Impact of Timing on the Functional Recovery Achieved With Platelet Supplementation After Treatment With Ticagrelor

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Background—American College of Cardiology/American Heart Association guidelines advise waiting 5 to 7 days before operating on $P2Y_{12}$ inhibitor-treated acute coronary syndrome patients, to allow dissipation of its antiplatelet effects. Platelet transfusion is often used to restore hemostasis during operations, but its effectiveness and optimal timing are unclear. We investigated the degree of functional gains obtained from platelet supplementation after loading and maintenance of dual antiplatelet therapy with ticagrelor and the influence of timing on this strategy.

Methods and Results—After baseline platelet testing (Multiplate Analyzer and VerifyNow), cardiovascular disease patients (n=20; 56.9±7.9 years; 65% men; 75% diabetic) received dual antiplatelet therapy as a single loading dose (ticagrelor 180 mg plus aspirin 325 mg) and as daily/maintenance treatment for 5 to 7 days (maintenance therapy: ticagrelor 90 mg BID plus aspirin 81 mg QD). At 4, 6, 24, and 48 hours from (last) dosing, patients' blood samples were supplemented with concentrated platelets from healthy donors in vitro, raising platelet counts by 0% (unsupplemented control), 25%, 50%, and 75%, and the function retested. Reactivity in supplemented samples was compared with respective 0% sample and with the pretreatment baseline. Results under loading dose and maintenance therapy regimens were nearly identical. Platelet reactivity was higher ($P<0.05$) in nearly all supplemented samples versus respective controls. Aggregations with supplementation were 59% to 79% of baseline at 24 hours and equal to baseline at 48 hours.

Conclusions—Platelet reactivity of ticagrelor-treated patients can be restored using concentrated platelets after a loading dose/maintenance therapy in a time-dependent manner under in vitro testing. Although statistically significant improvements are evident 6 hours after (last) dosing, ≥24 hours maybe needed for clinically meaningful restoration in platelet function.

Clinical Trial Registration—URL: https://clinicaltrials.gov. Unique identifier: NCT02201394.

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Key Words: aspirin ■ blood platelets ■ diabetes mellitus ■ humans ■ ticagrelor

Dual antiplatelet therapy (DAPT) is the standard of care in the management of acute coronary syndrome patients, with aspirin and clopidogrel combination being the current standard of care. Ticagrelor has demonstrated greater benefits than clopidogrel in reducing clinical events in acute coronary syndrome patients.[1–3] Although stronger platelet inhibition has clearly proven benefits in preventing ischemic events, it also complicates management of acute coronary syndrome patients by enhancing the risk of bleeding complications and the associated morbidity and mortality.[4] Repeat operation to control hemorrhage is almost 6× more likely if patients receive DAPT before coronary artery bypass grafting (CABG), and 20% of such patients require platelet transfusions.[5] The American College of Cardiology/American Heart Association guidelines for acute coronary syndrome patients requiring CABG surgery recommend discontinuation of any $P2Y_{12}$ receptor inhibitor therapy for at least 5 days before an elective operation and at least 24 hours before urgent CABG.[6] This treatment-devoid phase leaves these high-risk patients defenseless against recurrent ischemic events, and any means to shorten this vulnerable period would be of clinical value. Platelet transfusions are advised and frequently used to deal with bleeding complications during CABG surgery in patients on antiplatelet therapy,[7,8] and could potentially be used for the earlier restoration of hemostasis in DAPT-treated patients, thereby shortening their waiting time for operation. However, the degree to which the newly infused platelets restore patients' hemostatic function, and its relationship to the quantity of platelets infused, is unknown. More importantly, the effect of timing of transfusion relative to last drug intake—likely to be an important

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WHAT IS KNOWN

- The strategy of infusing platelets to reduce bleeding risk is frequently used during surgery with patients on antiplatelet therapy.
- Previous reports exploring the usefulness of platelet transfusion in countering the antiplatelet effects of ticagrelor show mixed results.

WHAT THE STUDY ADDS

- Use of homologous platelets to counter the antiplatelet effects of ticagrelor therapy in cardiovascular patients is a viable strategy.
- Time elapsed since ticagrelor dose administration/treatment cessation plays a critical role in determining the degree of functional gains obtained with platelet supplementations.

modulator of any benefits achieved—is also unclear. Timing is a critical variable in urgent scenarios because platelets transfused too soon after antiplatelet dosing may be rendered ineffective by any residual drug in circulation.

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We have previously reported that adding treatment-naive platelets can adequately reverse clopidogrel's inhibitory effects and restore hemostatic potential,[9] and similar results can also be achieved after prasugrel treatment at the appropriate time.[10] The possibility of normalizing platelet reactivity after ticagrelor therapy has also been investigated in a few small studies with mixed outcomes, ranging from no recovery at all[11,12] to some restoration of function.[13] Clopidogrel and prasugrel are members of thienopyridine class, binding irreversibly to the $P2Y_{12}$ receptor and exerting long acting inhibitory effects, whereas ticagrelor is the first member of the cyclopentyltriazolo-pyrimidine class, with reversible binding and short-acting effects, hence the BID administration. Given the limited and conflicting information on the topic, the present study was conducted to investigate the possibility of restoring platelet reactivity of ticagrelor-treated patients, using concentrated platelets from healthy donors, and the effect quantity and timing of infusion has on any functional improvement achieved. The design of the study was based on 2 clinical scenarios where the need for restoring hemostasis may arise: (1) patient having received a loading dose of DAPT with ticagrelor, and (2) patient on maintenance DAPT with ticagrelor. Study aim was to investigate whether the platelet function of a patient on DAPT could be restored to pretreatment levels within 48 hours of dosing, using fresh platelet concentrates.

Methods

Study Design and Population

The study was conducted using an open-label, 2 treatment-regimen design in patients with stable cardiovascular disease. Each patient received DAPT as a loading dose (LD) and as maintenance therapy, and each dosing modality was followed by a protocol for in vitro platelet supplementation and functional assessment performed at 4 prespecified time points (Figure 1).

Study candidates underwent screening that included medical history taking, physical examination, routine blood work, drugs of abuse testing and 12-lead ECG. Patients with unstable or acute cardiovascular disease, history of stroke or bleeding disorders, clinically significant abnormalities in screening test results, and those on treatments known to affect hemostasis (including anticoagulants, antiplatelets except aspirin, fibrinolytics, and NSAIDs) in the month before study participation, were excluded. A separate group of healthy volunteers underwent the same screening process and served as donors for freshly prepared concentrated platelets used in supplementation.

On the day of the study, patients reported to the Atherothrombosis Research Unit where after testing of baseline (pretreatment) platelet function, they received a LD of DAPT (ticagrelor 180 mg plus aspirin 325 mg). At 4, 6, 24, and 48 hours post dosing, patient's blood samples were collected and supplemented with concentrated platelets to increase the samples' platelet counts by 0% (control), 25%, 50%, and 75%. Platelet reactivity in each sample was tested using 2 methodologies (Multiplate Analyzer and VerifyNow PRU assay). On completion of the LD regimen part of the study, patients received DAPT as maintenance therapy (MT; ticagrelor 90 mg BID plus aspirin 81 mg QD ×5–7 days), and the same study protocol was performed 4, 6, 24, and 48 hours after the last dosing (Figure 1).

The study complied with the declaration of Helsinki and was approved by the Program for the Protection of Human Subjects (Institutional Review Board) of the Icahn School of Medicine at Mount Sinai. A written informed consent was obtained from each study patient and donor before initiating any study-related procedures.

Blood Sampling

Blood samples were drawn without stasis by clean venipuncture from an antecubital vein using a 21-gauge butterfly cannula system (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Samples from patients were collected in siliconized vacutainer tubes containing 3.2% sodium citrate at baseline before the administration of ticagrelor and at 4, 6, 24, and 48 hours after the LD/last maintenance dose.

Blood samples for isolating concentrated platelets for supplementation were drawn from nontreated, healthy donors (3 per subject) into acid citrate dextrose and 3.2% sodium citrate tubes. Platelets were isolated from the acid citrate dextrose samples using a 2-step centrifugation protocol and resuspended in citrated platelet-poor plasma as previously reported.[10]

Platelet Supplementation

Cell counts in the concentrated platelets used for supplementation averaged between 2200 and $2500\times10^3/\mu L$ at the 4 assessment time points. At each postdose time point, the concentrates were added to treated patients' blood in vitro targeting increases in the samples platelet counts of 25%, 50%, and 75%. For example, targeted counts for a blood sample with $200\times10^3/\mu L$ platelets would be 250 (↑25%), 300 (↑50%), and 350 (↑75%)$\times10^3/\mu L$, respectively. In clinical use, transfusion of 1 platelets apheresis unit is expected to increase platelet count by as much as $50\times10^3/\mu L$ in an average adult,[14] or ≈25% in an adult patient with a platelet count of $200\times10^3/\mu L$.

Volume of added concentrate was always kept at <10% of the sample volume to which it was being added, thereby preventing undue sample dilution. After gentle mixing by inversion, samples were allowed to stand at room temperature for 15 minutes before testing, and all experiments were completed within 2 hours of the collection of blood.

Assessment of Platelet Reactivity

Platelet function was assessed using 2 separate techniques: impedance aggregometry and light transmission aggregometry.

Multiplate Analyzer (DiaPharma, West Chester, OH) measures platelet function by impedance aggregometry and is one of the most

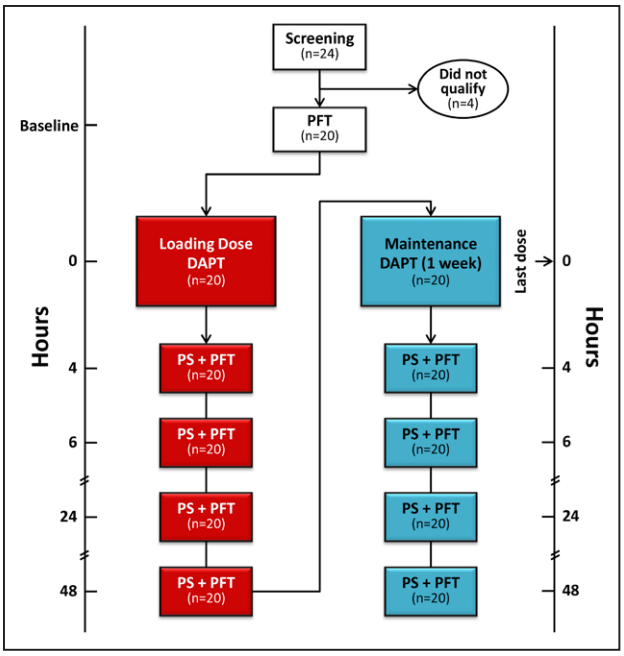

Figure 1. Study design. Platelet function tests (PFT): ADPtest in Multiplate Analyzer and PRUtest in VerifyNow. Platelet supplementation (PS): addition of concentrated platelets to dual antiplatelet therapy (DAPT)-treated patients' blood samples raising platelet counts by 0% (control), 25%, 50% and 75%.

widely used methodologies for platelet functional assessment in research. In addition to testing platelet reactivity in response to a wide variety of agonists, this methodology can also be used in the diagnosis of certain platelet disorders. Testing for study purposes was performed using the ADPtest assay, which stimulates platelet activation by adenosine diphosphate at a final concentration of 6.5 µmol/L, according to manufacturer's instructions. The most important adenosine diphosphate receptor ($P2Y_{12}$) is blocked by clopidogrel, ticagrelor, and prasugrel. Tests runs were for 6 minutes, and the parameter of area under the aggregation curve was recorded as units (U).

VerifyNow system (Accriva Diagnostics, San Diego, CA) is an Food and Drug Administration-approved, point-of-care device used in many catheterization laboratories. It is designed specifically for the testing of platelet inhibition by aspirin, $P2Y_{12}$ receptor inhibitors, and platelet glycoprotein IIb/IIIa inhibitors and is not suitable for diagnosing platelet disorders. VerifyNow, like the Multiplate, measures platelet aggregation in whole blood, but uses light transmission instead of impedance aggregometry for its assessment. The PRUtest kit, specific for assessing $P2Y_{12}$ receptor inhibition, was used for this study as per manufacturer's instructions, and the output of $P2Y_{12}$ reaction units (PRU) was recorded.

Statistical Analysis

The primary parameter was the platelet aggregation result area under the curve (U) generated by the Multiplate Analyzer. The pharmacodynamic parameters were listed and summarized using the standard statistics: mean±SD separately by each time point and the quantity of concentrated platelets added, unless specified otherwise.

Comparisons of platelet function across time points were performed using longitudinal mixed-effects model. Comparisons between supplementation levels within time points were tested using 1-way ANOVA. After testing for normality of distribution, pairwise comparisons were made using paired *t* test or Wilcoxon signed-rank test as appropriate, with Bonferroni correction. Baseline results were compared with each of the post-treatment time point means (in supplemented and nonsupplemented samples) using paired *t* test or Wilcoxon signed-rank test as appropriate. The last set of analyses was not corrected for multiple comparisons because the aim was to identify the earliest time point when a lack of statistical significance was observed.

The differences between the supplemented samples at each time point were visualized versus 100%, 90%, 80%, 70%, and 60% of baseline, using histograms. The threshold for statistical significance was set at the nominal P=0.05 level. All analyses were performed using STATA 14.2 software (StataCorp, College Station, TX).

Table 1. Baseline Demographic Characteristics of the Study Population

Baseline Characteristics (n=20)	
Age, y	56.9±7.9
Men, %	65%
BMI, kg/m^2	30.7±4.5
Type-II diabetes mellitus	75%
Hypertension	80%
Systolic blood pressure, mm Hg	127.2±17.5
Diastolic blood pressure, mm Hg	70.6±9.0
Hypercholesterolemia	95%
Cholesterol, mg/dL	158.7±62.0
Triglycerides, mg/dL	165.4±91.4
LDL, mg/dL	85.6±51.7
HDL, mg/dL	42.5±12.6
Smoking, %	
Current	10
Past	40
Never	50
Alcohol use, %	
Current	45
Past	15
Never	40

BMI indicates body mass index; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

Results

Twenty four patients underwent screening of which 4 were excluded for not meeting the inclusion/exclusion criteria. A total of 20 patients with cardiovascular disease (mean age of 56.9 years; 65% men and 75% diabetics) were enrolled in and completed both LD and MT parts of the study. Their demographic information is summarized in Table 1. No adverse events were reported by any of the study participants in the study.

Platelet Effects of Ticagrelor Loading Versus Maintenance Dosing

Platelet reactivity was significantly reduced after both LD and MT regimens and showed a nearly identical inhibitory pattern during the 48-hour testing period. The inhibitions were significant versus baseline at all 4 time points, with both testing methodologies (Figure 2). Peak inhibition was seen at 6 hours in both LD and MT dosing regimens with 82% and 80% inhibitions in multiplate results and 91% and 92% inhibitions

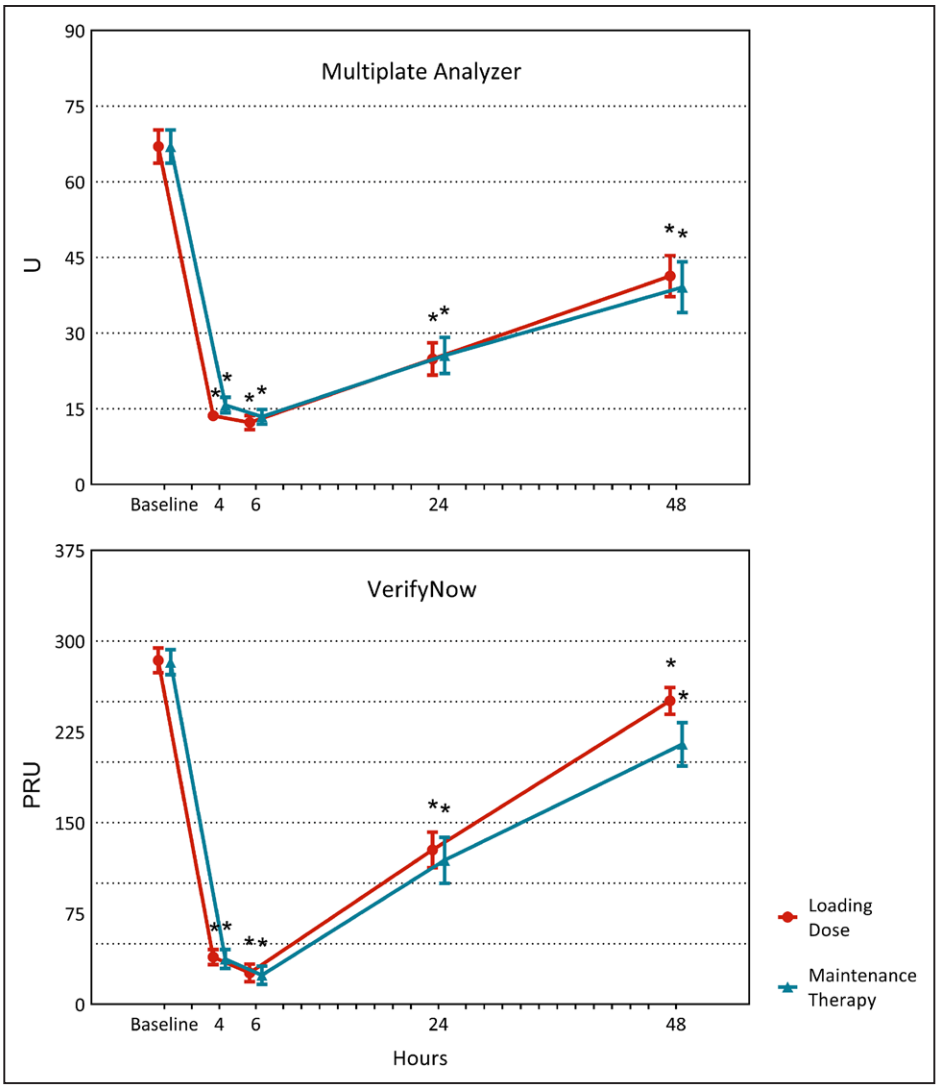

Figure 2. Time-dependent recovery of platelet function without supplementation after ticagrelor loading and maintenance therapy. Platelet reactivity (mean±SEM) is shown before (baseline) and after ticagrelor administration as a single loading dose and after maintenance therapy for 1 wk. A natural recovery in platelet reactivity was observed from 6 h onwards, in both Multiplate and VerifyNow testing. PRU indicates $P2Y_{12}$ reaction units. *P<0.05 vs baseline.

in PRU, respectively. Thereafter, a natural, time-dependent recovery in function was observed.

Platelet Function Normalization After Ticagrelor LD

Increases in platelet counts in the patients' samples after supplementation were consistent and close to specified targets of 25%, 50%, and 75% at each time point (Table I in the Data Supplement).

The addition of concentrated platelets produced a stepwise increase in platelet reactivity at each time point in Multiplate testing (Figure 3). These gains were statistically significant versus respective control (0%) samples even at the lowest supplementation level. Despite the statistically significant increases, platelet reactivity at 4 and 6 hours remained substantially lower than pretreatment value, peaking at 35% of the baseline aggregation. From 24 hours onwards, improvements in function from the addition of fresh platelets were more sizeable; with aggregations in 25%, 50%, and 75% supplemented samples reaching 59%, 74%, and 79% of baseline (Figure 3). By 48 hours, addition of fresh platelets even at the lowest tested level restored aggregation to where it was 85% of baseline (Table 2).

Results obtained with VerifyNow testing were less consistent than those with Multiplate. Platelet function after supplementation increased significantly versus respective control at 4, 6, and 24 hours, but not at 48 hours, and did not exhibit the consistent stepwise pattern observed with Multiplate testing (Figure 3). At 48 hours, PRU reached 90% of baseline in the control (0%) sample and the incremental addition of concentrated platelets produced an inverse effect on platelet reactivity (mean PRU of 251, 250, 226, and 204 with 0%, 25%, 50%, and 75% supplementations, respectively). Platelet function tested with VerifyNow could not be restored to baseline levels at any of the tested time points (Table 3).

Platelet Function Normalization After Ticagrelor MT

Increases in cell counts after the addition of concentrated platelets were consistently close to targets (Table I in the Data

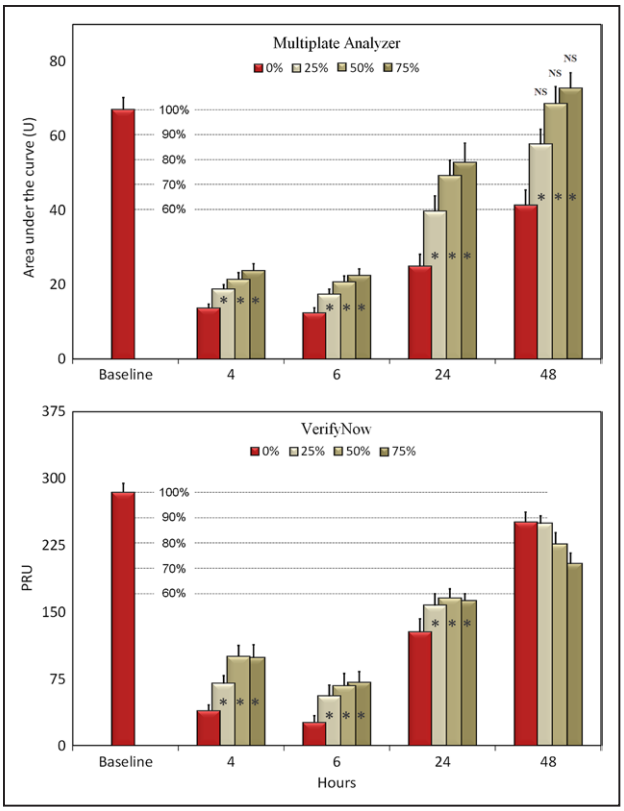

Figure 3. Restoring platelet function after ticagrelor loading dose. Platelet aggregation (mean±SEM) before (baseline) and after ticagrelor loading dose, with (25%, 50%, and 75%) and without (0%) platelet supplementation is shown. Reactivity was measured using Multiplate Analyzer ADPtest (U) and VerifyNow (PRU). Aggregation was significantly higher in all supplemented samples vs corresponding 0% sample except at 48 h in VerifyNow testing. At 48 hours, Multiplate testing showed aggregation in all supplemented samples to be statistically no different from baseline. NS indicates nonsignificant; and PRU, $P2Y_{12}$ reaction units. *P<0.05 vs corresponding 0% sample; NS: P>0.05 vs baseline.

Supplement). Functional improvements obtained with supplementation were stepwise and statistically significant versus the respective control at each time point in Multiplate testing (Figure 4). Supplementation did not improve function beyond 36% of baseline in the initial 6 hours. At 24 hours, addition of 25%, 50%, and 75% platelets produced results that reached 53%, 65%, and 71% of baseline (Table 2). By 48 hours, all 3 supplementation levels were able to restore function to baseline levels (mean aggregations of 56.8, 63.8, and 70.3 versus 67.0; P=0.051, 0.503, and 0.723, respectively; Figure 4; Table 2).

In VerifyNow testing, platelet function at 48 hours returned to 76% of baseline without the addition of any concentrated platelets. No amount of platelet supplementation could restore PRU to baseline levels at any of the tested time points (Figure 4; Table 3).

The platelet inhibitory effects of ticagrelor and the functional restoration achieved with supplementation after MT dosing regimen were nearly identical to those seen after LD regimen.

Discussion

Patients on DAPT with ticagrelor requiring surgical intervention are advised to stop treatment and wait 5 days for its antiplatelet effects to dissipate.[6] The ability of platelet concentrate supplementation to improve hemostasis in patients treated with clopidogrel and prasugrel has been previously reported.[9,10] In the present exploratory study, we found that concentrated platelets from healthy donors have the ability to restore platelet reactivity of ticagrelor-treated patients to pretreatment levels within 48 hours of LD administration or daily-therapy cessation.

The pharmacodynamic profile of ticagrelor after a loading versus maintenance dosing regimen seems almost identical in our study. Peak inhibitory effect was observed 6 hours after the dose administration in LD regimen and 6 hours after the last dose in MT regimen. In both regimens, an unaided physiological recovery in platelet function was evident beyond the 6-hour time point, resulting from a combination of gradual drug clearance from the body and the release of new platelets into circulation from the bone marrow. The pattern of natural recovery in platelet function observed in our study fits well with the guideline's recommendation on urgent CABG in $P2Y_{12}$ inhibitor-treated patients.

The addition of increasing levels of concentrated platelets from untreated, healthy donors caused a stepwise increase in platelet reactivity of ticagrelor-treated patients' blood. Although these gains were statistically significant at all tested time points, the clinical benefits of using platelet concentrates within the initial 6 hours of ticagrelor administration seem doubtful, given the diminutive absolute recovery attained this close to dosing. The lowest supplementation level (25%) used in this study corresponds to the increase in platelet count expected from transfusion of 1 platelets apheresis unit in an adult patient with a platelet count of $200\times10^3/\mu L$.[14] Even at 75%—a level impractical for most clinical scenarios—platelet supplementation fails to improve function beyond 36% of pretreatment baseline when administered within 6 hours of dosing. In other words, platelet inhibition at 6 hours even after supplementation is greater than what is typically observed after a 600-mg LD of clopidogrel.[15] This was equally true after both loading and maintenance dosing regimens. The muted hemostatic recovery within 4 to 6 hours of ticagrelor administration fits well with the half-life of ticagrelor at around 6.5 hours and its active metabolite AR-C124910XX at ≈9 hours.[16] It indicates inhibition of the newly added platelets by residual drug metabolite and is in concordance with the findings of earlier studies that had different patient populations and testing methodologies but assessment times identical or close to the 4- and 6-hour time points of our study.[11,13] By 24 hours, the improvements attained from adding fresh platelets seem much more substantial. The residual drug metabolite concentration around this time is too low to significantly affect new platelets,[16] and as a result, gains attained at 24 hours shift platelet function safely away from the cutoff associated with increased bleeding risk as per the Working Group on On-Treatment Platelet Reactivity.[17] By 48 hours, the substantial natural recovery in patients own platelet function combined with miniscule levels of drug metabolites in plasma allow platelet supplementation to restore reactivity back to pretreatment levels.

The overall results of the 2 testing methodologies used in our study—Multiplate and VerifyNow—were essentially analogous but displayed some differential characteristics

Table 2. Platelet Normalization Assessed Using Multiplate Analyzer (U)

	Loading Dose	Maintenance Therapy
	Aggregation (U, Mean±SD)	
Baseline	67.0±14.3	67.0±14.3
Postdose		
4 h		
0%	13.6±4.6	15.7±6.7
25%	18.7±5.2*	20.9±6.1*
50%	21.4±7.7*	22.1±5.9*
75%	23.6±8.4*	24.2±6.3*
6 h		
0%	12.3±5.9	13.4±6.0
25%	17.3±6.1*	16.5±5.5*
50%	20.7±6.5*	18.9±6.3*
75%	22.3±7.5*	20.4±6.2*
24 h		
0%	24.9±14.0	25.6±15.6
25%	39.7±18.1*	35.5±15.1*
50%	49.3±17.9*	43.3±16.7*
75%	52.8±22.7*	47.5±18.2*
48 h		
0%	41.3±17.7	39.1±21.3
25%	57.7±17.6*†	56.8±19.5*†
50%	68.6±20.4*†	63.8±19.1*†
75%	72.8±18.4*†	70.3±23.0*†

*P<0.05 vs corresponding 0%.
†P is not significant vs baseline.

Table 3. Platelet Normalization Assessed Using VerifyNow (PRU)

	Loading Dose	Maintenance Therapy
	Aggregation (PRU, Mean±SD)	
Baseline	284.1±42.2	284.1±42.2
Postdose		
4 h		
0%	39.1±25.9	37.5±32.4
25%	69.6±35.6*	72.2±38.5*
50%	100.3±49.6*	88.4±42.9*
75%	98.6±54.4*	85.9±49.4*
6 h		
0%	25.9±28.9	24.0±30.3
25%	55.3±50.2*	46.0±37.3*
50%	67.8±53.8*	55.4±41.8*
75%	71.2±42.8*	67.7±45.3*
24 h		
0%	127.6±58.8	119.0±78.0
25%	157.3±52.5*	147.6±65.2*
50%	165.9±40.5*	158.0±43.5*
75%	162.5±29.4*	154.0±51.0
48 h		
0%	250.6±45.8	214.8±74.0
25%	249.5±35.1	219.7±52.2
50%	226.2±51.6	221.2±44.2
75%	204.2±45.9	203.9±41.5

PRU indicates $P2Y_{12}$ reaction units.
*P<0.05 vs corresponding 0%.
†P is not significant vs baseline.

that confirm previous findings.[18] VerifyNow, designed primarily as a point-of-care device for assessing the inhibitory effects of antiplatelet agents, may not be ideally suited for measuring restoration of function using platelet concentrates because it uses light transmittance for its testing and may be susceptible to interpreting sample dilutions as functional changes. This assumption is supported by our data from the 48-hour time point, when the patients' PRU has recovered on its own to a level where platelet supplementation contributes no additional functionality in VerifyNow testing. With no opposing functional changes to mask it, the effects of dilution, thus, become apparent, and the addition of increasing levels (volumes) of platelet concentrates produces corresponding decreases in PRU. Impedance aggregometry in the Multiplate analyzer seem impervious to the effects of dilution and in fact makes it part of its standard testing protocol.

Based on the results of our study, it is apparent there is a short refractory period after ticagrelor treatment during which the ability of platelet transfusion to restore functionality is substantially lower. The availability of an antidote for ticagrelor would be desirable for the restoration of hemostasis in emergency situations.[19]

Conclusions

Platelet reactivity of patients treated with ticagrelor can be restored using concentrated platelets from healthy donors in a dose-dependent manner in in vitro testing. This is true whether the drug is administered as a LD or MT. Restoration of function is strongly dependent on the time elapsed since (last) dosing. Although statistically significant improvements in platelet function can be attained as early as 6 hours from treatment, these gains seem unlikely to be of substantial clinical value; therefore, a ticagrelor antidote could prove valuable for restoring platelet function in this period. The benefit quotient of platelet transfusion is likely to be significantly higher if administered 24 hours after the last drug intake, be it loading or MT.

Study Limitations

The study used ex vivo platelet supplementation as a proxy to gauge the functional gains obtained with in vivo platelet transfusions. Our overall study design is as close as one can get to the clinical scenario of a patient population receiving platelet transfusion, but it cannot truly reflect the actual clinical setting. This limitation, however, is more a part of the

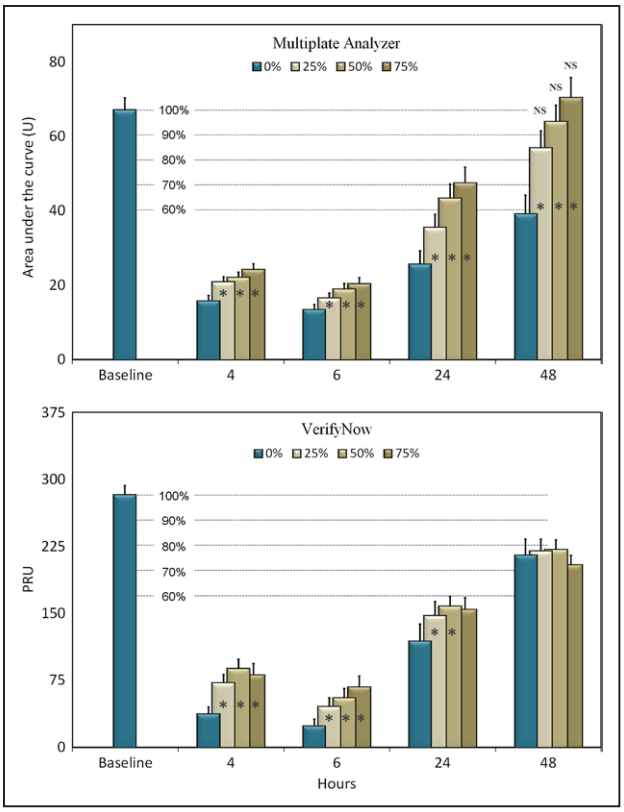

Figure 4. Restoring platelet function after ticagrelor maintenance therapy. Platelet aggregation (mean±SEM) before (baseline) and after 1 wk of ticagrelor maintenance therapy, with (25%, 50%, and 75%) and without (0%) platelet supplementation is shown. Reactivity was measured using Multiplate Analyzer ADPtest (U) and VerifyNow ($P2Y_{12}$ reaction units [PRU]). Aggregation was significantly higher in all supplemented samples vs corresponding 0% sample except at 48 h in VerifyNow testing. At 48 h, Multiplate testing showed aggregation in all supplemented samples to be statistically no different from baseline. NS indicates nonsignificant. *P<0.05 vs corresponding 0% sample; NS: P>0.05 vs baseline.

clinical setting than the study itself. A DAPT-treated surgical patient receiving platelet transfusion represents a serious situation, where clinical indications and ethical considerations hamper the standardization of critical study variables of timing and quantity of platelet infusion. The heterogeneity of variables associated with such a setup substantially raise the likelihood of inconclusive findings, leaving our chosen study design as the most viable option to investigate the specific aims of this study.

Despite the use of a surrogate measure, the findings of this study are significant for clinical practice. A previous study with in vivo platelet transfusion reported results consistent with this study, but because of the limitations inherent to a setup with in vivo transfusion, included no assessment of the effect of timing or quantity of transfusion.[13] Taken together, it is safe to assume that the overall findings of this study would be applicable in a clinical setting.

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Disclosures

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References

1. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA, Freij A, Thorsén M; PLATO Investigators. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2009;361:1045–1057. doi: 10.1056/NEJMoa0904327.
2. Steg PG, James S, Harrington RA, Ardissino D, Becker RC, Cannon CP, Emanuelsson H, Finkelstein A, Husted S, Katus H, Kilhamn J, Olofsson S, Storey RF, Weaver WD, Wallentin L, Group PS. Ticagrelor versus clopidogrel in patients with ST-elevation acute coronary syndromes intended for reperfusion with primary percutaneous coronary intervention: a platelet inhibition and patient outcomes (PLATO) trial subgroup analysis. *Circulation*. 2010;122:2131–2141. doi: 10.1161/CIRCULATIONAHA.109.927582.
3. Steg PG, Harrington RA, Emanuelsson H, Katus HA, Mahaffey KW, Meier B, Storey RF, Wojdyla DM, Lewis BS, Maurer G, Wallentin L, James SK; PLATO Study Group. Stent thrombosis with ticagrelor versus clopidogrel in patients with acute coronary syndromes: an analysis from the prospective, randomized PLATO trial. *Circulation*. 2013;128:1055–1065. doi: 10.1161/CIRCULATIONAHA.113.002589.
4. Eikelboom JW, Mehta SR, Anand SS, Xie C, Fox KA, Yusuf S. Adverse impact of bleeding on prognosis in patients with acute coronary syndromes. *Circulation*. 2006;114:774–782. doi: 10.1161/CIRCULATIONAHA.106.612812.
5. Yende S, Wunderink RG. Effect of clopidogrel on bleeding after coronary artery bypass surgery. *Crit Care Med*. 2001;29:2271–2275.
6. Hillis LD, Smith PK, Anderson JL, Bittl JA, Bridges CR, Byrne JG, Cigarroa JE, Disesa VJ, Hiratzka LF, Hutter AM, Jr, Jessen ME, Keeley EC, Lahey SJ, Lange RA, London MJ, Mack MJ, Patel MR, Puskas JD, Sabik JF, Selnes O, Shahian DM, Trost JC, Winniford MD; American College of Cardiology Foundation; American Heart Association Task Force on Practice Guidelines; American Association for Thoracic Surgery; Society of Cardiovascular Anesthesiologists; Society of Thoracic Surgeons. 2011 ACCF/AHA guideline for coronary artery bypass graft surgery. A report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. Developed in collaboration with the American Association for Thoracic Surgery, Society of Cardiovascular Anesthesiologists, and Society of Thoracic Surgeons. *J Am Coll Cardiol*. 2011;58:e123–e210. doi: 10.1016/j.jacc.2011.08.009.
7. Stainsby D, MacLennan S, Thomas D, Isaac J, Hamilton PJ; British Committee for Standards in Haematology. Guidelines on the management of massive blood loss. *Br J Haematol*. 2006;135:634–641. doi: 10.1111/j.1365-2141.2006.06355.x.
8. Sousa-Uva M, Storey R, Huber K, Falk V, Leite-Moreira AF, Amour J, Al-Attar N, Ascione R, Taggart D, Collet JP; ESC Working Group on Cardiovascular Surgery and ESC Working Group on Thrombosis. Expert position paper on the management of antiplatelet therapy in patients undergoing coronary artery bypass graft surgery. *Eur Heart J*. 2014;35:1510–1514. doi: 10.1093/eurheartj/ehu158.
9. Vilahur G, Choi BG, Zafar MU, Viles-Gonzalez JF, Vorchheimer DA, Fuster V, Badimon JJ. Normalization of platelet reactivity in clopidogrel-treated subjects. *J Thromb Haemost*. 2007;5:82–90. doi: 10.1111/j.1538-7836.2006.02245.x.
10. Zafar MU, Santos-Gallego C, Vorchheimer DA, Viles-Gonzalez JF, Elmariah S, Giannarelli C, Sartori S, Small DS, Jakubowski JA, Fuster V, Badimon JJ. Platelet function normalization after a prasugrel loading-dose: time-dependent effect of platelet supplementation. *J Thromb Haemost*. 2013;11:100–106. doi: 10.1111/jth.12058.

11. Bonhomme F, Bonvini R, Reny JL, Poncet A, Fontana P. Impact of non-inhibited platelet supplementation on platelet reactivity in patients treated with prasugrel or ticagrelor for an acute coronary syndrome: an ex vivo study. *Platelets*. 2015;26:324–330. doi: 10.3109/09537104.2015.1035247.
12. Martin AC, Berndt C, Calmette L, Philip I, Decouture B, Gaussem P, Gouin-Thibault I, Samama CM, Bachelot-Loza C, Godier A. The effectiveness of platelet supplementation for the reversal of ticagrelor-induced inhibition of platelet aggregation: an in-vitro study. *Eur J Anaesthesiol*. 2016;33:361–367. doi: 10.1097/EJA.0000000000000348.
13. O'Connor SA, Amour J, Mercadier A, Martin R, Kerneis M, Abtan J, Brugier D, Silvain J, Barthélémy O, Leprince P, Montalescot G, Collet JP; ACTION Study Group. Efficacy of ex vivo autologous and in vivo platelet transfusion in the reversal of P2Y12 inhibition by clopidogrel, prasugrel, and ticagrelor: the APTITUDE study. *Circ Cardiovasc Interv*. 2015;8:e002786. doi: 10.1161/CIRCINTERVENTIONS.115.002786.
14. Practice parameter for the use of fresh-frozen plasma, cryoprecipitate, and platelets. Fresh-frozen plasma, cryoprecipitate, and platelets administration practice guidelines development task force of the college of American pathologists. *JAMA*. 1994;271:777–781.
15. L'Allier PL, Ducrocq G, Pranno N, Noble S, Ibrahim R, Gregoire JC, Azzari F, Nozza A, Berry C, Doucet S, Labarthe B, Theroux P, Tardif JC; PREPAIR Study Investigators. Clopidogrel 600-mg double loading dose achieves stronger platelet inhibition than conventional regimens: results from the PREPAIR randomized study. *J Am Coll Cardiol*. 2008;51:1066–1072. doi: 10.1016/j.jacc.2007.12.013.
16. Butler K, Teng R. Pharmacokinetics, pharmacodynamics, safety and tolerability of multiple ascending doses of ticagrelor in healthy volunteers. *Br J Clin Pharmacol*. 2010;70:65–77. doi: 10.1111/j.1365-2125.2010.03669.x.
17. Tantry US, Bonello L, Aradi D, Price MJ, Jeong YH, Angiolillo DJ, Stone GW, Curzen N, Geisler T, Ten Berg J, Kirtane A, Siller-Matula J, Mahla E, Becker RC, Bhatt DL, Waksman R, Rao SV, Alexopoulos D, Marcucci R, Reny JL, Trenk D, Sibbing D, Gurbel PA; Working Group on On-Treatment Platelet Reactivity. Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding. *J Am Coll Cardiol*. 2013;62:2261–2273. doi: 10.1016/j.jacc.2013.07.101.
18. Vries MJ, Bouman HJ, Olie RH, Veenstra LF, Zwaveling S, Verhezen PW, Ten Cate-Hoek AJ, Ten Cate H, Henskens YM, van der Meijden PE. Determinants of agreement between proposed therapeutic windows of platelet function tests in vulnerable patients. *Eur Heart J Cardiovasc Pharmacother*. 2017;3:11–17. doi: 10.1093/ehjcvp/pvw026.
19. Buchanan A, Newton P, Pehrsson S, Inghardt T, Antonsson T, Svensson P, Sjogren T, Oster L, Janefeldt A, Sandinge AS, Keyes F, Austin M, Spooner J, Gennemark P, Penney M, Howells G, Vaughan T, Nylander S. Structural and functional characterization of a specific antidote for ticagrelor. *Blood*. 2015;125:3484–3490. doi: 10.1182/blood-2015-01-622928.

Impact of Timing on the Functional Recovery Achieved With Platelet Supplementation After Treatment With Ticagrelor

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SUPPLEMENTAL MATERIAL

Supplemental Table: Average platelet counts in patients' whole blood and percent increases after supplementation

		Platelet count in un-supplemented samples (x 10^3/µl)	Average increase in platelet counts achieved for the targeted supplementation levels		
			25%	50%	75%
Loading Dose	4 hours	245.1 (± 52.6)	24.7% (± 7.9%)	50.1% (± 6.0%)	74.3% (± 10.5%)
	6 hours	246.9 (± 44.9)	25.6% (± 5.9%)	49.2% (± 7.9%)	74.8% (± 6.8%)
	24 hours	246.5 (± 57.0)	24.5% (± 7.9%)	51.3% (± 4.7%)	74.9% (± 13.3%)
	48 hours	238.6 (± 58.0)	27.2% (± 6.4%)	50.1% (± 5.3%)	71.7% (± 6.8%)
Daily Dose	4 hours	240.7 (± 46.1)	26.9% (± 5.2%)	49.6% (± 6.0%)	71.0% (± 5.4%)
	6 hours	243.1 (± 51.2)	25.1% (± 5.3%)	47.9% (± 7.3%)	72.0% (± 11.4%)
	24 hours	238.7 (± 44.0)	28.5% (± 4.5%)	48.1% (± 7.0%)	70.4% (± 10.4%)
	48 hours	242.4 (± 52.8)	25.6% (± 5.4%)	50.9% (± 6.2%)	71.7% (± 6.7%)

Impact of Timing on the Functional Recovery Achieved With Platelet Supplementation After Treatment With Ticagrelor

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Background—American College of Cardiology/American Heart Association guidelines advise waiting 5 to 7 days before operating on $P2Y_{12}$ inhibitor-treated acute coronary syndrome patients, to allow dissipation of its antiplatelet effects. Platelet transfusion is often used to restore hemostasis during operations, but its effectiveness and optimal timing are unclear. We investigated the degree of functional gains obtained from platelet supplementation after loading and maintenance of dual antiplatelet therapy with ticagrelor and the influence of timing on this strategy.

Methods and Results—After baseline platelet testing (Multiplate Analyzer and VerifyNow), cardiovascular disease patients (n=20; 56.9±7.9 years; 65% men; 75% diabetic) received dual antiplatelet therapy as a single loading dose (ticagrelor 180 mg plus aspirin 325 mg) and as daily/maintenance treatment for 5 to 7 days (maintenance therapy: ticagrelor 90 mg BID plus aspirin 81 mg QD). At 4, 6, 24, and 48 hours from (last) dosing, patients' blood samples were supplemented with concentrated platelets from healthy donors in vitro, raising platelet counts by 0% (unsupplemented control), 25%, 50%, and 75%, and the function retested. Reactivity in supplemented samples was compared with respective 0% sample and with the pretreatment baseline. Results under loading dose and maintenance therapy regimens were nearly identical. Platelet reactivity was higher ($P<0.05$) in nearly all supplemented samples versus respective controls. Aggregations with supplementation were 59% to 79% of baseline at 24 hours and equal to baseline at 48 hours.

Conclusions—Platelet reactivity of ticagrelor-treated patients can be restored using concentrated platelets after a loading dose/maintenance therapy in a time-dependent manner under in vitro testing. Although statistically significant improvements are evident 6 hours after (last) dosing, ≤24 hours maybe needed for clinically meaningful restoration in platelet function.

Clinical Trial Registration—URL: https://clinicaltrials.gov. Unique identifier: NCT02201394.

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Key Words: aspirin ■ blood platelets ■ diabetes mellitus ■ humans ■ ticagrelor

Dual antiplatelet therapy (DAPT) is the standard of care in the management of acute coronary syndrome patients, with aspirin and clopidogrel combination being the current standard of care. Ticagrelor has demonstrated greater benefits than clopidogrel in reducing clinical events in acute coronary syndrome patients.[1–3] Although stronger platelet inhibition has clearly proven benefits in preventing ischemic events, it also complicates management of acute coronary syndrome patients by enhancing the risk of bleeding complications and the associated morbidity and mortality.[4] Repeat operation to control hemorrhage is almost 6× more likely if patients receive DAPT before coronary artery bypass grafting (CABG), and 20% of such patients require platelet transfusions.[5] The American College of Cardiology/American Heart Association guidelines for acute coronary syndrome patients requiring CABG surgery recommend discontinuation of any $P2Y_{12}$ receptor inhibitor therapy for at least 5 days before an elective operation and at least 24 hours before urgent CABG.[6] This treatment-devoid phase leaves these high-risk patients defenseless against recurrent ischemic events, and any means to shorten this vulnerable period would be of clinical value. Platelet transfusions are advised and frequently used to deal with bleeding complications during CABG surgery in patients on antiplatelet therapy,[7,8] and could potentially be used for the earlier restoration of hemostasis in DAPT-treated patients, thereby shortening their waiting time for operation. However, the degree to which the newly infused platelets restore patients' hemostatic function, and its relationship to the quantity of platelets infused, is unknown. More importantly, the effect of timing of transfusion relative to last drug intake—likely to be an important

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WHAT IS KNOWN

- The strategy of infusing platelets to reduce bleeding risk is frequently used during surgery with patients on antiplatelet therapy.
- Previous reports exploring the usefulness of platelet transfusion in countering the antiplatelet effects of ticagrelor show mixed results.

WHAT THE STUDY ADDS

- Use of homologous platelets to counter the antiplatelet effects of ticagrelor therapy in cardiovascular patients is a viable strategy.
- Time elapsed since ticagrelor dose administration/treatment cessation plays a critical role in determining the degree of functional gains obtained with platelet supplementations.

modulator of any benefits achieved—is also unclear. Timing is a critical variable in urgent scenarios because platelets transfused too soon after antiplatelet dosing may be rendered ineffective by any residual drug in circulation.

See Editorial by Kruger et al

We have previously reported that adding treatment-naive platelets can adequately reverse clopidogrel's inhibitory effects and restore hemostatic potential,[9] and similar results can also be achieved after prasugrel treatment at the appropriate time.[10] The possibility of normalizing platelet reactivity after ticagrelor therapy has also been investigated in a few small studies with mixed outcomes, ranging from no recovery at all[11,12] to some restoration of function.[13] Clopidogrel and prasugrel are members of thienopyridine class, binding irreversibly to the $P2Y_{12}$ receptor and exerting long acting inhibitory effects, whereas ticagrelor is the first member of the cyclopentyltriazolo-pyrimidine class, with reversible binding and short-acting effects, hence the BID administration. Given the limited and conflicting information on the topic, the present study was conducted to investigate the possibility of restoring platelet reactivity of ticagrelor-treated patients, using concentrated platelets from healthy donors, and the effect quantity and timing of infusion has on any functional improvement achieved. The design of the study was based on 2 clinical scenarios where the need for restoring hemostasis may arise: (1) patient having received a loading dose of DAPT with ticagrelor, and (2) patient on maintenance DAPT with ticagrelor. Study aim was to investigate whether the platelet function of a patient on DAPT could be restored to pretreatment levels within 48 hours of dosing, using fresh platelet concentrates.

Methods

Study Design and Population

The study was conducted using an open-label, 2 treatment-regimen design in patients with stable cardiovascular disease. Each patient received DAPT as a loading dose (LD) and as maintenance therapy, and each dosing modality was followed by a protocol for in vitro platelet supplementation and functional assessment performed at 4 prespecified time points (Figure 1).

Study candidates underwent screening that included medical history taking, physical examination, routine blood work, drugs of abuse testing and 12-lead ECG. Patients with unstable or acute cardiovascular disease, history of stroke or bleeding disorders, clinically significant abnormalities in screening test results, and those on treatments known to affect hemostasis (including anticoagulants, antiplatelets except aspirin, fibrinolytics, and NSAIDs) in the month before study participation, were excluded. A separate group of healthy volunteers underwent the same screening process and served as donors for freshly prepared concentrated platelets used in supplementation.

On the day of the study, patients reported to the Atherothrombosis Research Unit where after testing of baseline (pretreatment) platelet function, they received a LD of DAPT (ticagrelor 180 mg plus aspirin 325 mg). At 4, 6, 24, and 48 hours post dosing, patient's blood samples were collected and supplemented with concentrated platelets to increase the samples' platelet counts by 0% (control), 25%, 50%, and 75%. Platelet reactivity in each sample was tested using 2 methodologies (Multiplate Analyzer and VerifyNow PRU assay). On completion of the LD regimen part of the study, patients received DAPT as maintenance therapy (MT; ticagrelor 90 mg BID plus aspirin 81 mg QD ×5–7 days), and the same study protocol was performed 4, 6, 24, and 48 hours after the last dosing (Figure 1).

The study complied with the declaration of Helsinki and was approved by the Program for the Protection of Human Subjects (Institutional Review Board) of the Icahn School of Medicine at Mount Sinai. A written informed consent was obtained from each study patient and donor before initiating any study-related procedures.

Blood Sampling

Blood samples were drawn without stasis by clean venipuncture from an antecubital vein using a 21-gauge butterfly cannula system (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Samples from patients were collected in siliconized vacutainer tubes containing 3.2% sodium citrate at baseline before the administration of ticagrelor and at 4, 6, 24, and 48 hours after the LD/last maintenance dose.

Blood samples for isolating concentrated platelets for supplementation were drawn from nontreated, healthy donors (3 per subject) into acid citrate dextrose and 3.2% sodium citrate tubes. Platelets were isolated from the acid citrate dextrose samples using a 2-step centrifugation protocol and resuspended in citrated platelet-poor plasma as previously reported.[10]

Platelet Supplementation

Cell counts in the concentrated platelets used for supplementation averaged between 2200 and $2500\times10^3/\mu L$ at the 4 assessment time points. At each postdose time point, the concentrates were added to treated patients' blood in vitro targeting increases in the samples platelet counts of 25%, 50%, and 75%. For example, targeted counts for a blood sample with $200\times10^3/\mu L$ platelets would be 250 (↑25%), 300 (↑50%), and 350 (↑75%)$\times10^3/\mu L$, respectively. In clinical use, transfusion of 1 platelets apheresis unit is expected to increase platelet count by as much as $50\times10^3/\mu L$ in an average adult,[14] or ≈25% in an adult patient with a platelet count of $200\times10^3/\mu L$.

Volume of added concentrate was always kept at <10% of the sample volume to which it was being added, thereby preventing undue sample dilution. After gentle mixing by inversion, samples were allowed to stand at room temperature for 15 minutes before testing, and all experiments were completed within 2 hours of the collection of blood.

Assessment of Platelet Reactivity

Platelet function was assessed using 2 separate techniques: impedance aggregometry and light transmission aggregometry.

Multiplate Analyzer (DiaPharma, West Chester, OH) measures platelet function by impedance aggregometry and is one of the most

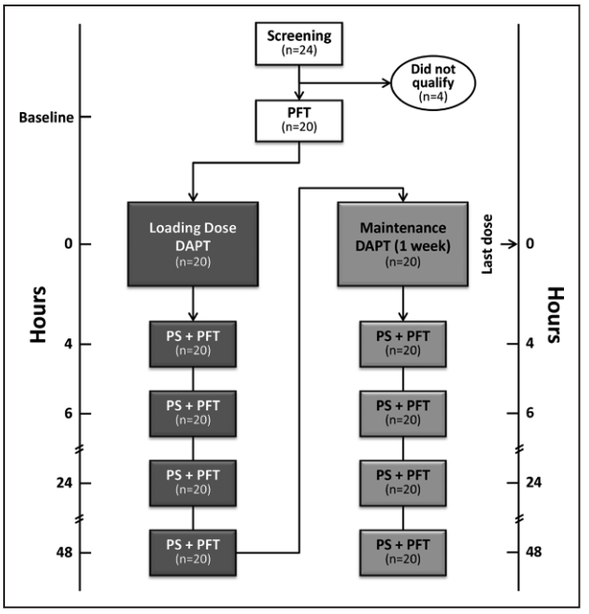

Figure 1. Study design. Platelet function tests (PFT): ADPtest in Multiplate Analyzer and PRUtest in VerifyNow. Platelet supplementation (PS): addition of concentrated platelets to dual antiplatelet therapy (DAPT)-treated patients' blood samples raising platelet counts by 0% (control), 25%, 50% and 75%.

widely used methodologies for platelet functional assessment in research. In addition to testing platelet reactivity in response to a wide variety of agonists, this methodology can also be used in the diagnosis of certain platelet disorders. Testing for study purposes was performed using the ADPtest assay, which stimulates platelet activation by adenosine diphosphate at a final concentration of 6.5 µmol/L, according to manufacturer's instructions. The most important adenosine diphosphate receptor ($P2Y_{12}$) is blocked by clopidogrel, ticagrelor, and prasugrel. Tests runs were for 6 minutes, and the parameter of area under the aggregation curve was recorded as units (U).

VerifyNow system (Accriva Diagnostics, San Diego, CA) is an Food and Drug Administration-approved, point-of-care device used in many catheterization laboratories. It is designed specifically for the testing of platelet inhibition by aspirin, $P2Y_{12}$ receptor inhibitors, and platelet glycoprotein IIb/IIIa inhibitors and is not suitable for diagnosing platelet disorders. VerifyNow, like the Multiplate, measures platelet aggregation in whole blood, but uses light transmission instead of impedance aggregometry for its assessment. The PRUtest kit, specific for assessing $P2Y_{12}$ receptor inhibition, was used for this study as per manufacturer's instructions, and the output of $P2Y_{12}$ reaction units (PRU) was recorded.

Statistical Analysis

The primary parameter was the platelet aggregation result area under the curve (U) generated by the Multiplate Analyzer. The pharmacodynamic parameters were listed and summarized using the standard statistics: mean±SD separately by each time point and the quantity of concentrated platelets added, unless specified otherwise.

Comparisons of platelet function across time points were performed using longitudinal mixed-effects model. Comparisons between supplementation levels within time points were tested using 1-way ANOVA. After testing for normality of distribution, pairwise comparisons were made using paired *t* test or Wilcoxon signed-rank test as appropriate, with Bonferroni correction. Baseline results were compared with each of the post-treatment time point means (in supplemented and nonsupplemented samples) using paired *t* test or Wilcoxon signed-rank test as appropriate. The last set of analyses was not corrected for multiple comparisons because the aim was to identify the earliest time point when a lack of statistical significance was observed.

The differences between the supplemented samples at each time point were visualized versus 100%, 90%, 80%, 70%, and 60% of baseline, using histograms. The threshold for statistical significance was set at the nominal P=0.05 level. All analyses were performed using STATA 14.2 software (StataCorp, College Station, TX).

Table 1. Baseline Demographic Characteristics of the Study Population

Baseline Characteristics (n=20)	
Age, y	56.9±7.9
Men, %	65%
BMI, kg/m^2	30.7±4.5
Type-II diabetes mellitus	75%
Hypertension	80%
Systolic blood pressure, mm Hg	127.2±17.5
Diastolic blood pressure, mm Hg	70.6±9.0
Hypercholesterolemia	95%
Cholesterol, mg/dL	158.7±62.0
Triglycerides, mg/dL	165.4±91.4
LDL, mg/dL	85.6±51.7
HDL, mg/dL	42.5±12.6
Smoking, %	
Current	10
Past	40
Never	50
Alcohol use, %	
Current	45
Past	15
Never	40

BMI indicates body mass index; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

Results

Twenty four patients underwent screening of which 4 were excluded for not meeting the inclusion/exclusion criteria. A total of 20 patients with cardiovascular disease (mean age of 56.9 years; 65% men and 75% diabetics) were enrolled in and completed both LD and MT parts of the study. Their demographic information is summarized in Table 1. No adverse events were reported by any of the study participants in the study.

Platelet Effects of Ticagrelor Loading Versus Maintenance Dosing

Platelet reactivity was significantly reduced after both LD and MT regimens and showed a nearly identical inhibitory pattern during the 48-hour testing period. The inhibitions were significant versus baseline at all 4 time points, with both testing methodologies (Figure 2). Peak inhibition was seen at 6 hours in both LD and MT dosing regimens with 82% and 80% inhibitions in multiplate results and 91% and 92% inhibitions

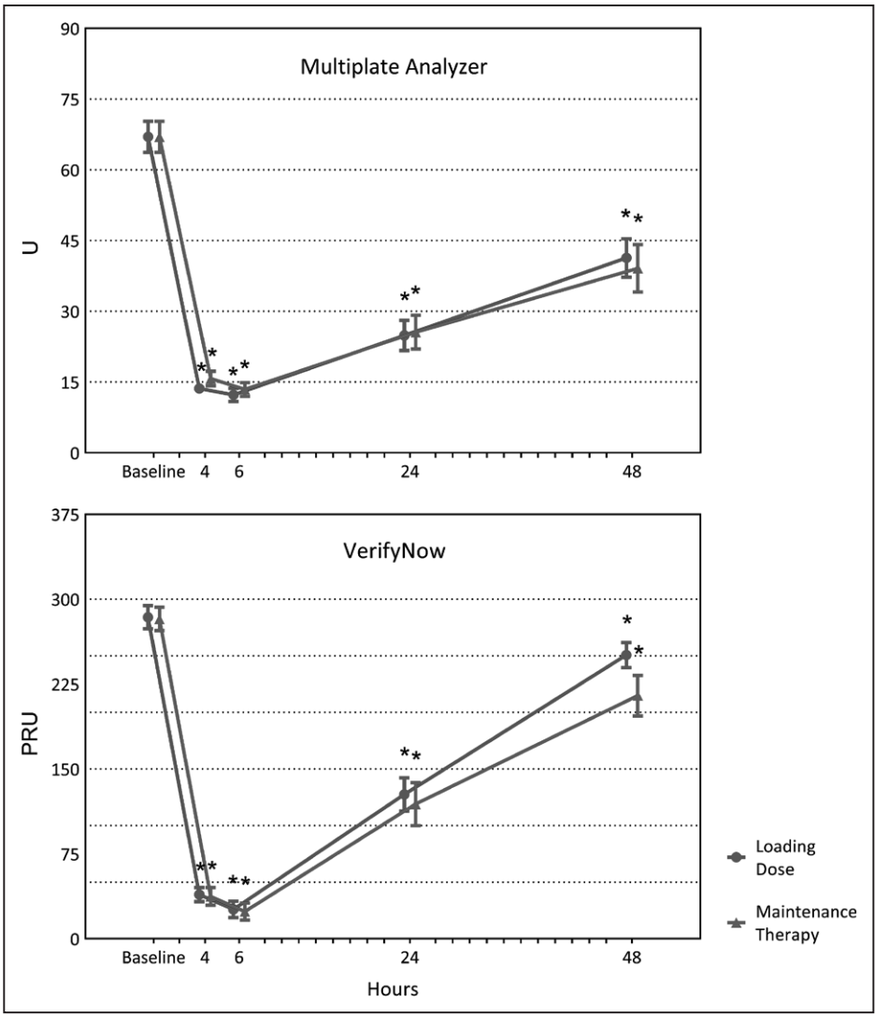

Figure 2. Time-dependent recovery of platelet function without supplementation after ticagrelor loading and maintenance therapy. Platelet reactivity (mean±SEM) is shown before (baseline) and after ticagrelor administration as a single loading dose and after maintenance therapy for 1 wk. A natural recovery in platelet reactivity was observed from 6 h onwards, in both Multiplate and VerifyNow testing. PRU indicates $P2Y_{12}$ reaction units. *P<0.05 vs baseline.

in PRU, respectively. Thereafter, a natural, time-dependent recovery in function was observed.

Platelet Function Normalization After Ticagrelor LD

Increases in platelet counts in the patients' samples after supplementation were consistent and close to specified targets of 25%, 50%, and 75% at each time point (Table I in the Data Supplement).

The addition of concentrated platelets produced a stepwise increase in platelet reactivity at each time point in Multiplate testing (Figure 3). These gains were statistically significant versus respective control (0%) samples even at the lowest supplementation level. Despite the statistically significant increases, platelet reactivity at 4 and 6 hours remained substantially lower than pretreatment value, peaking at 35% of the baseline aggregation. From 24 hours onwards, improvements in function from the addition of fresh platelets were more sizeable; with aggregations in 25%, 50%, and 75% supplemented samples reaching 59%, 74%, and 79% of baseline (Figure 3). By 48 hours, addition of fresh platelets even at the lowest tested level restored aggregation to where it was 85% of baseline (Table 2).

Results obtained with VerifyNow testing were less consistent than those with Multiplate. Platelet function after supplementation increased significantly versus respective control at 4, 6, and 24 hours, but not at 48 hours, and did not exhibit the consistent stepwise pattern observed with Multiplate testing (Figure 3). At 48 hours, PRU reached 90% of baseline in the control (0%) sample and the incremental addition of concentrated platelets produced an inverse effect on platelet reactivity (mean PRU of 251, 250, 226, and 204 with 0%, 25%, 50%, and 75% supplementations, respectively). Platelet function tested with VerifyNow could not be restored to baseline levels at any of the tested time points (Table 3).

Platelet Function Normalization After Ticagrelor MT

Increases in cell counts after the addition of concentrated platelets were consistently close to targets (Table I in the Data

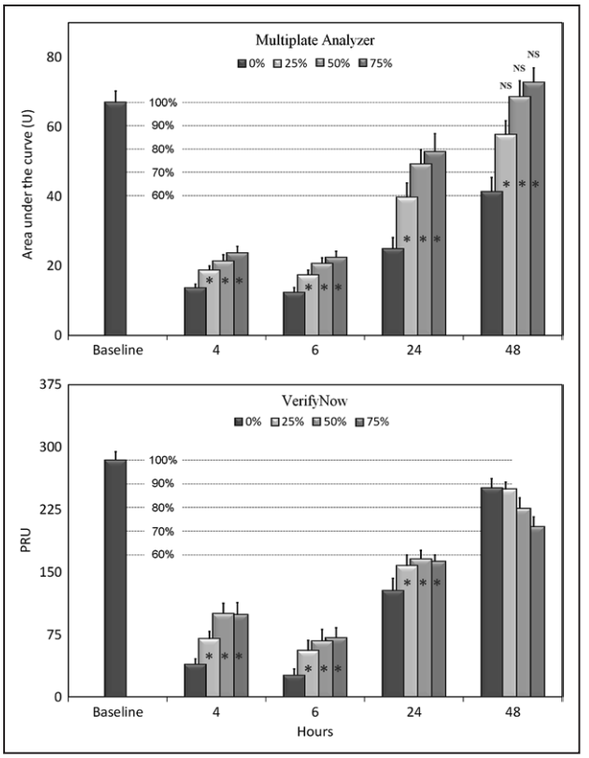

Figure 3. Restoring platelet function after ticagrelor loading dose. Platelet aggregation (mean±SEM) before (baseline) and after ticagrelor loading dose, with (25%, 50%, and 75%) and without (0%) platelet supplementation is shown. Reactivity was measured using Multiplate Analyzer ADPtest (U) and VerifyNow (PRU). Aggregation was significantly higher in all supplemented samples vs corresponding 0% sample except at 48 h in VerifyNow testing. At 48 hours, Multiplate testing showed aggregation in all supplemented samples to be statistically no different from baseline. NS indicates nonsignificant; and PRU, $P2Y_{12}$ reaction units. *P<0.05 vs corresponding 0% sample; NS: P>0.05 vs baseline.

Supplement). Functional improvements obtained with supplementation were stepwise and statistically significant versus the respective control at each time point in Multiplate testing (Figure 4). Supplementation did not improve function beyond 36% of baseline in the initial 6 hours. At 24 hours, addition of 25%, 50%, and 75% platelets produced results that reached 53%, 65%, and 71% of baseline (Table 2). By 48 hours, all 3 supplementation levels were able to restore function to baseline levels (mean aggregations of 56.8, 63.8, and 70.3 versus 67.0; P=0.051, 0.503, and 0.723, respectively; Figure 4; Table 2).

In VerifyNow testing, platelet function at 48 hours returned to 76% of baseline without the addition of any concentrated platelets. No amount of platelet supplementation could restore PRU to baseline levels at any of the tested time points (Figure 4; Table 3).

The platelet inhibitory effects of ticagrelor and the functional restoration achieved with supplementation after MT dosing regimen were nearly identical to those seen after LD regimen.

Discussion

Patients on DAPT with ticagrelor requiring surgical intervention are advised to stop treatment and wait 5 days for its antiplatelet effects to dissipate.[6] The ability of platelet concentrate supplementation to improve hemostasis in patients treated with clopidogrel and prasugrel has been previously reported.[9,10] In the present exploratory study, we found that concentrated platelets from healthy donors have the ability to restore platelet reactivity of ticagrelor-treated patients to pretreatment levels within 48 hours of LD administration or daily-therapy cessation.

The pharmacodynamic profile of ticagrelor after a loading versus maintenance dosing regimen seems almost identical in our study. Peak inhibitory effect was observed 6 hours after the dose administration in LD regimen and 6 hours after the last dose in MT regimen. In both regimens, an unaided physiological recovery in platelet function was evident beyond the 6-hour time point, resulting from a combination of gradual drug clearance from the body and the release of new platelets into circulation from the bone marrow. The pattern of natural recovery in platelet function observed in our study fits well with the guideline's recommendation on urgent CABG in $P2Y_{12}$ inhibitor-treated patients.

The addition of increasing levels of concentrated platelets from untreated, healthy donors caused a stepwise increase in platelet reactivity of ticagrelor-treated patients' blood. Although these gains were statistically significant at all tested time points, the clinical benefits of using platelet concentrates within the initial 6 hours of ticagrelor administration seem doubtful, given the diminutive absolute recovery attained this close to dosing. The lowest supplementation level (25%) used in this study corresponds to the increase in platelet count expected from transfusion of 1 platelets apheresis unit in an adult patient with a platelet count of $200\times10^3/\mu L$.[14] Even at 75%—a level impractical for most clinical scenarios—platelet supplementation fails to improve function beyond 36% of pretreatment baseline when administered within 6 hours of dosing. In other words, platelet inhibition at 6 hours even after supplementation is greater than what is typically observed after a 600-mg LD of clopidogrel.[15] This was equally true after both loading and maintenance dosing regimens. The muted hemostatic recovery within 4 to 6 hours of ticagrelor administration fits well with the half-life of ticagrelor at around 6.5 hours and its active metabolite AR-C124910XX at ≈9 hours.[16] It indicates inhibition of the newly added platelets by residual drug metabolite and is in concordance with the findings of earlier studies that had different patient populations and testing methodologies but assessment times identical or close to the 4- and 6-hour time points of our study.[11,13] By 24 hours, the improvements attained from adding fresh platelets seem much more substantial. The residual drug metabolite concentration around this time is too low to significantly affect new platelets,[16] and as a result, gains attained at 24 hours shift platelet function safely away from the cutoff associated with increased bleeding risk as per the Working Group on On-Treatment Platelet Reactivity.[17] By 48 hours, the substantial natural recovery in patients own platelet function combined with miniscule levels of drug metabolites in plasma allow platelet supplementation to restore reactivity back to pretreatment levels.

The overall results of the 2 testing methodologies used in our study—Multiplate and VerifyNow—were essentially analogous but displayed some differential characteristics

Table 2. Platelet Normalization Assessed Using Multiplate Analyzer (U)

	Loading Dose	Maintenance Therapy
	Aggregation (U, Mean±SD)	
Baseline	67.0±14.3	67.0±14.3
Postdose		
4 h		
0%	13.6±4.6	15.7±6.7
25%	18.7±5.2*	20.9±6.1*
50%	21.4±7.7*	22.1±5.9*
75%	23.6±8.4*	24.2±6.3*
6 h		
0%	12.3±5.9	13.4±6.0
25%	17.3±6.1*	16.5±5.5*
50%	20.7±6.5*	18.9±6.3*
75%	22.3±7.5*	20.4±6.2*
24 h		
0%	24.9±14.0	25.6±15.6
25%	39.7±18.1*	35.5±15.1*
50%	49.3±17.9*	43.3±16.7*
75%	52.8±22.7*	47.5±18.2*
48 h		
0%	41.3±17.7	39.1±21.3
25%	57.7±17.6*†	56.8±19.5*†
50%	68.6±20.4*†	63.8±19.1*†
75%	72.8±18.4*†	70.3±23.0*†

*$P<0.05$ vs corresponding 0%.
†P is not significant vs baseline.

Table 3. Platelet Normalization Assessed Using VerifyNow (PRU)

	Loading Dose	Maintenance Therapy
	Aggregation (PRU, Mean±SD)	
Baseline	284.1±42.2	284.1±42.2
Postdose		
4 h		
0%	39.1±25.9	37.5±32.4
25%	69.6±35.6*	72.2±38.5*
50%	100.3±49.6*	88.4±42.9*
75%	98.6±54.4*	85.9±49.4*
6 h		
0%	25.9±28.9	24.0±30.3
25%	55.3±50.2*	46.0±37.3*
50%	67.8±53.8*	55.4±41.8*
75%	71.2±42.8*	67.7±45.3*
24 h		
0%	127.6±58.8	119.0±78.0
25%	157.3±52.5*	147.6±65.2*
50%	165.9±40.5*	158.0±43.5*
75%	162.5±29.4*	154.0±51.0
48 h		
0%	250.6±45.8	214.8±74.0
25%	249.5±35.1	219.7±52.2
50%	226.2±51.6	221.2±44.2
75%	204.2±45.9	203.9±41.5

PRU indicates $P2Y_{12}$ reaction units.
*$P<0.05$ vs corresponding 0%.
†P is not significant vs baseline.

that confirm previous findings.[18] VerifyNow, designed primarily as a point-of-care device for assessing the inhibitory effects of antiplatelet agents, may not be ideally suited for measuring restoration of function using platelet concentrates because it uses light transmittance for its testing and may be susceptible to interpreting sample dilutions as functional changes. This assumption is supported by our data from the 48-hour time point, when the patients' PRU has recovered on its own to a level where platelet supplementation contributes no additional functionality in VerifyNow testing. With no opposing functional changes to mask it, the effects of dilution, thus, become apparent, and the addition of increasing levels (volumes) of platelet concentrates produces corresponding decreases in PRU. Impedance aggregometry in the Multiplate analyzer seem impervious to the effects of dilution and in fact makes it part of its standard testing protocol.

Based on the results of our study, it is apparent there is a short refractory period after ticagrelor treatment during which the ability of platelet transfusion to restore functionality is substantially lower. The availability of an antidote for ticagrelor would be desirable for the restoration of hemostasis in emergency situations.[19]

Conclusions

Platelet reactivity of patients treated with ticagrelor can be restored using concentrated platelets from healthy donors in a dose-dependent manner in in vitro testing. This is true whether the drug is administered as a LD or MT. Restoration of function is strongly dependent on the time elapsed since (last) dosing. Although statistically significant improvements in platelet function can be attained as early as 6 hours from treatment, these gains seem unlikely to be of substantial clinical value; therefore, a ticagrelor antidote could prove valuable for restoring platelet function in this period. The benefit quotient of platelet transfusion is likely to be significantly higher if administered 24 hours after the last drug intake, be it loading or MT.

Study Limitations

The study used ex vivo platelet supplementation as a proxy to gauge the functional gains obtained with in vivo platelet transfusions. Our overall study design is as close as one can get to the clinical scenario of a patient population receiving platelet transfusion, but it cannot truly reflect the actual clinical setting. This limitation, however, is more a part of the

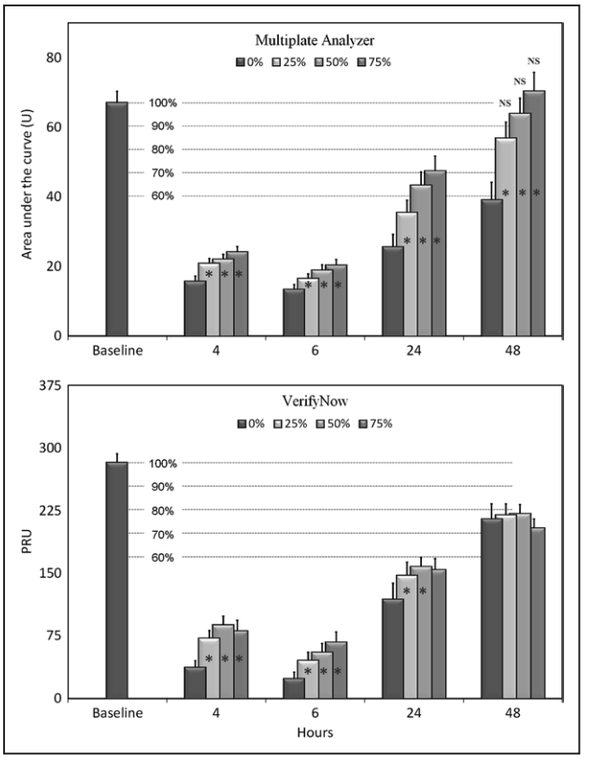

Figure 4. Restoring platelet function after ticagrelor maintenance therapy. Platelet aggregation (mean±SEM) before (baseline) and after 1 wk of ticagrelor maintenance therapy, with (25%, 50%, and 75%) and without (0%) platelet supplementation is shown. Reactivity was measured using Multiplate Analyzer ADPtest (U) and VerifyNow ($P2Y_{12}$ reaction units [PRU]). Aggregation was significantly higher in all supplemented samples vs corresponding 0% sample except at 48 h in VerifyNow testing. At 48 h, Multiplate testing showed aggregation in all supplemented samples to be statistically no different from baseline. NS indicates nonsignificant. *P<0.05 vs corresponding 0% sample; NS: P>0.05 vs baseline.

clinical setting than the study itself. A DAPT-treated surgical patient receiving platelet transfusion represents a serious situation, where clinical indications and ethical considerations hamper the standardization of critical study variables of timing and quantity of platelet infusion. The heterogeneity of variables associated with such a setup substantially raise the likelihood of inconclusive findings, leaving our chosen study design as the most viable option to investigate the specific aims of this study.

Despite the use of a surrogate measure, the findings of this study are significant for clinical practice. A previous study with in vivo platelet transfusion reported results consistent with this study, but because of the limitations inherent to a setup with in vivo transfusion, included no assessment of the effect of timing or quantity of transfusion.[13] Taken together, it is safe to assume that the overall findings of this study would be applicable in a clinical setting.

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References

1. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA, Freij A, Thorsén M; PLATO Investigators. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2009;361:1045–1057. doi: 10.1056/NEJMoa0904327.
2. Steg PG, James S, Harrington RA, Ardissino D, Becker RC, Cannon CP, Emanuelsson H, Finkelstein A, Husted S, Katus H, Kilhamn J, Olofsson S, Storey RF, Weaver WD, Wallentin L, Group PS. Ticagrelor versus clopidogrel in patients with ST-elevation acute coronary syndromes intended for reperfusion with primary percutaneous coronary intervention: a platelet inhibition and patient outcomes (PLATO) trial subgroup analysis. *Circulation*. 2010;122:2131–2141. doi: 10.1161/CIRCULATIONAHA.109.927582.
3. Steg PG, Harrington RA, Emanuelsson H, Katus HA, Mahaffey KW, Meier B, Storey RF, Wojdyla DM, Lewis BS, Maurer G, Wallentin L, James SK; PLATO Study Group. Stent thrombosis with ticagrelor versus clopidogrel in patients with acute coronary syndromes: an analysis from the prospective, randomized PLATO trial. *Circulation*. 2013;128:1055–1065. doi: 10.1161/CIRCULATIONAHA.113.002589.
4. Eikelboom JW, Mehta SR, Anand SS, Xie C, Fox KA, Yusuf S. Adverse impact of bleeding on prognosis in patients with acute coronary syndromes. *Circulation*. 2006;114:774–782. doi: 10.1161/CIRCULATIONAHA.106.612812.
5. Yende S, Wunderink RG. Effect of clopidogrel on bleeding after coronary artery bypass surgery. *Crit Care Med*. 2001;29:2271–2275.
6. Hillis LD, Smith PK, Anderson JL, Bittl JA, Bridges CR, Byrne JG, Cigarroa JE, Disesa VJ, Hiratzka LF, Hutter AM, Jr, Jessen ME, Keeley EC, Lahey SJ, Lange RA, London MJ, Mack MJ, Patel MR, Puskas JD, Sabik JF, Selnes O, Shahian DM, Trost JC, Winniford MD; American College of Cardiology Foundation; American Heart Association Task Force on Practice Guidelines; American Association for Thoracic Surgery; Society of Cardiovascular Anesthesiologists; Society of Thoracic Surgeons. 2011 ACCF/AHA guideline for coronary artery bypass graft surgery. A report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. Developed in collaboration with the American Association for Thoracic Surgery, Society of Cardiovascular Anesthesiologists, and Society of Thoracic Surgeons. *J Am Coll Cardiol*. 2011;58:e123–e210. doi: 10.1016/j.jacc.2011.08.009.
7. Stainsby D, MacLennan S, Thomas D, Isaac J, Hamilton PJ; British Committee for Standards in Haematology. Guidelines on the management of massive blood loss. *Br J Haematol*. 2006;135:634–641. doi: 10.1111/j.1365-2141.2006.06355.x.
8. Sousa-Uva M, Storey R, Huber K, Falk V, Leite-Moreira AF, Amour J, Al-Attar N, Ascione R, Taggart D, Collet JP; ESC Working Group on Cardiovascular Surgery and ESC Working Group on Thrombosis. Expert position paper on the management of antiplatelet therapy in patients undergoing coronary artery bypass graft surgery. *Eur Heart J*. 2014;35:1510–1514. doi: 10.1093/eurheartj/ehu158.
9. Vilahur G, Choi BG, Zafar MU, Viles-Gonzalez JF, Vorchheimer DA, Fuster V, Badimon JJ. Normalization of platelet reactivity in clopidogrel-treated subjects. *J Thromb Haemost*. 2007;5:82–90. doi: 10.1111/j.1538-7836.2006.02245.x.
10. Zafar MU, Santos-Gallego C, Vorchheimer DA, Viles-Gonzalez JF, Elmariah S, Giannarelli C, Sartori S, Small DS, Jakubowski JA, Fuster V, Badimon JJ. Platelet function normalization after a prasugrel loading-dose: time-dependent effect of platelet supplementation. *J Thromb Haemost*. 2013;11:100–106. doi: 10.1111/jth.12058.

11. Bonhomme F, Bonvini R, Reny JL, Poncet A, Fontana P. Impact of non-inhibited platelet supplementation on platelet reactivity in patients treated with prasugrel or ticagrelor for an acute coronary syndrome: an ex vivo study. *Platelets*. 2015;26:324–330. doi: 10.3109/09537104.2015.1035247.
12. Martin AC, Berndt C, Calmette L, Philip I, Decouture B, Gaussem P, Gouin-Thibault I, Samama CM, Bachelot-Loza C, Godier A. The effectiveness of platelet supplementation for the reversal of ticagrelor-induced inhibition of platelet aggregation: an in-vitro study. *Eur J Anaesthesiol*. 2016;33:361–367. doi: 10.1097/EJA.0000000000000348.
13. O'Connor SA, Amour J, Mercadier A, Martin R, Kerneis M, Abtan J, Brugier D, Silvain J, Barthélémy O, Leprince P, Montalescot G, Collet JP; ACTION Study Group. Efficacy of ex vivo autologous and in vivo platelet transfusion in the reversal of P2Y12 inhibition by clopidogrel, prasugrel, and ticagrelor: the APTITUDE study. *Circ Cardiovasc Interv*. 2015;8:e002786. doi: 10.1161/CIRCINTERVENTIONS.115.002786.
14. Practice parameter for the use of fresh-frozen plasma, cryoprecipitate, and platelets. Fresh-frozen plasma, cryoprecipitate, and platelets administration practice guidelines development task force of the college of American pathologists. *JAMA*. 1994;271:777–781.
15. L'Allier PL, Ducrocq G, Pranno N, Noble S, Ibrahim R, Gregoire JC, Azzari F, Nozza A, Berry C, Doucet S, Labarthe B, Theroux P, Tardif JC; PREPAIR Study Investigators. Clopidogrel 600-mg double loading dose achieves stronger platelet inhibition than conventional regimens: results from the PREPAIR randomized study. *J Am Coll Cardiol*. 2008;51:1066–1072. doi: 10.1016/j.jacc.2007.12.013.
16. Butler K, Teng R. Pharmacokinetics, pharmacodynamics, safety and tolerability of multiple ascending doses of ticagrelor in healthy volunteers. *Br J Clin Pharmacol*. 2010;70:65–77. doi: 10.1111/j.1365-2125.2010.03669.x.
17. Tantry US, Bonello L, Aradi D, Price MJ, Jeong YH, Angiolillo DJ, Stone GW, Curzen N, Geisler T, Ten Berg J, Kirtane A, Siller-Matula J, Mahla E, Becker RC, Bhatt DL, Waksman R, Rao SV, Alexopoulos D, Marcucci R, Reny JL, Trenk D, Sibbing D, Gurbel PA; Working Group on On-Treatment Platelet Reactivity. Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding. *J Am Coll Cardiol*. 2013;62:2261–2273. doi: 10.1016/j.jacc.2013.07.101.
18. Vries MJ, Bouman HJ, Olie RH, Veenstra LF, Zwaveling S, Verhezen PW, Ten Cate-Hoek AJ, Ten Cate H, Henskens YM, van der Meijden PE. Determinants of agreement between proposed therapeutic windows of platelet function tests in vulnerable patients. *Eur Heart J Cardiovasc Pharmacother*. 2017;3:11–17. doi: 10.1093/ehjcvp/pvw026.
19. Buchanan A, Newton P, Pehrsson S, Inghardt T, Antonsson T, Svensson P, Sjogren T, Oster L, Janefeldt A, Sandinge AS, Keyes F, Austin M, Spooner J, Gennemark P, Penney M, Howells G, Vaughan T, Nylander S. Structural and functional characterization of a specific antidote for ticagrelor. *Blood*. 2015;125:3484–3490. doi: 10.1182/blood-2015-01-622928.