Use of Thromboelastography Platelet Mapping for Assessment of Individual Platelet Response Secondary to Oral Antiplatelet Therapy after Percutaneous Coronary Intervention: An Attempt to Start Personalized Antiplatelet Therapy in India

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Abstract

High on-treatment platelet reactivity (HPR) with P2Y12 receptor antagonists in patients treated with dual antiplatelet therapy (DAPT) is strongly associated with adverse ischemic events after percutaneous coronary intervention (PCI). This prospective study was conducted to assess individual platelet response and HPR to antiplatelet medications in post-PCI cases by thromboelastography platelet mapping (TEG-PM). Total 82 patients who were on aspirin and on either clopidogrel, prasugrel, or ticagrelor were evaluated. The percentage of platelet inhibition to arachidonic acid (AA) and adenosine diphosphate (ADP) was calculated by \[100 - \frac{(MA_{AA} - MA_{Fibrin})}{(MA_{Thrombin} - MA_{Fibrin})} \times 100\], taking 50% response as cut-off for HPR. HPR to clopidogrel and prasugrel was 14.29 and 12.5%, respectively. No HPR was detected to aspirin and ticagrelor. The mean percentage of platelet inhibition was significantly higher in patients with ticagrelor 82.99, 95% confidence interval (CI) of [77.3, 88.7] as compared with clopidogrel 72.21, 95% CI of [65.3, 79.1] and prasugrel 64.2, 95% CI of [52.5, 75.9] (\(p\)-value of 0.041 and 0.003, respectively). Aspirin along with ticagrelor is associated with a higher mean percentage of platelet inhibition, and lower HPR as compared with the usage of aspirin combined with clopidogrel or prasugrel. Additionally, it might also be concluded that TEG-PM could be used effectively to measure the individual platelet functions which would make oral antiplatelet therapy more personalized for cardiac patients.

Keywords
► thromboelastography
► platelet mapping
► high on-treatment platelet reactivity
► antiplatelet therapy
► personalized medicine

Introduction

Platelet aggregation is considered the most important factor in the development of ischemic complications after percutaneous coronary intervention (PCI).1 Dual antiplatelet therapy (DAPT) with acetylsalicylic acid and a P2Y12 receptor antagonist reduces the risk of thrombosis after PCI in
Materials and Methods

Study Details

This was a prospective, observational, single-center cohort study where TEG-PM was performed in a group of patients to assess the platelet functions after starting DAPT. All these patients underwent PCI due to coronary artery disease (CAD) from June 2016 to December 2018 at a cardiac center in Eastern India. Institutional review board (ECR/587/Inst/WB/2014/RR-17) approval was obtained for this study. Informed consent was taken from each patient before PCI.
After discarding the first 2 to 3 mL of blood, the Vacutainer tubes were filled to capacity and gently inverted 3 to 5 times to ensure complete mixing of the anticoagulant. Blood samples were analyzed in the transfusion medicine laboratory by trained personnel within an hour of receiving the blood sample as per the manufacturer instructions. Both analyzer (series 5000) and the reagents were from Haemoscope Corporation (Niles, Illinois, United States). The following TEG parameters were studied: reaction time (R), time to initial fibrin formation up to 2 mm; K time (K), time to clot formation up to 20 mm; α angle (α), speed of clot formation; and maximum amplitude (MA), measurement of clot strength. The normal reference values of TEG parameters (as provided by the manufacturer) for kaolin-activated citrated samples were taken as R = 2 to 8 minutes, K = 1 minute to 3 minutes, α = 55 to 78 degrees, and MA = 51 to 69 mm. Heparin was used as an anticoagulant to eliminate thrombin activity in the sample during assay. Reptilase and activated factor XIII (activator F) were used to generate a cross-linked fibrin clot to isolate the fibrin contribution in clot strength. For maximal clot strength (MA\textsuperscript{thrombin}) 1 mm of citrate stabilized blood was transferred to a vial containing kaolin and mixed by gentle inverter. Kaolin activated blood (340 μL) was added to a TEG cup containing 20 μL of 0.2 M CaCl\textsubscript{2}. As MA, which is representing the maximal clot strength, can be ascertained by the binding of activated platelets to a fibrin mesh, 360 μL of heparinized blood was added to 10 μL of activator F (reptilase and factor XIIIa) and the contribution of each fibrin meshwork to clot strength (MA\textsuperscript{Fibrin}) was assessed. The contribution of P2Y\textsubscript{12} receptor or cyclooxygenase pathways to the clot formation can be measured by the addition of the appropriate agonist, ADP, or arachidonic acid (AA). Therefore, heparinized blood (360 μL) was added to a TEG cup in the presence of the activator F and agonist, 10 μL: ADP (2 μM, final concentration), yielding the (MA\textsuperscript{AA} or 10 μL AA (1 μM, final concentration) for the MA\textsuperscript{AA}. The percentage of platelet inhibition to agonist was calculated by TEG-PM software as [100−((MA\textsuperscript{ADP/AA}−MA\textsuperscript{Fibrin}) / (MA\textsuperscript{Thrombin}−MA\textsuperscript{Fibrin}) X 100)].

**Assessment of Outcome**

The percentage of HPR to clopidogrel, prasugrel, and ticagrelor was assessed and considered as the primary outcome of the study. The cut-off for HPR was set at 50% in response to the individual drug similar to the cut-off followed in platelet aggregometry. The percentage of platelet inhibition to each P2Y\textsubscript{12} receptor antagonist was also assessed during this study.

**Statistical Analysis**

Data were collected and analyzed by using Microsoft Excel statistics software package. Continuous variables were expressed as mean with standard deviation and categorical data were expressed as frequencies or percentages. Student’s t-test was applied for comparison of means and significance was established with p-values level of 0.05 (p < 0.05).

**Results**

All the demographic variables and baseline data were found similar in studied population (►Table 1). The mean age of patients was 63.5 years (range: 44.5–73 years) and the mean weight was 58.3 kg (range: 47–81 kg). All patients were male. All 82 patients were treated with aspirin, 42 among them were treated with clopidogrel in combination, 16 with prasugrel, and 24 with ticagrelor in combination, respectively (►Table 2). Fig. 1 is showing the number of HPR cases to each P2Y\textsubscript{12} receptor antagonist. Table 2 and Fig. 2 are showing that the mean percentage of platelet inhibition was significantly higher in patients with ticagrelor 82.99, 95% confidence interval (CI) of [77.3, 88.7] as compared with clopidogrel 72.21, 95% CI of [65.3, 79.1] and prasugrel 64.2, 95% CI of [52.5, 75.9] (p-value of 0.041 and 0.003, respectively). No bleeding manifestation was observed in any patient due to administration of DAPT.

**Table 1** Demographic details and baseline test parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>63.5 ± 4.22</td>
<td>44.5–73</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>58.3 ± 4.6</td>
<td>47–81</td>
</tr>
<tr>
<td>Baseline R (min)</td>
<td>4.17 ± 0.96</td>
<td>2–7.3</td>
</tr>
<tr>
<td>Baseline K (min)</td>
<td>1.8 ± 0.29</td>
<td>1.05–2.95</td>
</tr>
<tr>
<td>Baseline α (degrees)</td>
<td>64.17 ± 3.98</td>
<td>55.2–74.6</td>
</tr>
<tr>
<td>Baseline MA (MA&lt;sub&gt;K&lt;/sub&gt; in mm)</td>
<td>61.9 ± 2.71</td>
<td>55.5–68</td>
</tr>
<tr>
<td>AA (MA&lt;sub&gt;AA&lt;/sub&gt;)</td>
<td>16.3 ± 5.73</td>
<td>4.3–29.2</td>
</tr>
<tr>
<td>Platelets inhibition% with AA</td>
<td>80.69 ± 9.14</td>
<td>61.1–100</td>
</tr>
</tbody>
</table>

**Table 2** Details of P2Y<sub>12</sub> receptor antagonists studied

<table>
<thead>
<tr>
<th>Receptor Antagonist</th>
<th>Clopidogrel</th>
<th>Prasugrel</th>
<th>Ticagrelor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients tested</td>
<td>42</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>Patients with HPR</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>HPR (%)</td>
<td>14.29</td>
<td>12.50</td>
<td>0</td>
</tr>
<tr>
<td>Mean platelet inhibition (%)</td>
<td>72.21</td>
<td>64.20</td>
<td>82.99</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>22.85</td>
<td>23.80</td>
<td>14.19</td>
</tr>
<tr>
<td>Standard error of means</td>
<td>3.52</td>
<td>5.95</td>
<td>2.89</td>
</tr>
<tr>
<td>p-Value of means</td>
<td>0.041</td>
<td>0.003</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: AA, arachidonic acid; MA, maximal amplitude; R, K and α are standard thromboelastography parameters.
Although HPR to prasugrel has not been well reported, it was found 12.5% in our study which is in agreement with the results of Bonello et al\textsuperscript{21} where VASP method was used for assessment of platelet response. It was previously reported that ticagrelor has a mean percentage platelet inhibition of 80 to 90% when tested by platelets aggregometry\textsuperscript{22} which is again consistent with the current study finding of a mean platelet inhibition of 82.99% with ticagrelor. Average percentage of platelet inhibition in patients treated with prasugrel (64.2%) was lower than clopidogrel (72.21%) in our study which is in agreement with the TRILOGY-ACS trial (targeted platelet inhibition to clarify the optimal strategy to medically manage acute coronary syndromes) where prasugrel was not found superior to clopidogrel in patients who had acute coronary syndromes (ACS).\textsuperscript{23} Although, meta-analysis\textsuperscript{24} of several studies showed a stronger antiplatelet effect of ticagrelor over prasugrel which is in favor of the present study; the recent ISAR-REACT 5 trial (intracoronary stenting and antithrombotic regimen 5)\textsuperscript{25} showed that a stronger antiplatelet effect is not always associated with better composite end results in ACS cases. There was no bleeding manifestation with ticagrelor therapy despite higher platelet inhibition which is similar to the results of the PLATO trial (platelet inhibition and patient outcomes).\textsuperscript{26} Also, no HPR to aspirin was found in our study which is in agreement with the results of Tantry et al.\textsuperscript{27} There were no ischemic complications observed after 6-month follow-up in patients with HPR who were switched over to another DAPT combination depending on TEG-PM results.

Limitations
The major limitation of our study is the nonrandomized design. The sample size is small because of the poor affordability to this relatively costly test. Also, the follow-up test after switch over to a new DAPT combination was not possible due to the increase cost of the assay. Lack of gender distribution of results could be considered as another limitation as only male patients were evaluated. We did not have any provision to compare our test results with a more sensitive testing platforms like VerifyNow which has a defined HPR value for P2Y\textsubscript{12} reaction assessment. Furthermore, the pharmacokinetic analysis of drug levels in blood was not performed in this study. Hence, precise correlation with the observed effects of the drugs is not possible.

Conclusion
Although, measurements of platelet reactivity can vary over time in a significant proportion of patients and treatment adjustment according to a single PFT at a single time point might not be sufficient for guiding antiplatelet therapy, but it is nearly impossible to use “gold-standard” technologies like CYP2C19 genotyping, platelets aggregometry, or flowcytometry routinely to monitor the effect of antiplatelet therapy in resource-limited health care facilities. Therefore, we conclude that aspirin along with ticagrelor is associated with a higher mean percentage of platelet inhibition and lower
HPR as compared with the usage of aspirin with clopidogrel or prasugrel. In addition to that, it might also be concluded that TEG-PM could be used effectively to measure the individual platelet functions in response to antiplatelet drugs and the decision of switching to another combination of DAPT in HPR cases could be taken based on this assessment. This treatment approach might be useful to make oral antiplatelet therapy more personalized for cardiac patients in developing countries.

**Ethics Approval**
Ethical approval was obtained from the Institution Ethics Committee of The Mission Hospital (ECR/587/Inst/WB/2014/RR-17) for this study.

**Consent**
Individual informed written consent was obtained from each patient before PCI.

**Data Transparency**
Raw data are available; data sheet can be shared on request.

**Authors’ Contributions**
S.S.D. and N.A.M. designed the study; S.S.D. performed the tests; collected data and prepared the draft. D.D. provided support during data analysis. S.S.D. prepared the manuscript. All authors reviewed the manuscript before submission.

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**Conflict of Interest**
The authors declare that they have no conflict of interest.

**Acknowledgment**
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**References**
27 Tantry US, Bliden KP, Gurbel PA. Overestimation of platelet aspirin resistance detection by thrombelastograph platelet mapping and validation by conventional aggregometry using arachidonic acid stimulation. J Am Coll Cardiol 2005;46(9):1705–1709