Variability of Individual Platelet Reactivity Over Time in Patients Treated With Clopidogrel

Insights From the ELEVATE-TIMI 56 Trial

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ABSTRACT

BACKGROUND The degree of antiplatelet response to clopidogrel has been associated with clinical outcomes. Studies have investigated whether adjustment of antiplatelet therapies based on a single platelet function test is beneficial.

OBJECTIVES The aim of the study was to test the stability of platelet reactivity measurements over time among patients treated with standard and double doses of clopidogrel.

METHODS The ELEVATE-TIMI 56 (Escalating Clopidogrel by Involving a Genetic Strategy–Thrombolysis In Myocardial Infarction 56) investigators genotyped 333 patients with coronary artery disease and randomized them to various clopidogrel regimens. Patients with at least 2 platelet function results on the same maintenance dose of clopidogrel (75 mg or 150 mg) were analyzed. Platelet aggregation was measured using P2Y12 reaction units (PRU).

RESULTS In total, the mean platelet reactivity and the total number of nonresponders (PRU $>$230) with clopidogrel did not change between 2 periods for the 75-mg (22.4% vs. 21.9%; p = 0.86) and 150-mg doses of clopidogrel (11.5% vs. 11.5%; p = 1.00). In contrast, when evaluating each patient individually, 15.7% of patients taking clopidogrel 75 mg and 11.4% of patients taking 150 mg had a change in their responder status when tested at 2 different time points (p < 0.001). Despite being treated with the same dose of clopidogrel, >40% of patients had a change in PRU >40 on serial sampling, which approximates the average PRU difference caused by increasing the clopidogrel dose from 75 mg to 150 mg.

CONCLUSIONS Measurements of platelet reactivity vary over time in a significant proportion of patients. Thus, treatment adjustment according to platelet function testing at a single time point might not be sufficient for guiding antiplatelet therapy in clinical or research settings. (Escalating Clopidogrel by Involving a Genetic Strategy–Thrombolysis In Myocardial Infarction 56 [ELEVATE-TIMI 56]; NCT01235351) (J Am Coll Cardiol 2014;64:361–8) © 2014 by the American College of Cardiology Foundation
Treatment with aspirin and clopidogrel after percutaneous coronary intervention (PCI) is frequently used to prevent ischemic complications, including stent thrombosis (1), and this approach has been shown to reduce cardiovascular events in patients with acute coronary syndromes (ACS) (2). Studies have demonstrated variability in the pharmacodynamic response to clopidogrel (3,4). This variability can be explained in part by genetic polymorphisms (5), as well as by demographic, cellular, and clinical factors (6–9). Some clinical studies have demonstrated that patients with high on-clopidogrel platelet reactivity are at increased risk of stent thrombosis and other major cardiac complications (10–12).

Further studies have investigated whether dose adjustment of clopidogrel can overcome this high on-treatment platelet reactivity (13–16) and whether such an adjustment based on platelet function testing can improve clinical outcomes (17,18). Most of these studies have used a single platelet function test result for treatment stratification, often obtained early after the start of antiplatelet treatment with clopidogrel. Although data have demonstrated that the mean platelet function across a population is constant with serial samples (13–15), these findings do not exclude significant changes among individual patients over time.

The ELEVATE-TIMI 56 (Escalating Clopidogrel by Involving a Genetic Strategy—Thrombolysis In Myocardial Infarction 56) trial investigated the effect of escalating maintenance doses of clopidogrel on platelet reactivity in patients with coronary artery disease, taking into account the cytochrome P450 2C19 (CYP2C19) genotype (16). Participants underwent serial platelet function testing under stable conditions using a point-of-care adenosine diphosphate-mediated platelet aggregation assay (VerifyNow, Accumetrics, San Diego, California), as well as a vasodilator-stimulated phosphoprotein (VASP) assay. Thus, the study offers an opportunity to assess the stability of measurements of platelet reactivity over time in cardiovascular patients treated with standard- and double-dose clopidogrel in whom clinical factors, genotype, and compliance have been taken into account.

METHODS

STUDY POPULATION. ELEVATE-TIMI 56 was a multicenter, randomized, double-blind trial that enrolled and genotyped 333 patients across 32 sites with known cardiovascular disease taking 75 mg of clopidogrel daily (16). To be eligible, patients had an indication for the use of clopidogrel (myocardial infarction and/or PCI ≥4 weeks and ≥6 months before enrollment) and be clinically stable. All patients took aspirin, 81 to 325 mg daily, and they were requested to keep taking a stable dose during the study, if medically appropriate. Key exclusion criteria were the use of anticoagulants or proton pump inhibitors, current smoking, previous stent thrombosis, heightened risk of bleeding, end-stage renal or hepatic disease, or a procedure or hospitalization planned within the next 12 weeks.

The study design and flow of patients for the present post-hoc analysis are illustrated in Online Figure A. Of the 333 patients genotyped, 86 patients carried the CYP2C19*2 loss-of-function polymorphism and were not included in the present analysis because they did not have serial platelet function results for the
same dose. The remaining 247 patients did not carry the CYP2C19*2 loss-of-function polymorphism and were therefore randomized to a blinded sequence of maintenance doses of clopidogrel for 4 treatment periods in various sequences, each ~14 days. Two of these treatment periods were clopidogrel 75 mg and 2 were clopidogrel 150 mg. At the end of each period, compliance was assessed by pill counting; comedication, including aspirin intake, was recorded; platelet function testing was performed; and clinical adverse events were ascertained. Patients were instructed to take their clopidogrel study medication in the morning, and on the study visit days, they were instructed to take their dose after blood sampling. In total, 210 patients had 2 platelet function tests for the 75-mg dose and 209 for the 150-mg dose, along with a confirmed compliance of >80% at each visit. For the 75-mg dose, 35 patients were excluded due to not having a second sample at the same dose and 2 due to compliance <80%; for the 150-mg dose, 36 patients were excluded due to not having a second sample at the same dose and 2 due to compliance <80%.

The trial was approved by the institutional review board of each participating site, and participants provided written informed consent.

PLATELET FUNCTION STUDIES. Blood samples for platelet function testing were collected at the trough level before intake of the next maintenance dose and ~24 h after the last dose of clopidogrel by direct venipuncture using a 21-gauge (or higher diameter) needle, taking care to avoid hemolysis or contamination by tissue factors. Samples with signs of hemolysis or clotting were to be redrawn.

Platelet aggregation testing was conducted at each site with an encrypted point-of-care device (VerifyNow P2Y12 test, Accumetrics) and run between 10 min and 4 h after sampling according to manufacturer instructions. Results were reported as P2Y12 reaction units (PRU), indicating the amount of adenosine diphosphate-mediated platelet aggregation. The prespecified definition of nonresponder status was based on the VerifyNow assay and defined as ≥230 PRU (19). Because an additional nonresponder definition of ≥208 PRU also has been described (20,21), this cutoff was used in a sensitivity analysis. The coefficient of variation of this assay in patients treated with clopidogrel has been previously reported as 3.2% (22).

Additionally, platelet function was assessed by flow cytometric assessment of the phosphorylation status of VASP and expressed as the platelet reactivity index. The VASP platelet reactivity index was determined from blood samples sent with an isolated ambient shipping system to a central core laboratory (Center for Platelet Research Studies, Boston Children’s Hospital, Boston, Massachusetts), which was blinded to patient treatment group (23). Samples were to be tested within 72 h (88% within 24 h). Between sample receipt and analysis on the day of receipt, samples were stored at room temperature. All samples were allowed to stand at room temperature shielded from light for 30 min after staining before analysis by flow cytometry. Flow cytometric analysis was performed between 30 and 90 min after completion of staining.

STATISTICAL ANALYSIS. For intraindividual correlation analyses, all patients with at least 2 platelet function results at the same dose of clopidogrel and with a drug compliance >80% on both occasions were included. No imputation was applied for missing data. Discrete variables are reported as percentage and continuous variables as mean ± SD. Paired discrete variables were analyzed by a paired χ2 test, and paired categorical data by McNemar’s test unless otherwise specified. In the 2-sided test, a p value <0.05 was regarded as significant. Cohen’s kappa coefficients were used to describe the agreement of responder status between periods. The proportion of individuals with a change in responder status was evaluated using the alternative hypothesis of the proportion exceeding 5%. The distributions of change in platelet reactivity were generated, and SDs were reported. Analyses were run in SAS software version 9.1.3 Service Pack 2 (SAS Institute, Cary, North Carolina).

RESULTS

The mean age in the study population was 60.2 ± 9.9 years, 75% were male, 57% had a history of myocardial infarction, and 97% had a history of PCI. When assessing patients with serial measurements, the mean platelet reactivity as determined by the VerifyNow P2Y12 test and VASP assays did not differ between serial sampling with the 75-mg and 150-mg doses of clopidogrel (Table 1). Likewise, the total proportion of nonresponders to clopidogrel did not change between both periods at the same dose of clopidogrel (Table 1).

In contrast, analyzing each patient individually, 15.7% of patients taking clopidogrel 75 mg had a change in their nonresponder-status (PRU ≥230) when tested at 2 different time points (Table 2). Based on the coefficient of variation of the assay used, only 2.9% of patients would have been expected to change their nonresponder-status if platelet function would be stable over time. Using the definition of PRU ≥208, 1 in 5 patients experienced a change in status with
75 mg. Cohen’s kappa yielded values between 0.44 and 0.54, suggesting only moderate agreement, and a change in responder status was found in a significant proportion of patients irrespective of nonresponder definition or tested dose of clopidogrel ($p < 0.001$ for each) (Fig. 1). The number of patients changing from responder to nonresponder status was similar to the number of patients switching in the opposite direction.

Because even a minor change in platelet reactivity could alter the responder status (i.e., from 229 PRU to 230 PRU), data were also analyzed in a continuous fashion. The individual change in platelet reactivity over time approximated a Gaussian distribution with equal changes in both directions being found (Fig. 2). Notably, the SDs for the change in platelet reactivity over serial time points with the same clopidogrel dose were 68 PRU for 75 mg and 59 PRU for 150 mg. When analyzing various cutpoints ranging from 80 to 20 PRU, between 15.8% and 63.8% of patients demonstrated a change in platelet reactivity status over time, with parallel results seen with the VASP assay (Table 3, Online Fig. B). The number of patients with a given absolute change in platelet reactivity was similar for both the 75-mg and the 150-mg doses at all cutoffs.

Diabetes mellitus and body mass index were the variables most consistently associated with a change in platelet function over time across the assays and doses of clopidogrel used (Online Table A).

### DISCUSSION

A variable patient response to clopidogrel has been demonstrated and high on-treatment platelet reactivity, as measured by several different platelet function tests, has been associated with ischemic events (10,11). Additionally, low on-treatment platelet reactivity has been related to hemorrhagic events (24). The present analysis of ELEVATE-TIMI 56 data demonstrates that, in a cohort, mean on-treatment platelet reactivity did not differ over time. However, on an individual basis, a significant number of patients exhibited discordant values (Central Illustration), with approximately 1 in 5 patients experiencing a change in their clopidogrel responder status over time, despite similar clinical conditions. Importantly, the 150-mg dose of clopidogrel was not able to overcome this degree of variability in serial platelet reactivity measurements. These findings have implications for the potential clinical implementation of these tests, as well as for the design of future studies.

Some studies have demonstrated only minor changes in platelet phenotype or nonresponder status over time among patients treated with clopidogrel (23,25,26). Other data have shown that platelet function in individual healthy subjects who are not on
Antiplatelet therapy can vary significantly over time (27,28). One study that enrolled patients undergoing PCI reported that 27% of patients had a change in nonresponder status within 1 month (29). These latter findings, however, could have been explained by the large proportion of patients with ACS at the time of enrollment, a factor known to be associated with poor response to clopidogrel (6), and the direction of change was predominantly from nonresponder to responder status, consistent with the metabolic changes after ACS. Notably, even within the stable population in ELEVATE–TIMI 56, 1 in 4 patients still experienced a change in PRU >60 while being treated with the same dose of clopidogrel. This magnitude of change would, for example, be sufficient to shift an entire risk category in a meta-analysis that assessed PRU quartiles and rates of adverse cardiovascular outcomes (19). Moreover, in the present study, despite being treated with the same dose of clopidogrel, ~40% of patients had a change in PRU >40 with serial sampling, which approximates the average PRU difference caused by increasing the clopidogrel dose from 75 mg to 150 mg.

The time-dependent variability in individual platelet reactivity in ELEVATE–TIMI 56 was detected even within the context of a carefully monitored, double-blind clinical trial, with uniform assessment of platelet function among stable patients with cardiovascular disease. Additionally, the study controlled for potential biasing factors such as drug compliance, timing of the dose, and/or drugs interacting with clopidogrel bioactivation. The variability in platelet reactivity measurements would be expected to be even more pronounced in clinical practice where such close monitoring and genetic data might not be routine. We can only speculate which mechanisms contribute to this intraindividual variability. Potential factors might include either true alterations in platelet reactivity due to fluctuations in platelet production and expression of the P2Y12 receptor, changes in hepatic metabolism altering the level of clopidogrel bioactivation, unrecognized noncompliance, or artifactual changes in measured platelet reactivity due to biological or technical issues affecting the assay.

The significant intraindividual variability in response to antiplatelet therapy in a large proportion of patients, as demonstrated by the present analysis, could be integral for understanding the negative results observed in 3 randomized trials that evaluated the impact of personalized antiplatelet strategies guided by platelet function testing on clinical outcome (18,21,30). Two of these studies enrolled stable patients, and 1 trial included patients with and without ACS. In these studies, patients identified as nonresponders to clopidogrel received
additional loading doses and higher maintenance doses of clopidogrel (150 mg) or were switched to prasugrel. Dose adjustment was mainly based on a single platelet function test obtained early after the initial loading dose of clopidogrel. In 1 trial, an additional treatment adjustment was allowed, but not mandatory, if a limited response to antiplatelet therapy was found at 14 to 30 days after enrollment (30). The main finding of all 3 trials was that personalized antiplatelet treatment, compared with standard treatment with clopidogrel 75 mg daily, did not reduce major cardiovascular events. Several potential reasons for these findings have been discussed such as limited effectiveness of the intervention compared with the standard treatment strategy (150 mg vs. 75 mg clopidogrel) and limited power due to lower-than-expected event rates.

Given the results of the present analysis, intra-individual variability in platelet phenotype might have been another factor leading to these results because strategies using only platelet function results from a single time point might not detect the majority of patients with a suboptimal response to clopidogrel.

**STUDY LIMITATIONS.** There are some limitations to the present analysis. Each treatment period consisted of ~14 days. Although platelet phenotype becomes more stable during long-term treatment, this time frame allowed for a reasonable window to achieve a steady-state antiplatelet effect with each regimen, with no significant carryover effect from the previous treatment. Second, variability between the timing of study drug administration and blood sampling could have occurred, as well as non-adherence. In an attempt to address these concerns, sites and patients were instructed to draw samples at trough levels and conduct pill counts. Third, patients with the CYP2C19*2 loss-of-function polymorphism were not included in the present study because they were not tested twice at the same dose. Fourth, given the post-hoc design of the present analysis, these data need confirmation in further studies. Finally, this trial cannot determine

### TABLE 3   Proportion of Individual Subjects With a Change in Platelet Reactivity Between Periods by Clopidogrel Dose and Assay

<table>
<thead>
<tr>
<th></th>
<th>75-mg Clopidogrel</th>
<th>150-mg Clopidogrel</th>
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<tbody>
<tr>
<td>VerifyNow Assay (change in PRU)</td>
<td></td>
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</tr>
<tr>
<td>&gt;20 PRU</td>
<td>63.8 (56.9–70.3)</td>
<td>61.7 (54.8–68.3)</td>
</tr>
<tr>
<td>&gt;40 PRU</td>
<td>41.4 (34.7–48.4)</td>
<td>40.7 (33.9–47.7)</td>
</tr>
<tr>
<td>&gt;60 PRU</td>
<td>28.6 (22.6–35.2)</td>
<td>24.9 (19.2–31.3)</td>
</tr>
<tr>
<td>&gt;80 PRU</td>
<td>16.2 (11.5–21.9)</td>
<td>15.8 (11.1–21.5)</td>
</tr>
<tr>
<td>VASP assay (change in PRI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5 PRI</td>
<td>65.2 (58.4–71.7)</td>
<td>70.8 (64.1–76.9)</td>
</tr>
<tr>
<td>&gt;10 PRI</td>
<td>42.4 (35.6–49.4)</td>
<td>52.2 (45.2–59.1)</td>
</tr>
<tr>
<td>&gt;15 PRI</td>
<td>27.6 (21.7–34.2)</td>
<td>29.2 (23.1–35.9)</td>
</tr>
<tr>
<td>&gt;20 PRI</td>
<td>20.5 (15.2–26.6)</td>
<td>18.7 (13.6–24.6)</td>
</tr>
</tbody>
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Values are % (95% confidence interval). Abbreviations as in Table 1.
the significance of the intraindividual variability in clinical practice because it was not powered for clinical outcomes, and intraindividual variability might be even greater in real life without the present strict control for compliance and other factors affecting the antiplatelet effect of clopidogrel. To eliminate the documented alteration in platelet reactivity that occurs in the setting of ACS, we studied stable patients. The variability we observed, assuming it would also be present in ACS patients, also would be of clinical significance in that high-risk setting.

CONCLUSIONS

Measurements of platelet reactivity vary over time in a significant proportion of patients, even when higher maintenance doses of clopidogrel are used. Based on these results, treatment adjustment according to platelet function testing at a single time point might not be sufficient for guiding antiplatelet therapy in either clinical or research settings.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Even while maintaining a stable dose of clopidogrel, >40% of patients exhibit variations in platelet reactivity similar to those caused by doubling the dose from 75 to 150 mg daily.

TRANSLATIONAL OUTLOOK: In designing future studies that use measurements of platelet inhibition in patients exposed to clopidogrel, investigators should consider the limitations of testing at a single time point and the value of serial data.

REFERENCES


KEY WORDS clopidogrel, nonresponder, platelet reactivity, variability

APPENDIX For a supplemental table and figures, please see the online version of this article.