogrel (difference between pre- and posttreatment values) and a high posttreatment platelet activity, which was proposed as a better estimate of thrombotic risk.\textsuperscript{13,14,16-19}

Therefore we performed one test per patient to assess the clopidogrel effect with ADP-induced platelet aggregation, from one single blood sample just before PCI, without baseline determination. Patients were stratified into quartiles according to their post therapy ADP induced platelet aggregation represented by the AUC value obtained with the ADPtest using multiple electrode aggregometry performed with the Multiplate\textsuperscript{®} analyzer. The patients of the fourth quartile were characterized as “clopidogrel low responders” (AUC > 700 AU*min). In addition, we considered the first quartile (Q1) of patients “clopidogrel high-responders” (AUC < 200 AU*min), the second quartile of patients (Q2) “clopidogrel responders” (200 AU*min < AUC < 500 AU*min), the third quartile (Q3) “clopidogrel intermediate responders” (500 AU*min < AUC < 700 AU*min). Similarly, in other studies the patients from the fourth quartile were defined as low responders and were characterized by a maximal intensity of ADP-induced platelet aggregation > 70% (Gurbel et al,\textsuperscript{16} Cuisset et al).\textsuperscript{20}

A relation between clopidogrel resistance and recurrence of clinical CV ischemic events is emerging. Correlation of ADP-induced platelet aggregation with clinical outcomes was showed for the first time in ST elevation ACS by Mateszky et al.\textsuperscript{14} These data and a lot of results from clinical trials strongly suggested that the clopidogrel resistance might be associated with increased risk of recurrent CV events.\textsuperscript{21-28}

In this study, a number of 10 CV ischemic events occurred in the 1-month post PCI follow-up period. We demonstrated a correlation between clopidogrel response defined on a single blood sample before the PCI and the recurrence of CV ischemic events for NSTE ACS patients undergoing coronary stenting; in addition, according with the quartile stratification, a number of 8 events (80%) occurred in the 4th quartile patients, characterized as “clopidogrel low-responders”, and 2 events (20%) in the third quartile patients - “clopidogrel intermediate responders”. No CV events were registered to the patients from the first and second quartile, characterized as “clopidogrel high-responders” and “clopidogrel responders”.

**CONCLUSION**

The results of the present study are encouraging. We found out a correlation between clopidogrel platelet response and a subgroup of patients at higher risk of recurrent ischemic CV events after stenting for NSTE ACS. In addition, we used a single blood sample per patient for testing posttreatment ADP-induced platelet aggregation and a new, faster, simple and accurate method to assess the platelet aggregation. The cut-off value of AUC using multiple electrodes aggregation (MEA) was useful to identify the “clopidogrel low-responders.”
PERSPECTIVES

MEA seems to be a non-complex method to identify the clopidogrel low responders. These high-risk patients may potentially benefit from a more aggressive antithrombotic therapy (higher clopidogrel doses, alternative molecules, combined antiplatelet therapy).29

REFERENCES

11. Cattaneo M. Laboratory detection of ‘aspirin resistance’: what test should be used (if any)? Eur Heart J 2007;28:1673-5.