Does the VerifyNow P2Y12 assay overestimate “therapeutic response” to Clopidogrel?

Insights using short thrombelastography

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Summary

In contrast to short thrombelastography (s-TEG) which utilises adenosine diphosphate (ADP) alone, the VerifyNow P2Y12 assay (VN-P2Y12) additionally uses prostaglandin E1 (PGE1) as agonist to assess response to P2Y12 inhibitors. Based upon previous observations, we hypothesised that VN-P2Y12 overestimates the therapeutic effects of clopidogrel. Simultaneous assay with s-TEG and VN-P2Y12 was performed in 43 healthy volunteers and 170 patients either on or off clopidogrel. Furthermore, in 27 patients on clopidogrel 75 mg we compared the effects of adding 22 nM PGE1 to ADP on platelet aggregation in s-TEG to ADP alone. A higher proportion of individuals had a result indicating high platelet reactivity (HPR) with s-TEG than VN-P2Y12 in (i) 43 clopidogrel naive volunteers (95.3% vs 81.4%, p = NS); (ii) 28 volunteers loaded with clopidogrel 600 mg (39.3% vs 10.7%, p = < 0.01); (iii) 123 clopidogrel naive patients (93.5% vs 78%, p = < 0.001); (iv) 47 patients on clopidogrel 75 mg (42.6% vs 4.3%, p = < 0.0001). In 59 patients loaded with clopidogrel 600 mg/900 mg, a greater proportion had a “therapeutic response” with VN-P2Y12 compared to s-TEG, regardless of the threshold for defining HPR with VN-P2Y12 (P2Y12 reaction units ≥ 230 or 208). Furthermore, adding PGE1 to ADP in s-TEG potentiated the anti-aggregatory effects of clopidogrel compared with ADP alone. In conclusion, VN-P2Y12 overestimates the functional effects of clopidogrel in some individuals, possibly because it utilises PGE1 in addition to ADP. This could have implications for the ability of VN-P2Y12 to stratify patients as “responders” or “non-responders” to clopidogrel.

Keywords

Clopidogrel, high platelet reactivity, thrombelastography, VerifyNow P2Y12 assay, prostaglandin E1

Introduction

Platelet activation and aggregation play a key role in the pathophysiological development of myocardial infarction (MI) and stent thrombosis (ST). In patients undergoing percutaneous coronary intervention (PCI) with stents, a combination of aspirin and a P2Y12 receptor inhibitor, most commonly clopidogrel, represents essential therapy. However, response to clopidogrel between individuals is heterogeneous (1, 2). Furthermore, there is an association between poor response to clopidogrel (also known as high platelet reactivity, HPR) and subsequent adverse ischaemic events (3-7). These factors have been a driver for the introduction of stronger, faster acting P2Y12 inhibitors such as prasugrel and ticagrelor (8, 9). Although these agents confer reduced risk of ischaemic events in some patients, they are also associated with increased rates of bleeding (10).

One management strategy in PCI patients would therefore be to give all patients clopidogrel, but assess individual platelet reactivity with a view to tailoring therapy with stronger agents in those patients with HPR. This strategy is dependent upon the availability of a simple, accurate point-of-care test of platelet function. Recent large randomised trials including GRAVITAS (Gauging Responsiveness with A VerifyNow assay – Impact on Thrombosis And Safety trial) (11), ARCTIC (Assessment by a Double Randomization of a Conventional Antiplatelet Strategy versus a Monitoring- guided Strategy for Drug-Eluting Stent Implantation and of Treatment Interruption versus Continuation one Year after Stenting) (12) and TRIGGER-PCI (Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel) (13) have all employed the VerifyNow™ P2Y12 (VN-P2Y12) assay (Accumetrics Inc., San Diego, CA USA) to detect HPR in order to modify therapy. VN-P2Y12 is a turbidimetric whole blood assay that measures agglutination of fibrinogen-coated beads in response to a combination of prostaglandin E1 (PGE1) and the platelet agonist adenosine diphosphate (ADP) (14). All the randomised trials of tailoring ther-
apy using VN-P2Y12 to detect and stratify patients with HPR have failed to demonstrate a positive outcome for this strategy.

Thrombelastography (TEG) is a test of whole blood clotting. This group has introduced a modification of TEG using the agonist ADP that allows for the assessment of platelet function using a parameter called area under the curve at 15 minutes (min) (AUC15), which incorporates both speed and strength of clot formation in whole blood. A further parameter, known as percentage clotting inhibition (% CIn), compares clotting responses to agonists such as ADP with maximal platelet-activation in response to thrombin. The parameters AUC15 and % CIn are known as short TEG (s-TEG), and the method and its reproducibility are described in detail elsewhere (15-17).

This group has previously observed in a population who have had drug-eluting ST that some patients exhibited HPR on P2Y12 inhibitors using s-TEG (18), yet had a response in the "therapeutic range" for VN-P2Y12. As a result of this observation we therefore speculated that VN-P2Y12 may be overestimating the degree of inhibition of ADP-induced blood clotting in some individuals, possibly as a result of the presence of PGE1 (in addition to ADP) in the VN-P2Y12 assay. The aims of the experiments reported here were as follows:-

1. To compare simultaneous responses to ADP in s-TEG and VN-P2Y12 in healthy volunteers on and off clopidogrel.
2. To compare simultaneous responses to ADP in s-TEG and VN-P2Y12 in patients on and off clopidogrel.
3. To test whether adding PGE1 to the ADP in s-TEG would lead to an increase in the apparent "therapeutic response" to clopidogrel in patients compared to ADP stimulation alone.

Methods

Study population

Studies were approved by the Southampton and South West Hampshire Research Ethics Committee and conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent prior to inclusion.

Study exclusion criteria were as follows:-

- Healthy volunteers:
  - an antiplatelet agent or non-steroidal anti-inflammatory (NSAID) within 14 days
  - a history of peptic ulceration, asthma, or bleeding.
- Patients:
  - a NSAID or antiplatelet agents other than those specified in the study
  - anticoagulation medication in the preceding 28 days
  - a history of clopidogrel intolerance, bleeding diathesis or major haematological disturbance precluding the use of dual antiplatelet therapy (DAPT)
  - planned use of glycoprotein IIb/IIIa inhibitors during PCI
  - ST-elevation acute coronary syndrome

Simultaneous assay with s-TEG and VN-P2Y12 was performed in 43 patients and 170 patients either on or off clopidogrel. A breakdown of the participants in each group, their antiplatelet regimen and the time-points at which sampling was undertaken is provided in Table 1.

Venesection

Venesection was performed from the anticubital fossa and the first 2mls were discarded. Blood was then drawn into a 6-ml Lithium Heparin Vacutainer® for TEG® analysis and a 2 ml 3.2% sodium citrate Vacutainer® for VerifyNow™ analysis.

Sample analysis

Modified TEG®

Samples were analysed using the modified TEG® platelet mapping system (Haemonetics Corp, Braintree, MA, USA) according to the

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Population</th>
<th>Clopidogrel therapy</th>
<th>Aspirin co-administered</th>
<th>Sampling</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15</td>
<td>Healthy volunteers</td>
<td>None</td>
<td>No</td>
<td>No</td>
<td>Sambu et al. (16)</td>
</tr>
<tr>
<td>B</td>
<td>28</td>
<td>Healthy volunteers</td>
<td>Loading with Clopidogrel 600mg</td>
<td>No</td>
<td>Pre-loading dose and 6 hours after loading dose</td>
<td>Hobson et al. (17)</td>
</tr>
<tr>
<td>C</td>
<td>64</td>
<td>Patients with stable coronary artery disease</td>
<td>None</td>
<td>75mg once daily</td>
<td></td>
<td>Sambu et al. (16)</td>
</tr>
<tr>
<td>D</td>
<td>59</td>
<td>Patients being considered for elective PCI</td>
<td>Loading with clopidogrel 600mg (30) or 900mg (29)</td>
<td>75mg once daily</td>
<td>Pre-loading dose and 1, 2, 6 and 24 hours after loading dose</td>
<td>Hobson et al. (17)</td>
</tr>
<tr>
<td>E</td>
<td>20</td>
<td>Patients post PCI and stent insertion</td>
<td>Clopidogrel 75mg once daily</td>
<td>75 – 150 mg once daily</td>
<td></td>
<td>Sambu et al. (16)</td>
</tr>
<tr>
<td>F</td>
<td>27</td>
<td>Patients following angiography +/- PCI</td>
<td>Clopidogrel 75mg once daily</td>
<td>75 – 150 mg once daily</td>
<td>24 hours post-angiography</td>
<td>Ongoing study in its follow-up phase</td>
</tr>
</tbody>
</table>
manufacturer’s instructions. As described previously, modified TEG® utilises four channels to detect the effects of antiplatelet therapy acting via the AA and ADP pathways (15, 16). The four channels comprise: (i) the thrombin channel which contains Kaolin that causes maximal activation of thrombin and therefore maximal platelet activation, (ii) the fibrin channel which contains Activator F® (a mixture of reptilase and factor XIIIa), which generates a fibrin clot without platelet activation, (iii) the ADP channel which contains Activator F® and ADP (2 µM), and (iv) the AA channel which contains Activator F® and AA (1 mM).

In addition, in Group F an extra channel was used, which contained not only standard concentrations of Activator F® and ADP, but also 22 nM PGE1, the same concentration of PGE1 used in the VN-P2Y12 assay.

Whole blood (360 µl) was then pipetted into each channel which consists of an oscillating cup into which a pin is suspended. As blood clots, changes in its viscoelasticity are transmitted to the pin and the resulting torque generates an electrical signal producing a TEG® trace (15). The maximum amplitude (MA), which is representative of maximal clot strength, has been shown to correlate well with light transmittance aggregometry (LTA) in assessment of responses to antiplatelet therapy (19-21).

Short TEG

AUC15 is calculated using bespoke software (Areafinder 2:1) developed using National Instrument Labview 7.0. AUC15 has been shown to correlate well with the traditional TEG® parameter MA in assessment of responses to antiplatelet therapy and is reproducible. The methodology for AUC15 and %Cln are described in detail elsewhere (15, 16).

VerifyNow™

Samples were analysed using the VerifyNow™ P2Y12 assay according to the manufacturer’s instructions. Results were expressed as P2Y12 reaction units (PRU).

Table 2: Summary of key results including MA, AUC15, PRU and rates of HPR with VN-P2Y12 and s-TEG in different populations of healthy volunteers and patient either on or off clopidogrel.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>Mean +/- 95% confidence interval of the mean</th>
<th>High Platelet Reactivity (HPR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MA ADP</td>
<td>AUC15 ADP</td>
</tr>
<tr>
<td>Clopidogrel naïve volunteers (Groups A and B)</td>
<td>43</td>
<td>61.5 +/- 2.4</td>
<td>1000.3 +/- 65</td>
</tr>
<tr>
<td>Volunteers after taking clopidogrel (Group B)</td>
<td>28</td>
<td>34.8 +/- 6.9</td>
<td>511.9 +/- 118.3</td>
</tr>
<tr>
<td>Clopidogrel naïve patients (Groups C and D)</td>
<td>123</td>
<td>60.9 +/- 1.7</td>
<td>1045.1 +/- 39.7</td>
</tr>
<tr>
<td>Patients taking clopidogrel (Groups E and F)</td>
<td>47</td>
<td>46.4 +/- 3.9</td>
<td>687.5 +/- 79.5</td>
</tr>
</tbody>
</table>

Table 3: Correlation, agreement, concordance and discordance between results from s-TEG and VN-P2Y12 in different populations of healthy volunteers and patients either on or off clopidogrel. The shaded column represents subjects with discordant results who had a response in the “therapeutic range” with VN-P2Y12 but not s-TEG.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>Correlation</th>
<th>Agreement</th>
<th>Concordant results</th>
<th>Discordant results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PRU and AUC15</td>
<td>PRU and %Cln ADP (AUC15)</td>
<td>Kappa (k)</td>
<td>p value</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coeff.</td>
<td>p value</td>
<td>Coeff.</td>
<td>p value</td>
</tr>
<tr>
<td>Clopidogrel naïve volunteers (Groups A and B)</td>
<td>43</td>
<td>0.222</td>
<td>NS</td>
<td>0.255</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Volunteers after taking clopidogrel (Group B)</td>
<td>28</td>
<td>0.74</td>
<td>&lt; 0.001</td>
<td>-0.765</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Clopidogrel naïve patients (Groups C and D)</td>
<td>123</td>
<td>0.424</td>
<td>&lt; 0.001</td>
<td>-0.223</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Patients taking clopidogrel (Groups E and F)</td>
<td>47</td>
<td>0.372</td>
<td>&lt; 0.005</td>
<td>-0.207</td>
<td>NS</td>
</tr>
</tbody>
</table>
Definitions

HPR was defined by s-TEG as a % Cln ADP (AUC15) ≤ 30% (15, 21). For VN-P2Y12, a subject was defined as having HPR for a measured PRU of ≥ 230. This cut-off was reported as the optimal cut-off to define HPR by VN-P2Y12 in a collaborative meta-analysis (22). This cut-off was subsequently used in the GRAVITAS trial for defining HPR (5, 11). In addition an alternative cut-off of PRU ≥ 208 was also evaluated in our study because this lower level more recently has been reported as being predictive of future adverse cardiovascular events (ACE) in patients with stable CAD (13, 23, 24).

Statistical methods

Continuous variables are presented as the mean ± 95% confidence interval (CI) of the mean, unless stated otherwise. Correlation between continuous variables was assessed using Pearson’s correlation. Categorical variables are presented as percentages. Agreement between the two assays was assessed by Kappa statistics.
Table 4: AUC15 in the ADP channel and PRU at baseline and four time-points after loading 59 patients with clopidogrel 600 mg/900 mg. Results are represented as mean ± 95% CI of the mean. The p value given is for comparison with the baseline time-point before administration of clopidogrel.

<table>
<thead>
<tr>
<th>Loading with Clopidogrel 600 mg</th>
<th></th>
<th>Loading with Clopidogrel 900 mg</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>AUC15</td>
<td>PRU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean +/- 95% CI</td>
<td>p value</td>
</tr>
<tr>
<td>Baseline</td>
<td>30</td>
<td>1010.9 +/- 80.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1 hour</td>
<td>30</td>
<td>904.3 +/- 77.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2 hour</td>
<td>30</td>
<td>738.9 +/- 119</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 hours</td>
<td>27</td>
<td>738.7 +/- 101.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24 hours</td>
<td>17</td>
<td>822.5 +/- 128.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Patients loaded with clopidogrel 600 mg/900 mg

Simultaneous assay by s-TEG and VN-P2Y12 was undertaken in 59 patients being loaded with clopidogrel 600 mg/900 mg (Group D) as summarised in Table 4. Data was available for analysis in 89.3% (clopidogrel 600 mg) and 90.3% (clopidogrel 900 mg) of the planned data points. Missing data were due either to early hospital discharge or to having received abciximab/bivalirudin during PCI. Following the loading dose of clopidogrel 600 mg/900 mg, both AUC15 in the ADP channel and PRU from VN-P2Y12 reduced significantly in a time-dependent manner (Table 4).

In patients being loaded with clopidogrel 600 mg a response in the “therapeutic range” was seen at baseline (i.e. pre-loading) in eight patients (26.7%) by VN-P2Y12 compared to three patients (10%) with s-TEG (p = NS). In the clopidogrel 900 mg sub-group, three patients (10%) had a response in the “therapeutic range” with VN-P2Y12 at baseline in contrast to none with s-TEG.

Using the therapeutic threshold of % CIn ADP (AUC15) ≥30% and PRU ≤300, there was a statistically significant difference in the percentage of patients exhibiting a “therapeutic response” with VN-P2Y12 and s-TEG at all time-points subsequent to loading with clopidogrel 600 mg/900 mg (Figure 2A and Figure 3A). Even when using the PRU threshold of 208, this discrepancy persisted at the 2, 6 and 24 hour time-points in all patients being loaded with clopidogrel (Figure 2B and Figure 3B).

The effect of adding PGE1 to the ADP channel on Short TEG responses in patients on DAPT

In 27 patients on DAPT the ex vivo clotting response to ADP alone was compared in the s-TEG assay with responses to a combination of ADP and PGE1 (Group F). The mean MA (Figure 4B) and mean AUC15 (Figure 4C) were significantly lower and corre-
Khanna et al. Does VerifyNow overestimate response to clopidogrel?

Discussion

This paper describes the following key results. Firstly, that VN-P2Y12 finds a larger proportion of individuals taking clopidogrel are within its “therapeutic range” than s-TEG. Secondly, that this excess in reported clopidogrel “responders” with VN-P2Y12 compared to s-TEG is consistent in healthy volunteers as well as patients and is seen regardless of whether these populations were taking clopidogrel or not. Thirdly, even when the PRU threshold

spondingly the mean %CIn (▶ Figure 4C) was significantly higher in the PGE1/ADP channel compared to that in the ADP-only channel. Furthermore, the response of six patients lies in the “therapeutic range” of s-TEG when using % CIn AUC15 in the PGE1/ADP channel, but was in a range indicating HPR (i.e. hyporesponsive) when evaluated in the ADP-only channel. The degree of concordance between VN-P2Y12 and s-TEG improves from 70.4% to 92.6% when using AUC15 from the PGE1/ADP channel as opposed to the ADP-only channel.

Figure 2: Comparison of s-TEG and the VN-P2Y12 assay before and at four time-points after loading thirty patients with clopidogrel 600 mg. A) Percentage of patients above the therapeutic thresholds (% CIn ADP (AUC15) ≥ 30% – shaded grey, and PRU ≤ 230 – shaded black), B) Percentage of patients above therapeutic thresholds (% CIn ADP (AUC15) ≥ 30% – shaded grey, and PRU ≤ 208 – shaded black).
Khanna et al. Does VerifyNow overestimate response to clopidogrel?

denoting HPR is arbitrarily decreased from PRU ≥ 230 to PRU ≥ 208, an excess of subjects within the “therapeutic response” range of VN-P2Y12 was still observed. Finally, one possible mechanism for the observed excess of apparent responders to clopidogrel with VN-P2Y12 is that when PGE1 is added to ADP in s-TEG we observed a consistent reduction in whole blood clotting, thus simulating an apparently greater “therapeutic response” compared to ADP alone.

Given that there is a very well-described association between HPR and thrombotic events in patients requiring P2Y12 inhibitors (25), the options for reducing the risk for those individuals who do not achieve a therapeutic response are limited, especially since robust definitions of HPR remain contentious (26). The requirement for a simple, rapid point-of-care test of platelet function is clear cut if a strategy of personalised P2Y12 inhibitor therapy is to be delivered accurately. Amongst a range of candidates, VN-P2Y12 has emerged as the only truly point-of-care test that accurately de-
Figure 4: Assessing effect of adding PGE1 to ADP using s-TEG in patients on DAPT with clopidogrel 75 mg as the P2Y12 inhibitor. A) Line graph comparing individual clotting responses in the PGE1/ADP channel to the ADP channel, B) Error bar graphs comparing MA in the ADP and PGE1/ADP channels, C) Error bar graphs comparing AUC15 in the ADP and PGE1/ADP channels, D) Error bar graphs comparing % Cln (AUC15) in the ADP and PGE1/ADP channel. Data are represented as mean ± 95% CI of the mean.

scibes, in a snapshot fashion, the platelet reactivity response of an individual to P2Y12 therapy. However, for any such test, establishing a scientifically and statistically well-validated cut-off value for the definition of therapeutically “adequate” or “inadequate” that relates to both the risk of thrombotic events at one end of the therapeutic spectrum, and bleeding at the other, is recognised to be highly challenging (27). In this context, it is notable that recent randomised trials employing VN-P2Y12 have not shown any benefit for this “tailoring of therapy” approach (11-13). However, these trials have been critically dependent for the intervention at randomisation upon a binary allocation of each patient into two arms of “adequate responder” or “inadequate responder” to clopidogrel. It is clear that if the employed assay does not, in fact, accurately reflect the true clotting response of some patients to ADP-
What is known about this topic?
- In patients on clopidogrel, high platelet reactivity is associated with increased risk of future ischaemic events. However, most patients on clopidogrel have an adequate therapeutic response and in that group the bleeding risk and cost are lower than for patients on stronger P2Y12 inhibitors.
- A number of large randomised trials including GRAVITAS, ARCTIC and TRIGGER-PCI have stratified patients into categories of "responders" and "non-responders" to clopidogrel using the VN-P2Y12 assay. These trials have universally failed to show a positive outcome for a strategy of tailored antiplatelet therapy.
- The VN-P2Y12 assay uses PGE1 is used in addition to ADP as agonists to make the test more specific for assessing P2Y12 blockade by reducing the contribution of the P2Y1 pathway on ADP-mediated platelet aggregation.

What does this paper add?
- In a heterogeneous population of healthy volunteers and patients, both on and off clopidogrel, we showed that the VN-P2Y12 assay overestimates response in some individuals as compared to s-TEG.
- Furthermore, in a group of patients on dual antiplatelet therapy we showed that the addition of PGE1 to ADP in s-TEG potentiates the anti-aggregatory effects of clopidogrel.
- These observations raise the possibility that VN-P2Y12 produces an overestimate of the functional effects of clopidogrel in some individuals, possibly because it utilises PGE1 as an agonist in addition to ADP.

stimulation (even if it does reflect the degree of P2Y12 inhibition), then a proportion of the trial population would be allocated incorrectly at randomisation.

The VN-P2Y12 assay has been compared with several platelet function assays including vasodilator-stimulated phosphoprotein (VASP) phosphorylation (28), whole-blood aggregometry (29), multiple electrode platelet aggregometry (MEA) (30), as well as the platelet function analyser (PFA-100). The outcome of these studies indicates modest correlation at best. Although there are some studies showing a good correlation (correlation coefficients ranging from 0.62 to 0.86) between LTA and VN-P2Y12 (31-34), this has not been consistent in patients with stable CAD (29).

The consistency of our data presents a persuasive case that VN-P2Y12 overestimates response to clopidogrel in some subjects. Inevitably this leads us to speculate about a possible mechanistic explanation for these observations. The main difference between the assays is that s-TEG uses ADP as the sole agonist, whereas VN-P2Y12 uses a combination of ADP and PGE1. The inclusion of PGE1 is based on the theoretical justification that this makes the test more specific for P2Y12 blockade by reducing the contribution of the P2Y1 pathway on ADP-mediated platelet aggregation. ADP-mediated activation of the P2Y1 receptor causes platelet aggregation by increasing phospholipase C-mediated mobilisation of calcium (Ca++) from intracellular stores. PGE1 inhibits this pathway, and thus platelet aggregation, by stimulating adenylylate cyclase-mediated production of cyclic-AMP. As P2Y12 antagonists block ADP-mediated inhibition of adenylylate cyclase, PGE1 would be expected to potentiate the anti-aggregatory effects of a P2Y12 inhibitor. Indeed, Fox et al. have described the synergistic effects of combining PGE1 with a P2Y12 blocker on inhibiting ADP-mediated increases in intracellular Ca++ levels (35). More pertinently, however, they demonstrated that combining PGE1 with a P2Y12 inhibitor (clopidogrel or cangrelor) produces a greater inhibitory effect on platelet aggregation than that seen with either agent on its own. Thus, the addition of PGE1 to ADP may inadvertently exaggerate the apparent therapeutic effects of clopidogrel observed by VN-P2Y12.

Based upon this potential mechanism, we assessed the effects of running simultaneous s-TEG channels with ADP alone or ADP plus PGE1 at a concentration (22 nM) replicating that in the VN-P2Y12 assay. The results confirm that adding PGE1 to ADP leads to reduced clotting and therefore to an apparently greater therapeutic effect of clopidogrel than is observed in the ADP-only channel. These results do suggest that VN-P2Y12, whilst indeed providing a pure assessment of the effect of P2Y12 receptor inhibition, may not be providing a true indication of the actual blood clotting consequence of taking clopidogrel. The suitability of this assay as the sole arbiter at the point of randomisation in an interventional trial of tailored P2Y12 inhibitor therapy is therefore uncertain.

These experiments have several limitations. Firstly, the number of subjects is small. Secondly, the endpoints are assay-dependent rather than based on clinical outcomes. Thirdly, the studies were all conducted by a research team in a single centre. Finally, the investigators were unblinded. As such the data can be seen as hypothesis-generating only.

In conclusion, VN-P2Y12 produces an overestimate of the functional effects of clopidogrel in some individuals, possibly because it utilises PGE1 as an agonist in addition to ADP. This observation could have implications for the ability of the VN-P2Y12 assay to detect and stratify patients as “responders” or “non-responders” to clopidogrel. Further mechanistic and clinical outcomes data are required.

Conflicts of interest
NC has received unrestricted research grants from Haemonetics, Medtronic, St Jude Medical and speaker/consulting fees from Boston Scientific, Abbott Vascular, Haemonetics, Daiichi Sankyo and St Jude Medical. AH has received a travel bursary from Eli-Lilly/Daiichi Sankyo.

References


