Impact of Routine Platelet Reactivity Testing with VerifyNow Assay on Antiplatelet Choice After Percutaneous Coronary Intervention

Background: High on-treatment ADP platelet reactivity (HPR) measured by VerifyNow P2Y12 assay (VN) is an established risk factor for ischemic events after percutaneous coronary intervention (PCI). We hypothesized that routine use of VN at time of PCI in clinical practice may affect choice of P2Y12 antiplatelet therapy at discharge.

Methods: In a single center retrospective analysis, we examined the influence of VN testing on choice of P2Y12 inhibitor post PCI in routine clinical practice. Assessment of HPR was used routinely in clinical care during the time period of analysis at discretion of clinical providers. Subjects with PRU>208 after the loading dose of clopidogrel or during clopidogrel steady state were switched to alternate P2Y12 inhibitors.

Results: We identified 1001 patients with PCI during the time period specified. A total of 252 subjects underwent VN testing. Among those, 43% were found to have HPR on clopidogrel and were switched to alternate therapies (prasugrel [n=60], ticagrelor [n=48]). Patients who had VN platelet function testing were more likely to be discharged on clopidogrel as compared to those who did not have VN assay done (57% vs. 50%, p=0.039). There was no significant difference in 1-year net-MACE (CVD, MI, stent thrombosis, BARC 2 or higher bleeding) using tailored antiplatelet therapy (VN testing) as compared to standard of care group (adjusted HR:0.92, 95% CI: 0.54–1.5, p=0.74).

Conclusion: Routine use of VN assay in personalized antiplatelet treatment decision-making after PCI is associated with lower likelihood of using novel P2Y12 inhibitors.

Keywords: clopidogrel, prasugrel, ticagrelor, myocardial infarction

Introduction
Among available P2Y12 platelet inhibitors, clopidogrel continues to be widely used because of the decreased risk of bleeding, lower cost, and less likelihood of side effects such as dyspnea. Clopidogrel bioactivation is in part determined by inter-individual differences in pharmacogenetics, predominantly cytochrome P450 2C19 variants. Inadequate platelet inhibition increases risk of stent thrombosis and other adverse cardiovascular events.

High on treatment platelet reactivity (HPR) during treatment with clopidogrel has been consistently found to be strong risk factor for recurrent ischemic events after PCI. In the landmark study by Stone et al, HPR defined as PRU>208 measured by the VN P2Y12 assay, was associated with 2.49 fold increased risk...
Subjects who underwent PCI with subsequent placement of at least 1 drug eluting (DES) or bare metal stent (BMS) between 2012 and 2018 at Eskenazi hospital in Indianapolis. Platelet reactivity testing with VN P2Y12 assay was available bedside in the cardiac catheterization laboratory at the discretion of the clinical provider at Eskenazi Health during this time period. When used, VN P2Y12 assay was completed after administration of clopidogrel (at least 4 hrs after 600mg loading dose if not loaded previously), usually at the time of PCI. Pharmacogenetic testing was not routinely performed in our institution during the study period.

Study Design

Platelet reactivity was assessed using VN P2Y12 assay according to the manufacturer’s instructions. The VN point-of-care instrument measures platelet-induced aggregation of fibrinogen coated beads in response to 20μM ADP as an increase in light transmittance and uses a proprietary algorithm to report values in P2Y12 reaction units (PRU). The assay also contains prostaglandin E1 to minimize contribution of P2Y1 to platelet aggregation. HPR was defined as PRU>208 to maintain consistency with previous studies. Providers had been instructed on the use of the PRU cutoffs and were encouraged to continue patients on clopidogrel if they had low on treatment platelet reactivity. Patients identified with HPR (PRU>208) after administration of clopidogrel were switched to either prasugrel or ticagrelor. Percutaneous coronary interventions were performed according to established standards and guidelines. Subjects who had VN testing done were compared to subjects who did not have a platelet assay performed during their hospital stay at Eskenazi Health between 2012 and 2018. During the time period analyzed, we did not follow a protocol of de-escalation as used in the TROPICAL-ACS study with repeat platelet testing on clopidogrel 1 week after switching from prasugrel.

The primary outcome of the study was the prevalence of clopidogrel prescribed at hospital discharge. The main clinical endpoint was defined as combined net-MACE (cardiovascular death, MI, stent thrombosis, bleeding in Academic Research Consortium [BARC] 2 or higher bleeding) assessed at 1 year. Endpoints were evaluated by review of electronic medical records. Patients with stent thrombosis and cardiovascular deaths were further adjudicated using original source documents and angiographic images when available. Death was considered non-cardiac when an unequivocal non-cardiac cause was documented.

Statistical Analysis

Baseline variables were compared between groups using Pearson-Chi Square test, and continuous data by Student’s t-test. Binary outcome was compared by use of one-sided
Fisher’s exact test. Survival analysis was performed using Kaplan-Meier estimates and the Log rank test was used to evaluate differences between groups. Cox proportional hazards model regression analysis was performed with forward multivariate adjustment of clinically significant baseline co-variates (p<0.1). Statistical analysis was performed with the use of SPSS software, version 25.0 (SPSS Inc., Chicago, Illinois).

### Table 1 Clinical Variables

| Characteristics          | VerifyNow P2Y12 Platelet Assay Was Not Done (n = 749) | VerifyNow P2Y12 Platelet Assay Was Done (n = 252) | p value* |
|--------------------------|------------------------------------------------------|--------------------------------------------------|----------|
| Age (years)              | 61.7 ± 11                                            | 60.9 ± 10                                         | 0.33     |
| Gender                   |                                                      |                                                  |          |
| Female                   | 264/749 (35%)                                        | 101/252 (40%)                                     | 0.17     |
| Male                     | 485/749 (65%)                                        | 151/252 (60%)                                     |          |
| Race                     |                                                      |                                                  | 0.73     |
| Black or African American| 270/749 (36%)                                        | 93/252 (37%)                                      |          |
| White                    | 400/749 (53%)                                        | 130/252 (52%)                                     |          |
| Unknown/Not reported     | 60/749 (8%)                                          | 24/252 (9%)                                       |          |
| Body mass index (kg/m²)  | 31.8 ± 8                                             | 32 ± 8                                            | 0.75     |
| Angina                   |                                                      |                                                  | 0.14     |
| Stable                   | 149/749 (20%)                                        | 65/252 (26%)                                      |          |
| Unstable                 | 316/749 (42%)                                        | 100/252 (40%)                                     |          |
| Acute MI on Presentation |                                                      |                                                  | <0.001   |
| STEMI                    | 170/749 (23%)                                        | 16/252 (6%)                                       |          |
| NSTEMI                   | 321/749 (43%)                                        | 173/252 (69%)                                     |          |
| Medical History          |                                                      |                                                  |          |
| Diabetes mellitus        | 343/749 (46%)                                        | 121/252 (48%)                                     | 0.54     |
| Hypertension             | 563/749 (75%)                                        | 217/252 (86%)                                     | <0.001   |
| End stage renal disease  | 11/749 (1%)                                          | 6/252 (2%)                                        | 0.33     |
| Hyperlipidemia           | 312/749 (42%)                                        | 130/252 (52%)                                     | 0.006    |
| Peripheral vascular disease | 53/749 (7%)                                    | 21/252 (8%)                                       | 0.51     |
| Cerebrovascular accident | 67/749 (9%)                                          | 32/252 (13%)                                      | 0.08     |
| Prior myocardial infarction | 107/749 (14%)                        | 52/252 (21%)                                      | 0.17     |
| Coronary artery bypass graft | 79/749 (10%)                                | 31/252 (12%)                                      | 0.44     |
| Prior percutaneous coronary intervention | 127/749 (17%)                           | 69/252 (27%)                                      | <0.001   |
| Tobacco use              | 495/749 (66%)                                        | 177/252 (70%)                                     | 0.23     |
| Stent Type               |                                                      |                                                  | <0.001   |
| Drug eluting stent       | 628/749 (84%)                                        | 234/252 (93%)                                     |          |
| Bare metal stent         | 121/749 (16%)                                        | 17/252 (7%)                                       |          |
| Medication at Discharge  |                                                      |                                                  |          |
| ACEI/ARB                 | 585/749 (78%)                                        | 204/252 (81%)                                     | 0.34     |
| Aspirin                  | 729/749 (97%)                                        | 244/252 (97%)                                     | 0.67     |
| Statin                   | 724/749 (97%)                                        | 245/252 (97%)                                     | 0.66     |
| Beta blocker             | 688/749 (92%)                                        | 240/252 (95%)                                     | 0.074    |
| Proton pump inhibitor    | 145/749 (19%)                                        | 67/252 (27%)                                      | 0.015    |
| P2Y12 Inhibitor Pre-VN   |                                                      |                                                  |          |
| Clopidogrel              | 241/252 (96%)                                        |                                                  |          |
| Prasugrel                | 8/252 (3%)                                           |                                                  |          |
| Ticagrelor               | 3/252 (1%)                                           |                                                  |          |

Notes: *t*-test (continuous data), Chi-square (Binary data).
Results

A total of 1001 patients had PCI performed between 2012 and 2018. Among those, 252 had VN platelet function assay performed during their hospital stay. The majority of patients (96%) who had VN platelet testing performed had received pre-treatment with clopidogrel. Patients who did not have VN platelet reactivity testing performed were more likely to have presented with ST elevation myocardial infarction (STEMI) and to have a bare metal stent placed. Patients who did have VN platelet testing done were more likely to have a prior diagnosis of hypertension, hyperlipidemia, or prior PCI, present with a non-STEMI, and were more likely to be prescribed a proton-pump inhibitor (Table 1). Clinical baseline variables were otherwise well matched between groups.

Among patients who underwent platelet reactivity testing, 43% were found to be non-responders and were switched to alternate therapies (prasugrel [n=60], ticagrelor [n=48]). There was a wide range of on-treatment platelet reactivity (mean ± SD: 178 ± 88 PRU; range: 4–385 PRU).

Patients undergoing platelet function assay testing using VN were more likely to be discharged on clopidogrel vs. an alternate P2Y12 inhibitor in comparison to those who did not have this test done (57% vs. 50%, p=0.039) (Figure 1).

Use of VN in tailoring antiplatelet therapy after PCI compared to standard of care group was associated with no significant difference in risk of recurrent 1-year net-MACE (CVD, MI, stent thrombosis, BARC 2 or higher bleeding) (non-adjusted Hazard Ratio: 0.96 [95% CI: 0.57–1.6], p=0.87)). There were no significant differences in clinical outcomes after multivariate adjustment comparing VN platelet reactivity testing group vs. standard of care (no VN) group (Table 2, Figure 2).

Discussion

The results of our retrospective analysis demonstrate that routine use of VN assay in personalized antiplatelet treatment decision-making after PCI is associated with lower likelihood of using novel P2Y12 inhibitors as compared to standard treatment. Despite the higher prevalence of subjects with prior PCI and higher prevalence of NSTEMI in patients among the guided therapy group, there was no significant difference in clinical outcomes during 1-year follow up. Patients presenting with acute ST-elevation myocardial infarction were almost universally treated with either prasugrel or ticagrelor at time of primary PCI, making it unfeasible to use VN guidance for clopidogrel response at time of initial hospitalization. Upfront use of a novel P2Y12 inhibitor in our practice was routinely continued until discharge, and de-escalation of antiplatelet therapy using a platelet assay was not performed as a strategy in our institution.

Routine antiplatelet monitoring for high on-treatment platelet reactivity has been controversial due to lack of prospective trials showing superiority of such an approach compared to universal use of either prasugrel or ticagrelor. However the main focus of use in our practice was to reduce the use of more expensive novel P2Y12 inhibitors while minimizing the risk of thrombotic events by screening for HPR on clopidogrel in the periprocedural period. More recently the TROPICAL-ACS trial showed benefit of guided antiplatelet de-escalation after PCI using the multiplate assay. In that trial, patients who demonstrated low platelet reactivity after 7 days of clopidogrel 14 days post PCI, were switched to clopidogrel, whereas patients with HPR continued on prasugrel. The net-MACE benefit was driven mainly by a lower incidence of bleeding events, but also lower risk of combined ischemic endpoints. In contrast, in the much smaller study by Cayla et al VN guided change of antiplatelet therapy in elderly patients who were initially prescribed low dose prasugrel was not superior to continued treatment with prasugrel 5mg daily.

While clinical practice guidelines recommend the use of either ticagrelor or prasugrel over clopidogrel after PCI in patients presenting with ACS, more recent clinical trials have highlighted the increased risk of non-CABG bleeding with universal use of a potent P2Y12 inhibitor. Avoidance of a potent P2Y12 inhibitor in patients who...
have an acceptable pharmacodynamic response to clopidogrel and who may be at increased risk of bleeding may be a preferred strategy in post PCI dual antiplatelet therapy. Clinical risk scores have been developed to estimate bleeding risk on prolonged dual antiplatelet therapy, however they lack specificity and sensitivity and are less useful in assessing risk in regard to choice of potency of antiplatelet therapy. Clopidogrel bioactivation is dependent on activity of several cytochrome P450 (CYP) isoenzymes. In particular, variation in CYP 2C19 isoenzyme activity due to common single nucleotide polymorphisms significantly affects clopidogrel response and on treatment platelet reactivity. Several studies have demonstrated a reduction of net-MACE events by using pharmacogenetics guidance to tailor treatment with clopidogrel after PCI. On treatment platelet reactivity to ADP by VN correlates with active clopidogrel metabolite concentration and is an established pharmacodynamic measure of clopidogrel response. Thus, the use of VN assay to screen for HPR may be an alternative to a pharmacogenetic guided

Table 2 Clinical Events

| Clinical Events (1-Year) | VerifyNow Done | No VerifyNow Done | Adj. Hazard Ratio (95% Confidence Interval) | p-value*
|-------------------------|----------------|-------------------|---------------------------------------------|--------
| Net-MACE (combined death, myocardial infarction, stent thrombosis, BARC 2 or more bleeding) | 19/252 (7.5%) | 59/749 (7.9%) | 0.92 (0.54–1.5) | 0.74 |
| Cardiovascular death | 4/252 (1.6%) | 14/749 (1.9%) | 0.91 (0.29–2.9) | 0.87 |
| Myocardial infarction | 14/252 (5.6%) | 35/749 (4.7%) | 1.31 (0.67–2.7) | 0.42 |
| BARC 2 or more bleeding | 6/252 (2.4%) | 14/749 (1.9%) | 1.28 (0.49–3.3) | 0.61 |

Note: *Cox proportional hazards model analysis with forward multivariate adjustment of clinically significant baseline co-variates (p<0.1).
Abbreviation: BARC, Bleeding in Academic Research Consortium.

Figure 2 Clinical outcomes with Kaplan Meier cumulative survival curves for combined primary endpoint (Net-MACE: death, myocardial infarction, stent thrombosis, bleeding in Academic Research Consortium (BARC) 2 or more) (Panel [A]), myocardial infarction (Panel [B]), cardiovascular death (Panel [C]), and BARC 2 or more bleeding (Panel [D]). Analysis by log-rank model.
P2Y12 treatment strategy, and preferable in certain situations due to the ability to use the VN assay at the point-of-care with a very short turn-around time. In our study, there was no significant difference in occurrence of net-MACE or thrombotic events between groups post PCI, despite a higher prevalence of ACS in the VN guided group.

Limitations of our study include the retrospective, single center design of the study with a limited number of prescribers, and differences in baseline variables between patients with and without VN testing. The study was also not powered to evaluate clinical endpoints.

**Conclusions**

Tailoring of antiplatelet therapy by VN P2Y12 assay is feasible, and results in a lower likelihood of using a potent P2Y12 inhibitor post PCI in clinical practice. There was no significant association of VN P2Y12 assay use with clinical outcomes.

**Disclosure**

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**References**

1. Pereira NL, Rihal CS, So DY, et al. Clopidogrel pharmacogenetics. Circ Cardiovasc Interv. 2019;12(4):e007811. doi:10.1161/CIRCINTERVENTIONS.119.007811
2. Kreutz RP, Nystrom P, Kreutz Y, et al. Influence of paraoxonase-1 Q192R and cytochrome P450 2C19 polymorphisms on clopidogrel response. Clin Pharmacol. 2012;4:13–20. doi:10.2147/CPAA.S27822
3. Breet NJ, van Werkum JW, Bouman HJ, et al. Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. JAMA. 2010;303(8):754–762. doi:10.1001/jama.2010.181
4. Gurbel PA, Bliden KP, Guyer K, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STEMTING Study. J Am Coll Cardiol. 2005;46(10):1820–1826. doi:10.1016/j.jacc.2005.07.041
5. Gurbel PA, Bliden KP, Navickas IA, et al. Adenosine diphosphate-induced platelet-fibrin clot strength: a new thrombelastographic indicator of long-term post-stenting ischemic events. Am Heart J. 2010;160(2):346–354. doi:10.1016/j.amhe.2010.05.034
6. Stone G, Wittenbichler B, Weiss G, et al. Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents (ADAPT-DES): a prospective multicentre registry study. Lancet. 2013;382(9892):614–623. doi:10.1016/S0140-6736(13)61170-8
7. Bouman HJ, Parlag E, van Werkum JW, et al. Which platelet function test is suitable to monitor clopidogrel responsiveness? A pharmacokinetic analysis on the active metabolite of clopidogrel. J Thromb Haemost. 2010;8(3):482–488. doi:10.1111/j.1538-7836.2009.03733.x
8. Price MJ, Berger PB, Teirstein PS, et al. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. JAMA. 2011;305(11):1097–1105. doi:10.1001/jama.2011.290
9. Collet JP, Cuisset T, Rangé G, et al. Bedside monitoring to adjust antiplatelet therapy for coronary stenting. N Engl J Med. 2012;367(22):2100–2109. doi:10.1056/NEJMoa1209979
10. Trenk D, Stone GW, Gawaz M, et al. A randomized trial of prasugrel versus clopidogrel in patients with high platelet reactivity on clopidogrel after elective percutaneous coronary intervention with implantation of drug-eluting stents: results of the TRIGGER-PCI (Testing platelet reactivity in patients undergoing elective stent placement on clopidogrel to guide alternative therapy with prasugrel) study. J Am Coll Cardiol. 2012;59(24):2159–2164. doi:10.1016/j.jacc.2012.02.026
11. Sibbing D, Aradi D, Jacobshagen C, et al. Guided de-escalation of antiplatelet treatment in patients with acute coronary syndrome undergoing percutaneous coronary intervention (TROPICAL-ACS): a randomised, open-label, multicentre trial. Lancet. 2017;390(10104):1747–1757. doi:10.1016/S0140-6736(17)32155-4
12. Price MJ. Bedside evaluation of thienopyridine antiplatelet therapy. Circulation. 2009;2009(2119):2625–2632. doi:10.1161/CIRCULATIONAHA.107.696732
13. Levine GN, Bates ER, Blankenship JC, et al. American College of Cardiology Foundation; American Heart Association Task Force on practice guidelines; Society for Cardiovascular Angiography and Interventions. 2011 ACCF/AHA/SCAI guideline for percutaneous coronary intervention: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions. J Am Coll Cardiol. 2011;58(24):e44–122. doi:10.1016/j.jacc.2011.08.007
14. Thygesen K, Alpert JS, Jaffe AS, et al. Fourth universal definition of myocardial infarction (2018). J Am Coll Cardiol. 2018;72(18):2231–2264. doi:10.1016/j.jacc.2018.08.1038
15. Mehran R, Rao SV, Bhatt DL, et al. Standardized bleeding definitions for cardiovascular clinical trials: a consensus report from the Bleeding Academic Research Consortium. Circulation. 2011;123(23):2736–2747. doi:10.1161/CIRCULATIONAHA.110.009449
16. Academic Research Consortium, Cullip DE, Windecker S, Mehran R, et al. Clinical end points in coronary stent trials: a case for standardized definitions. Circulation. 2007;115(17):2344–2351. doi:10.1161/CIRCULATIONAHA.106.685313.
17. ANGEO investigators, Cayla G, Cuisset T, Silvain J, et al. Platelet function monitoring to adjust antiplatelet therapy in elderly patients stented for an acute coronary syndrome (ANGEO): an open-label, blinded-endpoint, randomised controlled superiority trial. Lancet. 2016;388(10055):2015–2022. doi:10.1016/S0140-6736(16)31323-X.
18. Gimbel ME. Randomised comparison of clopidogrel versus ticagrelor or prasugrel in patients of 70 years or older with non-ST-elevation acute coronary syndrome - Popular age. Paper presented at: European Society of Cardiology Congress; August 31; 2019; Paris, France.
19. Claassens DMF, Vos GJA, Bergmeijer TO, et al. A genotype-guided strategy for oral P2Y12 inhibitors in primary PCI. N Engl J Med. 2019;381:1621–1631. doi:10.1056/NEJMoa1907096
20. Levine GN, Bates ER, Bittl JA, et al. ACC/AHA guideline focused update on duration of dual antiplatelet therapy in patients with coronary artery disease: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2016;68(10):1082–1115. doi:10.1016/j.jacc.2016.03.513
21. Kreutz RP, Flockhart DA. Amlodipine—not a significant contributor to clopidogrel non-response? Heart. 2013;99(7):437–439. doi:10.1136/heartjnl-2012-303214
22. Mega JL, Hocholzer W, Frelinger AL, et al. Dosing clopidogrel based on CYP2C19 genotype and the effect on platelet reactivity in patients with stable cardiovascular disease. JAMA. 2011;306(20):2221–2228. doi:10.1001/jama.2011.1703
23. Shen DL, Wang B, Bai J, et al. Clinical value of CYP2C19 genetic testing for guiding the antiplatelet therapy in a Chinese population. *J Cardiovasc Pharmacol*. 2016;67(3):232–236. doi:10.1097/FJC.00000000000337

24. Cavallari LH, Lee CR, Beitelshes AL, et al. Multisite Investigation of outcomes with implementation of CYP2C19 genotype-guided antiplatelet therapy after percutaneous coronary intervention. *JACC Cardiovasc Interv*. 2018;11(2):181–191. doi:10.1016/j.jcin.2017.07.022

25. Notarangelo FM, Maglietta G, Bevilacqua P, et al. Pharmacogenomic approach to selecting antiplatelet therapy in patients with acute coronary syndromes: the PHARMCLO trial. *J Am Coll Cardiol*. 2018;71(17):1869–1877. doi:10.1016/j.jacc.2018.02.029